TIAGO TAIETE

“Molecular technologies in the study of periodontal diseases”

“Tecnologias moleculares no estudo das doenças periodontais”

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FACULDADE DE ODONTOLOGIA DE PIRACICABA

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“Molecular technologies in the study of periodontal diseases”

Orientador: Prof. Dr. Márcio Zaffalon Casati

“Tecnologias moleculares no estudo das doenças periodontais”

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Clínica Odontológica da Faculdade de Odontologia de Piracicaba da UNICAMP para obtenção do Título de Mestre em Clínica Odontológica, na área de Periodontia.

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Epígrafe

“O correr da vida embrulha tudo.

A vida é assim: esquenta e esfria,

aperta e daí afrouxa,

sossega e depois desinquieta.

O que ela quer da gente é coragem”

Guimarães Rosa

RESUMO

A periodontite agressiva é caraterizada por apresentar início precoce, rápida progressão e pobre resposta as abordagens terapêuticas quando comparado a periodontite crônica. Entretanto, os mecanismos responsáveis por essas diferenças ainda não são completamente compreendidos. Ferramentas como a genômica, proteômica ou transcriptômica podem esclarecer esses aspectos, gerando importantes informações a respeito da patogênese das doenças periodontais. Portanto, os objetivos do presente estudo foram: i) Apresentar uma revisão de literatura focada na aplicação da genômica, transcriptômica, proteômica e metabolômica no estudo das doenças periodontais. ii) Avaliar as diferenças no perfil de expressão gênica da mucosa mastigatória, sem a influência do biofilme subgengival, em indivíduos com histórico de periodontite agressiva e crônica, comparando-os entre si e também com o perfil de expressão de indivíduos sem histórico de periodontite visando à identificação de possíveis alterações constitutivas na expressão de genes que podem estar relacionadas com as diferenças entre as duas formas de periodontite. Para o primeiro objetivo, foi realizada uma revisão dos artigos que empregaram as tecnologias ômicas no estudo das doenças periodontais. Os estudos presentes na literatura indicaram que essas tecnologias podem levar a um melhor entendimento dos eventos moleculares envolvidos na patogênese das doenças periodontais. Para o segundo objetivo, foram obtidas amostras teciduais da mucosa mastigatória de 4 pacientes com histórico de periodontite crônica, 4 com periodontite agressiva generalizada e 4 indivíduos sem histórico de periodontite. As amostras foram manipuladas para a extração de RNA total, síntese de cDNA de fita dupla e a hibridização por microarranjo de DNA, avaliando a expressão de 45033 genes. Os programas R e Bioconductor foram empregados para realizar a análise de RMA (Robust-Multi-Array), calcular o valor de expressão (fold-change) e o valor de *p* para comparações múltiplas através do método de Benjamini-Hochberg. O enriquecimento dos dados, através de ontologia gênica e análise de

via, foi realizado com o programa de bioinformática DAVID (Database for Annotation, Visualization and Integrated Discovery). Três comparações foram realizadas: comparação 1 (periodontite agressiva x indivíduos sem histórico de periodontite); comparação 2 (periodontite crônica x indivíduos sem histórico de periodontite); e comparação 3 (periodontite crônica x periodontite agressiva). A comparação entre os grupos identificou: 192 genes e 50 grupos diferencialmente expressos na comparação 1, 43 genes e 27 grupos na comparação 2 e 168 genes e 75 grupos na comparação 3. Genes com funções no sistema imune, entre os quais receptores de linfócitos natural killer, foram mais expressos em periodontite agressiva; em contraste, genes envolvidos na proliferação e diferenciação de queratinócitos, assim como genes com função em processos neurais foram menos expressos. Já os indivíduos com periodontite crônica se caracterizaram por um aumento na expressão de genes com função na resposta a estímulos externos, e uma diminuição na expressão dos genes relacionados ao sistema imune. Dentro dos limites desse estudo, pode-se concluir que existem diferenças nos perfis de expressão gênica da mucosa mastigatória de indivíduos com histórico de periodontite agressiva e crônica, quando comparadas entre si e com indivíduos sem histórico de periodontite, que podem estar associadas às diferenças em suas patogêneses.

Palavras-chave: **Periodontite agressiva; Periodontite crônica; Biologia molecular; Expressão Gênica; Susceptibilidade a doenças.**

ABSTRACT

Aggressive periodontitis is characterized by early onset, rapid progression and poor response to treatment compared to chronic periodontitis. However, mechanisms responsible for these differences are not fully understood. Tools such as genomics, proteomics and transcriptomics can clarify these aspects, producing important information about the pathogenesis of periodontal diseases. Therefore, the aims of this study were: i) Provide a literature review focused on the application of genomics, transcriptomics, proteomics, and metabolomics in the study of periodontal diseases. ii) Evaluate the gene expression profile of tissue from masticatory mucosa, without the influence of biofilm, in patients with a history of generalized aggressive and chronic periodontitis, and also with gene expression profile of individuals without periodontitis, with the aim to identify possible constitutive alterations in gene expression, which may be related to the differences between both forms of periodontitis. For the first objective, it was performed a review of articles that employed omics technologies in the study of periodontal diseases. The studies in the literature indicated that theses technologies can lead to a better understanding of molecular events involved in the pathogenesis of periodontal diseases. For the second objective, masticatory mucosa tissue samples were obtained from 4 patients with a history of chronic periodontitis, 4 with generalized aggressive periodontitis, and 4 from subjects without history of periodontitis. Tissue samples were processed for total RNA extraction, double-strand cDNA synthesis for subsequent hybridization reaction on microarrays, which assessed the expression of 45033 genes. R and Bioconductor softwares used to perform the RMA analysis (Robust Multi-Array), calculate the value of expression (fold-change) and the *p*-value for multiple comparisons through Benjamini-Hochberg method. Enrichment analysis, through gene ontology (GO) and pathway analysis, was performed with DAVID (Database for Annotation, Visualization and Integrated Discovery). Three comparisons were performed: comparison 1 (aggressive periodontitis x healthy subjects); comparison 2 (chronic periodontitis x

healthy subjects); and comparison 3 (chronic periodontitis x aggressive periodontitis). Comparisons analysis found 192 genes and 50 groups differentially expressed in comparison 1, 43 genes and 27 groups in comparison 2, and 168 genes and 75 groups in comparison 3. Genes with function in the immune system, including natural killer cells receptors, were higher expressed in aggressive periodontitis; in contrast, genes involved in proliferation and differentiation of keratinocytes, as well as genes with function in neural process were lower expressed. Chronic periodontitis subjects were characterized by increased expression of genes related to response to external stimuli, and a decrease in the expression of genes related to the immune system. Within the limits of this study, it can be concluded that there are differences in the gene expression profile of masticatory mucosa of patients with a history of chonic and aggressive periodontitis, when compared among them and with healthy group, which may be associated with differences in their pathogenesis.

Key words: **Aggressive periodontitis; Chronic periodontitis; Molecular biology; Gene expression; Disease susceptibility.**

Sumário

Introdução

A periodontite é uma doença inflamatória de etiologia multifatorial, caracterizada pela destruição óssea e perda de inserção conjuntiva (Feng & Weinberg, 2006). A destruição do suporte periodontal, em sua maior parte, é resultado da resposta imune-inflamatória do hospedeiro aos periodontopatógenos (Socransky & Haffajee, 2005). Estudos epidemiológicos apontam que a periodontite crônica tem maior prevalência, afetando mais de 50% da população, enquanto que os indivíduos diagnosticados com periodontite agressiva representam 1 a 15% da população (Demmer & Papapanou, 2010).

Embora a periodontite crônica e a agressiva aparentemente derivam dos mesmos fatores etiológicos, elas apresentam diferenças claras no seu desenvolvimento e progressão (Tonetti & Mombelli, 1999). Enquanto a periodontite crônica apresenta uma progressão lenta, usualmente compatível com os fatores locais, a periodontite agressiva é definida como uma doença inflamatória de início precoce, acometendo geralmente indivíduos jovens, caracterizada pela rápida perda de inserção e destruição óssea (Armitage, 1999). Visando uma melhor compreensão dos fatores envolvidos na patogênese destas doenças e tentando explicar possíveis diferenças entre as formas crônica e agressiva da periodontite, aspectos microbiológicos, imunológicos e genéticos têm sido estudados; entretanto os mecanismos responsáveis por essas diferenças ainda não foram completamente explicados (Kinane *et al*., 1999; Armitage, 2002; Casarin *et al*., 2010).

Na tentativa de esclarecer essas dúvidas, novas abordagens como a genômica, transcriptômica, proteômica e metabolômica tem sido utilizadas para se avaliar os mecanismos envolvidos na patogênese da doença periodontal. De fato, alguns estudos avaliaram o transcriptoma do tecido gengival e de células mononucleares da circulação periférica de pacientes com periodontite (Papapanou *et al*., 2004; Beikler *et al*., 2008; Demmer *et al*., 2008; Sorensen *et al*., 2008; Papapanou *et al*., 2009). Sorensen *et al*. (2008) avaliaram a expressão gênica de

monócitos e linfócitos da circulação periférica de pacientes com periodontite agressiva localizada e generalizada, demonstrando que o perfil de expressão gênica na periodontite agressiva localizada foi homogêneo, enquanto na periodontite agressiva generalizada a expressão gênica foi heterogênea, indicando uma entidade patológica mais complexa e variável (Sorensen *et al*., 2008).

Em 2004, em um estudo piloto, Papapanou *et al*. utilizando a técnica de microarray (microarranjo de DNA) para avaliar a expressão gênica do tecido gengival inflamado de pacientes com periodontite crônica e agressiva, não encontraram diferenças significativas na expressão gênica entre os dois tipos de periodontite. Empregando a mesma tecnologia, Demmer *et al*. (2008) avaliaram a expressão gênica entre tecidos gengivais sadios e acometidos por doença periodontal, anterior a qualquer intervenção terapêutica, não fazendo distinção entre as formas crônica e agressiva. Os resultados mostraram que da análise do transcriptoma, 12744 genes foram diferencialmente expressos, sendo que 5295 foram mais expressos e 7449 foram menos expressos nos tecidos acometidos por periodontite. Da mesma maneira a análise por ontologia gênica revelou que 61 grupos (incluindo apoptose, resposta humoral antimicrobiana, apresentação de antígenos, regulação do processo metabólico e angiogênese) foram diferencialmente expressos nos tecidos doentes, indicando o envolvimento de um amplo número de genes e, consequentemente, de seus mecanismos intracelulares. A despeito desses estudos, genes específicos relacionados à doença periodontal ainda não foram identificados.

Entretanto, deve-se ressaltar que os resultados da análise de expressão gênica dos tecidos gengivais podem ser influenciados pela composição da microbiota, pelo grau de inflamação tecidual, por fatores ambientais, por fatores genéticos e epigenéticos (Demmer *et al*., 2008; Kornman, 2008; Papapanou *et al*., 2009). Dessa forma, para determinar diferenças entre distintos perfis de doença, devem-se considerar alterações constitutivas que podem estar presentes nesses indivíduos, ou seja, avaliar a expressão gênica quando essas variáveis estão

ausentes. Essa avaliação permitiria determinar alterações que podem estar relacionadas a alterações genéticas dos indivíduos ou alterações do controle celular individual (Kinane *et al*., 2006; Takashiba & Naruishi, 2006; Stabholz *et al*., 2010; Nahid *et al*., 2011). Além disso, é importante compreender que o perfil de expressão gênica dos tecidos sadios e intactos de indivíduos com periodontite pode apresentar diferenças se comparados aos sítios saudáveis de indivíduos que não tiveram experiência de periodontite (Demmer *et al*., 2008).

Nesse contexto, a avaliação do perfil de expressão gênica da mucosa mastigatória, sem a influência da microbiota na expressão gênica tecidual, pode permitir a identificação de possíveis alterações dos mecanismos da fisiologia tecidual, próprios dos indivíduos, que possam ser traduzidos em um perfil de maior susceptibilidade ao desenvolvimento das doenças periodontais. Esse tipo de análise se justifica uma vez que a presença de bactérias na região do sulco gengival, mesmo aquelas compatíveis com a saúde periodontal, exercem uma influência na expressão gênica tecidual (Papapanou *et al*., 2009), impossibilitando a análise das características relacionadas apenas com o indivíduo.

Portanto, os objetivos do presente estudo foram: i) apresentar uma revisão de literatura focada na aplicação da genômica, transcriptômica, proteômica e metabolômica no estudo das doenças periodontais. ii) analisar o perfil de expressão gênica da mucosa mastigatória, sem influência do biofilme, de pacientes com histórico de periodontite crônica e agressiva, comparando-os entre si e também com o perfil de expressão de indivíduos sem histórico de periodontite. Descrevendo os genes, as classes e as vias que tais genes pertencem, com expressão diferenciada entre os grupos.

Capítulo 1

The potential use of molecular approaches for diagnosis in periodontics: the role of omics knowledge

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Running title: Molecular diagnosis of periodontal disease

Keywords: periodontitis; biomarkers; genomics; transcriptomics; proteomics; metabolomics

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Abstract

Periodontal diseases are highly complex and have multifactorial etiology processes with the participation of several biological mechanisms. This review sought to discuss some of the advances in scientific research that use molecular biology methods to address the aspects of genomic, proteomic, transcriptomic, and metabolomic of periodontal diseases. Several studies in the literature show that these tools aim to promote a better understanding of the mechanisms involved in the pathogenesis and identification of new biomarkers of periodontal diseases that

may be used in future diagnostic tests to detect the presence of active disease, predict future progression, and evaluate the response to periodontal therapy.

Introduction

Periodontitis is a group of chronic inflammatory diseases affecting the teeth and supporting tissues, including the alveolar bone (Dennison and van Dyke, 1997; Sørensen *et al*., 2008). It is one of the most common causes of tooth loss in adults (Chung *et al*., 2009). The disease develops when there is an imbalance between the microbial challenge and host immune-inflammatory response, with its clinical expression altered by important environmental and host-modifying conditions (Quirynen *et al*., 2001; Armitage, 2002).

Although the knowledge about the pathology pathways has grown rapidly in recent decades, the underlying mechanisms of many diseases remain obscure (Burke, 2003), including periodontal diseases. Chronic and aggressive periodontitis diseases appear to derive from the same etiologic factors, they present clear differences in their development and progression (Tonetti and Mombelli, 1999); however, the mechanisms responsible for these differences remain unknown (Kinane *et al*., 1999; Casarin *et al*., 2010). In addition, it is highly probable that chronic periodontitis or aggressive periodontitis diseases are not a single entity, but constellations of polymicrobial and polygenic infections, and possibly with different mechanisms of pathogenesis (Armitage, 2002; Dentino *et al*., 2013). However, the clinical parameters currently used for diagnosis of periodontal diseases, such as pocket depth and bleeding on probing, are poor reflections of the pathobiology of the various forms of periodontitis and are not specific or reliable for diagnosis (Armitage, 2004; Taba *et al*., 2005; Demmer *et al*., 2008). In this respect, some studies have attempted to identify the characteristics indicative of the pathogenic process status with greater accuracy, i.e., biomarkers. These biomarkers could be used in order to facilitate the early diagnosis of the active phases of periodontal disease and the identification of patients at risk of

future development of the disease (de Souza and Taba, 2004; Taba *et al*., 2005; Demmer *et al*., 2008).

Biomarkers are defined by National Institutes of Health (NIH, USA) as 'a characteristic that is objectively measured and evaluated as an indicator of normal biologic process, pathogenis processes, or pharmacologic responses to a therapeutic intervention'. Many of the agents involved in the pathogenesis of periodontal diseases, including substances derived from host (such as cytokines, enzymes, antibodies, and products from tissue breakdown) and/or microbial factors (such as specific harboring-pathogens in the subgingival environment), may be identified at different levels (depending on the type, severity, and disease activity) in the gingival crevice fluid, saliva, and gingival tissue (Taba *et al*., 2005). All of them could be considered potential biomarkers. According to Taba *et al*. (2005) there are several diagnostic tools to measure periodontal disease at the molecular, cellular, and tissue levels, including polymerase chain reaction (PCR), DNA-DNA hybridization, and enzyme-linked immunoabsorbent assay (ELISA), among others. However, to date, there is no practical and accurate periodontal indicator based in these analyses (Uitto, 2003).

With the advances in scientific research and the development of new techniques providing the chance to study genomic, transcriptomic, proteomic, and metabolomic capabilities, our understanding of the onset, progression, and pathogenic process involved in periodontal diseases has greatly expanded, thus increasing our possibility of identifying biomarkers of periodontal diseases (Taba *et al*., 2005; Kormman, 2008). In this sense, this review summarizes some of these advances in research using molecular biology techniques, such as genomic, transcriptomic, proteomic, and metabolomics to evaluate the characteristics of the host. These techniques might eventually lead to new diagnostic tests for periodontal diseases.

Genomics

Genomics refers to the study of the entire genome of an organism, i.e., all the DNA of a single organism. Technological breakthroughs in genomic research, such as improvements in DNA-sequencing, SNPs microarray, and genome-wide association study, allow assessing thousands of genes and their association with disease-related events (Burke, 2003; Schäfer *et al*., 2011). Its development has the potential to change the practice of health sciences, particularly dentistry (Giacomelli and Covani, 2010; Grant, 2012).

Genetic variations are mainly the SNPs (single nucleotide polymorphisms), that can affect gene expression or the functions of protein produced (Takashiba and Naruishi, 2006). In periodontics, for some time genetic studies evaluated the relationship between the presence of individual gene polymorphisms of cytokines, immuno-receptors, human leukocyte antigens (HLA), and others and the risk of onset and severity of periodontal diseases, as well as the result of periodontal treatment (Takashiba and Naruishi, 2006; Grant, 2012).

Many studies have reported a positive association between polymorphisms of the interleukin (IL)-1 gene and periodontal diseases. Kornman *et al*. (1997) reported an association between polymorphisms in the genes encoding IL-1α (-889) and IL-1β (+3953) and an increased severity of periodontitis, with other studies showing similar results. Gore *et al*. (1998) also reported a possible role of IL-1α and IL-1β gene polymorphisms in the susceptibility to adult periodontitis**.** On the other hand, Papapanou *et al*. (2001) in a study of 132 periodontitis patients who were age- and sex-matched with controls did not show any association with the composite genotype and periodontitis. Meisel *et al*. (2002) stated that the composite genotype showed a strong interaction with smoking, whereas non-smokers, even genotype-positive, were not at increased risk, suggesting that genetic–environmental interaction is more important than genetic factors alone for the determination of susceptibility to periodontitis.

Schäefer *et al.* (2010) published the first genome-wide association study in periodontics. In this study, examining 500,568 potential SNPs in 141 generalized

aggressive periodontitis patients and 142 localized aggressive periodontitis patients independently identified 197 and 244, respectively, quality-controlled SNPs. However, when the results from both sets were compared, only one SNP rs 1537415 located whithin intron 2 of GLT6D1 gene remained significant, which was subsequently validated in a third set of patients. GLT6D1 gene encodes for an ezyme glycosiltransferase 6 family, single-pass transmembranes proteins. These enzymes contribute to the synthesis of histo-blood related antigens in the Golgi. GLT6D1 had strong expression in leukocytes and gingival connective tissue and may influence immune responses.

Divaris *et al*. (2012) performed a genome-wide association analysis investigating risk loci for a high colonization with pathogenic periodontal bacteria, in 1020 white subjects, and in a second step to validate their results, in samples of 123 African American. In white subjects 13 loci (including genes involved in MMP-13 expression, macrophage adhesion and migration, and interleukin—33 production) provide suggestive evidence for high colonization with red complex, orange complex, and *Aggregatibacter actinomycetemcomitans* (Aa). In African American associations reported for red and orange complex microbiota, but not for Aa, had the same effect direction. These findings lead to and improved understanding of the host-microbiome interactions in periodontitis.

To date, although some associations were identified between gene polymorphisms and periodontal disease, there is limited evidence for most of the genes studied (Kinane and Hart, 2003; Schäfer *et al*., 2011). Futures studies using high thoughput technologies, such as genome-wide association studies, in a larger numbers of patients and control subjects may yield more associations. Thereby, improving the understanding of disease process, and easing diagnostic, which may allow to stratify patients according to the disease risk (Schäfer *et al*., 2011; Grant, 2012; Sleiman and Termatchi, 2013).

Transcriptomic

Transcriptomic is defined as the study of the set of all RNAs transcribed from a cell or tissue in a particular condition that provides their gene expression profile (Kebschull and Papapanou, 2010). The study of pathological features of periodontal disease by analyzing the gene expression profile may increase the understanding of biological events involved in the development of periodontitis (Giacomelli and Covani, 2010; Kebschull and Papapanou, 2010). This form of technology and assessment has provided important information in the medical area, particularly in the research of the pathogenesis of tumors and cancer (Quackenbush, 2006), muscular dystrophy (Haslett and Kunkel, 2002), Alzheimer's disease and dementia (Colangelo *et al*., 2002), and other diseases. The microarray technology is the most common method used to study the gene expression profile (Wang *et al*., 2003), and it can be performed to investigate a group of genes with specific functions, ranging from a few hundred genes, to over 22000 to 44000 transcripts which covers the whole transcriptome.

In the context of periodontal diseases, studies have evaluated the gene expression profile of gingival tissue and peripheral blood mononuclear cells from patients with periodontitis. Sørensen *et al*. (2008) evaluated the gene expression profile from the peripheral blood mononuclear cells of patients with both localized aggressive periodontitis and generalized aggressive periodontitis, compared to healthy subjects (control group). Fifty-three genes had altered expression in patients with localized aggressive periodontitis compared to control, and 14 of these were involved in immune response. On the other hand, patients with generalized aggressive periodontitis showed a gene expression profile similar to control subjects, with only three genes with differential expression, indicating a more heterogeneous disease entity (Sørensen *et al*., 2008).

In a pilot study, Papapanou *et al*. (2004) reported that it was possible to separate patients with chronic and aggressive periodontitis through the gene expression profile of inflamed gingival tissue into two groups, regardless of clinical diagnosis. These groups had similar clinical periodontal status and subgingival

bacterial profiles, but differed significantly with respect to serum IgG levels against the important periodontal pathogens *Porphyromonas gingivalis*, *Tannerella forsythia* and *Campylobacter rectus*. Interestingly, differential diagnosis between chronic and aggressive periodontitis could not be done.

Demmer *et al*. (2008) evaluated the gene expression of inflammed gingival tissue adjacent to periodontal pockets of patients with chronic and aggressive periodontitis in comparison to healthy gingival tissue. Several genes, including those responsible for apoptosis, humoral response, antigen presentation and angiogenesis, were differently expressed in periodontal health and disease. It could be related to microbial profile in periodontal pocket, as observed by Papapanou *et al*. (2009). In this study, the analysis of the effect of the periodontal pathogens in the gene expression of adjacent gingival tissue, showed that the expression of certain genes were correlated to bacterial load, somehow modulating pathogenesis of disease. However, the pathway of this relationship still remained not fully explained.

Assessing possible factors that influenced the patient response to periodontal treatment, Kim *et al*. (2006) showed that 68 genes were positively and 6 genes were negatively modulated in sites with refractory periodontitis, compared to individuals who responded adequately to periodontal treatment. Most of these genes have immune-inflammatory functions. In this way, Beikler *et al*. (2008) investigated gene expression changes in gingival tissue in residual pockets ≥ 7mm, after a session of periodontal treatment of patients with chronic periodontitis. The authors found that periodontal treatment promotes a strong negative regulation of immune-inflammatory response and activates pathways that regulate tissue damage and repair. They suggested that these differentially expressed genes can be targets for new diagnostic and treatment strategies, and could be used to explain the negative clinical response of some specific periodontal conditions.

In summary, it has been postulated that different patterns of gene expression, as the measurement of various biomarkers, could define periodontal status with different clinical implications. However, more studies are needed to

elucidate this information (Offenbacher *et al*., 2007; Kormman, 2008). Complete information of the gene expression profile of tissues affected by the disease, which is influenced by genetic and constitutive characteristics from subjects, microbiota composition, environmental factors, and other modifying factors, can provide details of molecular networks involved in the affected tissues, concomitantly leading to the development of new preventive and therapeutic approaches (Hood *et al*., 2004; Kormman, 2008).

Proteomics

The goal of proteomics is to identify, quantify, and analyse the locations and interactions of the ensemble of expressed proteins in a cell, tissue or fluid in normal and diseased conditions (Garcia and Tabak, 2008). Proteomics should also be applied to reach a deeper understanding of the molecular mechanisms underlying oral disorders (Giacomelli and Covani, 2010), and could assume great importance during diagnosis, because proteins are the key functional components of biochemical systems and the cellular targets of therapeutics agents (Agrawal *et al*., 2012).

Proteomics development has been made possible by advances in protein sequencing by mass spectrometry and the accompanying separation techniques, including electrophoresis and non gel based techniques, such as liquid chromatography tandem mass spectrometry (LC-MS/MS), quantitative proteomics by mass spectrometry and isobaric tags for relative and absolute quantitation (iTRAQ), and fluorescence-based detection technologies (Tyers and Mann, 2003; Grant *et al*., 2010).

Analyses of salivary proteins have shown that differences in the salivary protein profile are associated with oral pathologies, such as chronic and aggressive periodontitis, oral lichen planus, and oral cancer (Gonçalves *et al*., 2011). Wu *et al*. (2009) investigated whether salivary proteomics technology can be used to evaluate the disease progression of periodontitis, and identified 11 proteins

showing a different expression in saliva of generalized aggressive periodontitis patients compared to healthy controls.

Using saliva proteome to understand the changes after periodontal treatment, Haigh *et al*. (2010) showed that 11 proteins were more abundant in individuals with active disease, while 4 proteins decreased on average. In this study, most of the altered proteins (haptoglobin, prolactin inducible protein, and parotid secretory protein) were associated with host defense.

The content of gingival crevice fluid can also be assessed by proteomic methods, indicating a more local analysis in periodontal disease, i.e., periodontal pocket. Kojima *et al*. (2000), through separation of proteins by two-dimensional electrophoresis and protein identification by mass spectrometry, evaluated proteins in gingival crevice fluid from periodontitis patients. They reported a distinct pattern of proteins in health and periodontal diseases, showing the presence of two calcium-binding proteins, calgranulin A (MRP8) and calgranulin B (MRP14) in periodontitis sites, which had previously been linked to neurological inflammation and neoplastic diseases (Kojima *et al*., 2000). Lundy *et al*. (2005) used mass spectrometry to assess the presence and quantity of human neutrophil α-defensins (human neutrophil peptides) in the gingival crevice fluid of patients with periodontal disease compared to healthy subjects. They found higher levels of defensins in sites with periodontal health. According the authors, there was a more intact defensin barrier in periodontal health related to some immune conditions against periodontitis.

Recent studies employed high-throughput proteomic techniques to enhance understanding of the proteins and molecular pathways involved in gingival inflammation. Grant *et al*. (2010) assessed quantitatively the changes in GCF proteome profiles in the experimental gingivitis using iTRAQ labeled samples and Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. They identified 186 human and 16 bacterial proteins throughout the period of experimentally induced gingivitis, including proteins displaying antibacterial properties, DNA binding, cytoskeleton, and neuronal and synapse associated

proteins, which were associated with changes to the clinical parameters. Similarly, Bostanci *et al*. (2013) using liquid chromatography-tandem mass spectrometry for label-free quantitative proteomics, analyzed sequential protein expression in gingival crevicular fluid samples during the induction and resolution of experimental gingivitis. Proteins involved in immune response and antimicrobial function were upregulated in induction of gingivitis, such as as alpha-amylase 1, calgranulin A, cystatins, lysozyme C, and Cathepsin G. In resolution period several histones, immune response-related and neutrophil-derived antibacterial proteins including cathepsin G, myeloperoxidase, alpha enolase, and defensin-1 were downregulated.

Bostanci *et al.* (2010) assessed the composition of GCF of generalized aggressive periodontitis subjects by liquid chromatography-mass spectrometry. The proportion of enzymes associated with polymorphonuclear neutrophils, such as matrix metalloproteinase-8 (MMP-8), cathepsin G, myeloperoxidase, and bacterial, viral, and yeast protein was increased in disease compared to health. While host defense-related proteins, such as Cystatin-B and defensins, were detected only in health.

Differing from the analysis of DNA and RNA that may benefit from the amplification stages of the molecules before the analytical procedures, in proteomics it is not often possible to analyze the whole proteome. In addition, it is often necessary the removal or separation of some proteins that are abundantly found, such as albumin, prior to analytical procedures (Grant, 2012), and its presence could mask analysis results. Regardless of the advances in analytical techniques, further technological improvements, organization of international proteomics projects, and open access to results are needed for proteomics to fulfill its potential.

Comprehensive proteomic analysis complements other “-omics” approaches allowing more detailed investigation to find composites of proteins that could be used as biomarkers of periodontitis in diseased tissue and fluids, which

would facilitate the diagnosis and therapeutic monitoring and understanding of host-pathogen interactions.

Metabolomics

Metabolomics is a discipline that monitors the quantities of all chemicals, such as lipids, sugars, and amino acids except, DNA, RNA, and proteins, involved within a sample in order to understand metabolic dynamics associated with conditions such as a disease or drug exposure (Garcia and Tabak, 2008; Giacomelli and Covani, 2010; Spielmann and Wong, 2011; Grant, 2012). Metabolomics methodologies are evolving (Garcia and Tabak, 2008), however, no single experimental technique can analyze all chemical structures. Therefore, samples are analyzed by several techniques, including nuclear magnetic resonance and mass spectrometry (Grant, 2012).

Metabolomics is a promising technique for improved disease screening, diagnosis, and prognosis, but there are very few articles that reported the assessment of the metabolome in periodontal disease (Garcia and Tabak, 2008). In others areas, this approach provide essential information about various chronic and multifactorial diseases such as cardio-vascular disease, diabetes, and diseases of the central nervous system (Tanke, 2007).

Barnes *et al*. (2009) performed a metabolomics profiling of gingival crevice fluid (GCF) collected from healthy, gingivitis, and periodontitis sites from 22 chronic periodontitis subjects by liquid and gas chromatography mass spectrometry. Based on the chemical reference library, 103 metabolites were detected and from them approximately 50% of the detected metabolites showed altered levels among the three periodontal conditions. The authors also demonstrated that the purine degradation pathway, a major biochemical source for reactive oxygen production, was significantly accelerated at the diseases' sites, suggesting that periodontal disease induces oxidative stress and inflammation (Barnes *et al*., 2009; Grant, 2012).

Gronert *et al*. (2004) used lipidomics, which is a type of metabolomics, to investigate the role of lipids in cellular function. They investigated and quantified diacylglycerol (DAGK) species in neutrophils from localized aggressive periodontitis patients and reported low levels of this molecule that could lead to an increase in superoxide anions production.

The concentration and fluxes of small molecules present in cells, tissues, organs, and biologic fluids, described by metabolomics, result from a complex interaction between the gene and protein expression and are influenced by environment interactions. The results from metabolomics analyses complement the information obtained from other "-omics" methodologies, which may improve the understanding of the biological basis of the disease (Sugimoto *et al*., 2010).

However, additional studies using advanced methods of metabolite quantification and more sophisticated data processing and analysis strategies for integrating the knowledge obtained from other "-omics" studies are needed to increase the understanding of the pathogenesis of periodontal diseases as well as to evaluate whether measurements provide a relevant impact on the disease diagnosis.

Systems Biology

Advances in the genomic, proteomic, transcriptomic, and metabolomic technologies enabled a better explanation of the molecular networks that are involved in specific gene-environment interactions. Analysis of massive amounts of information of all the molecules involved in a given process and the interactions between these molecules appears to be an enormous task (Kormman, 2008; Giacomelli and Covani, 2010). Bioinformatics can become an added value in this context. According to Giacomelli and Covani (2010) bioinformatics is defined as the application of information technology to the field of molecular biology through the development of original algorithms.

This approach is cited in the literature as system biology; that is, the computational integration of data generated by the set of genetic, transcriptomic,

proteomic, and metabolomic platforms to understand functions through different levels of biomolecular organization though the reconstruction of the complex network involved (Nicholson and Wilson, 2003; Fukushima *et al*., 2009; Grant, 2012). According to Hood (2004) in a review, system biology is a scientific discipline that endeavors to quantify all of the molecular elements of a biological system to assess their interactions and to integrate that information into graphical network models that serve as predictive hypothesis to explain emergent behaviors. Considering the idea that is beginning to revolutionize medicine is that disease may perturb the normal network structures of a system through genetic perturbations and/or by pathological and environmental changes. With this in mind, gene, protein, and metabolic expression profiling could reveal those changes (Papapanou *et al*., 2009).

The pathophysiology of periodontitis is characterized by the involvement of various biologic pathways (Covani *et al*., 2008). The identification and the characterization of candidate genes, proteins, and molecules involved in a given disease by the integrated use of "-omics" disciplines will probably represent one of the milestones of future health care (Giacomelli and Covani, 2010). The analysis of the complex network of connections between genes/proteins may allow the identification of potential molecular markers and targets (Giacomelli and Covani, 2010).

An example of this approach applied to periodontics is the study of Covani *et al*. (2008) that searched through the published articles and identified 61 genes potentially involved in periodontitis. Analysis was performed by bioinformatics approaches for predicting possible leader genes, i.e., genes that may be supposed to play an important role in the analyzed processes, reporting that five genes were identified as leader genes (NFκB1, RELA, PIK3R1, GRB2, and CBL). These genes are predominantly involved in receptor-mediated signaling of the host inflammatory-immune system.

The results achieved through "-omics" technologies, and the integration of the results by bioinformatics approaches will allow a better understanding of the

events that occur in the pathogenesis of periodontal disease, and may lead to the identification of new risk factors, biomarkers, and therapeutic targets.

Conclusion

To conclude, there is a vast literature on biomarker research in periodontal disease. However, there are still no biomarkers that can be used accurately in clinical practice. Improvements in the research techniques, as those that employ '-omics' disciplines, may lead to a better understanding of pathogenic events of periodontal disease, providing a comprehensive view of the disease process through identification of molecules and pathways involved in the shift from health to disease and in different pathogenic process. Consequently, they can be applied in future diagnosis of periodontal disease through identifying one or more biomarkers for the detection of the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients through personalized healthcare aiming at early prediction and intervention. However, more research is needed before these diagnostic tools become a reality in clinical practice.

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Capítulo 2

Gene expression profiles in healthy masticatory mucosa of aggressive and chronic periodontitis

Abstract

Background and Objective: Gene-expression profiling, a powerful tool to generate comprehensive genome-level data sets, have recently been applied to study the pathobiology of periodontitis, however, genes responsible for the aggressive periodontitis have not been identified. The aim of the present study was to investigate the gene expression profile of healthy masticatory mucosa in subjects with a history of generalized aggressive periodontitis, chronic periodontitis, and who have not experienced destructive periodontitis.

Material and Methods: Healthy masticatory mucosa were taken from four well-maintained patients with a history of generalized aggressive periodontitis, four with chronic periodontitis, and four subjects who have not experienced destructive periodontitis. Total RNA from 12 tissue samples were employed in order to generate genome wide transcriptome profiles. Data mining analysis, such as comparisons, gene ontology and pathway analyses were performed.

Results: Comparison analysis revealed that 192 probes sets were differentially expressed between aggressive periodontitis subjects and healthy subjects, 43 when comparing chronic periodontitis and healthy, and 168 when comparing chronic periodontitis and aggressive periodontitis. Comparative gene ontological analysis identified 50 differentially regulated ontological groups in aggressive periodontitis subjects compared to healthy subjects, 27 in chronic periodontitis and healthy subjects, and 75 groups in chronic periodontitis and aggressive periodontitis. Genes with function in the immune system, including natural killer cells receptors, were most expressed in aggressive periodontitis; in

contrast, genes involved in proliferation and differentiation of keratinocytes, as well as genes with function in neural process were less expressed. Chronic periodontitis subjects were characterized by increased expression of genes related to response to external stimuli, and a decrease in the expression of genes related to the immune system.

Conclusion: Microarray and data mining analysis demonstrate that chronic, and especially aggressive periodontitis presents differences in tissue gene expression profile between them and to healthy subjects, indicating that these diseases could present different pathways of periodontal destruction.

Key words Periodontitis; Gene expression; Microarray; Susceptibility

Introduction

Periodontitis is one of the most common chronic inflammatory diseases, and is characterized by the destruction of periodontal tissue (Feng & Weinberg, 2006). The attachment loss and bone destruction result from interactions between periodontal microorganisms and susceptible host, leading to the release of cytokines, chemokines and proteolytic enzymes that mediate tissue damage (Socransky & Haffajee, 2005). Epidemiologic studies suggest that up to 50% of the population is affected by chronic periodontitis while 1-15% may suffer from aggressive periodontitis (Demmer & Papapanou, 2010).

Aggressive periodontitis are challenging pathological entity, once it is characterized by early onset, rapid attachment loss and bone destruction, and poor response to periodontal therapy, while chronic disease presents a slower progression, compatible with local factors (Armitage, 1999). In order to explain possible differences between both forms of disease, microbiological and host immune response aspects, as well as genetic aspects, have been studied. However, mechanisms responsible for these differences are poorly understood and

no conclusive results has been described yet (Armitage, 2010; Casarin *et al*., 2010; Stabholz *et al*., 2010; Dentino *et al*., 2013). Looking for a better understanding of the impact of host characteristics in pathologies development, a new tool to investigate pathological pathways has been used for identify possible alterations on periodontal disease, the gene profile investigation (Demmer *et al*., 2008; Danavian *et al*., 2012).

To date, published studies investigating the gene expression profile in periodontitis have controversial results. Papapanou *et al*. (2004) reported no significant differences in gene expression at different pathological sites in patients with chronic and aggressive periodontitis, and limited differences between diseased and healthy sites. On the other hand, Kim *et al.* (2006), Demmer *et al.* (2008) and Danavian *et al.* (2012) showed a great number of genes that were differentially expressed in periodontitis compared to healthy sites. In addition, Beikler *et al.* (2008) demonstrated that in chronic periodontitis sites, the overall expression of immune and inflammatory genes was down-regulated following non-surgical therapy.

Despite researches investigating periodontitis gene expression profiles there is no conclusive results for chronic, and especially for aggressive periodontitis regarding the genes associated to periodontal destruction (Danavian *et al*., 2012). Perhaps this may be explained by the fact that most studies exclusively used healthy gingival tissues from individuals with periodontitis, which may not necessarily be identical to those healthy sites in subjects who have not experienced destructive periodontitis (Demmer *et al*., 2008), once gene expression is influenced due inherent or acquired characteristics (Krämer *et al*., 2013). Moreover, gene expression from healthy tissue reported in these studies may be influenced by subgingival microbiota (Papapanou *et al*., 2009; Kebschull & Papapanou, 2011). Thereby, the investigation of sites “free” of these variables, such as masticatory mucosa, could be an interesting form to identify intrinsic host alterations that could be associated to disease development and progression.

Therefore, the aim of the present study was to investigate the differences in gene expression profile of healthy masticatory mucosa in subjects with a history of generalized aggressive periodontitis, chronic periodontitis, and who not experienced destructive periodontitis.

Material and Methods

Subjects

The study design was approved by the Ethics Committee of the University of Campinas (017/2010), and informed written consent was granted by each subject after explanations were provided. A total of twelve subjects were recruited from the patient referred to implant placement to the Graduate Clinic of Piracicaba Dental School, University of Campinas, Piracicaba, Brazil, between March 2011 and January 2012. Patients with a past of chronic periodontitis (n=4) or generalized aggressive periodontitis (n=4) (Armitage, 1999), and who had received regular periodontal maintenance care at least 1 year following active periodontal therapy, presenting full-mouth plaque index (Ainamo & Bay, 1975), and full-mouth bleeding score (Mühlemann & Son, 1971) less than 30% were selected. Another 4 subjects, with no history of periodontal disease (free of interproximal attachment loss and had no probing pocket depths of >4mm) were also enrolled (Sørensen *et al.*, 2008; Jönsson *et al.*, 2011). The participants were non-smokers, had not received systemic antibiotics or anti-inflammatory drugs in the previous 6 months, did not have systemic diseases (e.g., cardiovascular, diabetes), and were not pregnant or lacting. The following clinical parameters were assessed in all participants before tissue collection: full-mouth plaque index, full-mouth bleeding score, probing pocket depth and clinical attachment level at six points around each tooth.

Biopsies

Healthy masticatory mucosa tissue samples were obtained from edentulous sites at implant placement moment. After local anesthesia (Articain with 1:100.000 epinephrine), performed distant from site of tissue collection in order to avoid possible effects on gene expression, two parallel linear incisions, 3 mm apart, were made through the soft tissue until bone contact was achieved. The two incisions were connected with perpendicular incisions delimiting a tissue sample of 3 x 3 mm, which was carefully dissected (Donati *et al*., 2009). The tissue specimens comprised the oral epithelium and the underlying connective tissue. After collection, the specimens were rinsed with sterile saline solution, and immediately stored overnight in a RNA stabilization reagent (RNAlater, Ambion Inc, Austin, TX) at 4^{o}C and then stored at -80^{o}C for subsequent RNA isolation.

Isolation of total RNA from tissue samples

Tissue specimens were disrupted by a motor pestle (Marconi, Piracicaba, SP, Brazil) and homogenized in 1 ml of Trizol Reagent (Ambion Inc, Austin, TX, USA) for 5 minutes. After incubation with 200 µL of chloroform and centrifugation at 12,000 x g, RNA collected in the upper aqueous phase was precipitated by mixing with 500 µL isopropyl alcohol and additional centrifugation, and it was washed in 1mL of 75% ethanol.

The remaining DNA was removed using RNase-Free DNase Kit (Turbo DNA-free, Ambion Inc, Austin, TX, USA) following the manufacturer's instructions. RNA quantity was evaluated spectrophotometrically using a Nanodrop 2000 device (Thermo Scientific, Wilmington, DE, USA). Quality control of total RNA samples were determined prior to the microarray experiments using the RNA 6000 NanoLabChip Kit of the Agilient 2100 Bioanalyzer (Agilent Technologies, Palo Alto,

CA, USA). Samples that had RIN values above 8 were used for further microarray analysis.

Double-stranded cDNA synthesis and purification

Double-stranded cDNA was synthesized from five micrograms of total RNA using the cDNA Synthesis System (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions. cDNA samples were cleaned with 20 µl of T_4 DNA Polymerase, 1,5 µl of RNase I, and 5 µl of proteinase K. Then, cDNA was purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, with a final volume of 40 µl.

Microarray hybridization

Each cDNA sample was random-primer labeled with Cy3-nonamers according to Roche NimbleGen's standard protocol using NimbleGen One-Color DNA Labeling Kits (Roche Diagnostics, Mannheim, Germany). Where possible, one microgram of cDNA was used in the labeling reaction. For those samples that did not yield one microgram of cDNA, the maximal volume (forty microliters) of the High Pure column-eluted cDNA was used. Using random assignment, each Cy3-labeled cDNA sample was applied to NimbleGen Human Gene Expression 12x135K Arrays (Roche Diagnostics, Mannheim, Germany). Each slide contains 12 independent arrays, each with 135,000 probes covering 45,033 genes, 3 probes/target gene. The array was then hybridized for 16 hours at 42°C, washed, dried, and scanned at 2 µm resolution using a NimbleGen MS 200 Microarray Scanner (Roche NimbleGen).

Microarray data analysis

The NimbleScan v2.6 software (Roche NimbleGen) was used to extract fluorescence intensity signals from the scanned images. The data was analyzed using different packages in the software R. All packages were available in the Bioconductor open source software project for analysis of genomic data. Expression data were normalized and summarized using the Robust Multi-Array (RMA) analysis.

Comparison analyses were then used to differentiate the real changes in gene expression levels between aggressive periodontitis group and healthy group (comparison I), chronic periodontitis group and healthy group (comparison II). Data were filtered to ensure both statistical and biological significance. Genes that showed at least two-fold changes in their gene expression and with adjusted $p < 0.05$ (corrected for multiple testing using Benjamini-Hochberg method) were identified as differentially expressed (Koshi *et al*., 2007; Shimizu *et al*., 2010). Then, genes differentially expressed in both comparisons were again further assessed to determine gene expression levels between chronic periodontitis group and aggressive periodontitis group (comparison III). Gene ontology (GO) Biological Process analysis and pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used for further investigation on the differential genes of interest. Enrichment analysis of the data was then performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang *et al*., 2009a; 2009b).

Results

Patients' characteristics

No differences were observed between groups regarding oral hygiene status (Full mouth plaque and bleeding indices). With regard to the clinical parameters the groups presented similar values of clinical attachment loss and

probing pocket depth. The mean age of aggressive periodontitis patients was 32.25 ± 5.85 years, which was statistically younger than that of chronic periodontitis patients (51.75 ± 3.30 years old). However, there was no difference regarding the age between aggressive group and healthy group, and chronic group and healthy group (table 1).

Microarray Analysis

The microarray data (raw and normalized) were processed according to Minimum Information About a Microarray Experiment (MIAME) guidelines. Applying the cutoff criteria (corrected *p* value ≤ 0.05, fold-change ≥ 2 or fold-change ≤ -2) transcriptome analysis revealed that 192 probes sets were differentially expressed between aggressive periodontitis group and healthy group (comparison I), 43 when comparing chronic periodontitis group and healthy group (comparison II), and 168 when comparing chronic periodontitis group and aggressive periodontitis group (comparison III). The complete list of genes differentially regulated can be viewed in the supplementary tables 1, 2, and 3.

Comparison I – Aggressive periodontitis group and Healthy group

The microarray analysis in aggressive periodontitis group compared to healthy group showed that 56 transcripts were increased, and 136 were decreased. Fold-changes in expression ranged between 6.10 and 2.62 for the top 15 probes sets, with increased expression, and between -6.36 to -3.10 for the top 15 probes sets with decreased expression (table 2). Several constituents of natural killer cell, such as killer cell immunoglobulin-like receptors and killer cell lectin-like receptors, and other genes such as formyl peptide receptor 1 (FPR1), which are known to play an important role in regulation of the immune response were detected as up-regulated genes. In contrast, genes involved in neural process,

such as internexin neuronal intermediate filament (INA); MAM domain containing glycosylphosphatidylinositol anchor 1 (MDGA1); semaphorin 7A (SEMA7A), and genes related to cellular growth, development and division, differentiation and cornification of keratinocytes, such as POU class 2 homeobox 3 (POU2F3), calmodulin-like 5 (CALML5), caspase 14, apoptosis-related cysteine peptidase (CASP14), early growth response 3 (EGR3), and DEAD (Asp-Glu-Ala-Asp) box polypeptide 12 were found to be down-regulated.

Gene ontological analysis identified 7 significantly and differentially up-regulated ontological groups in aggressive periodontitis group compared to healthy group, including cellular defense response, immune response, immune system process, defense response, response to stimulus, and natural killer cell activation (table 3 for top GOs). In contrast, 43 groups, including neurotransmitter transport, nervous system development, development process, multicellular organismal development were found to be down-regulated. The complete list of gene ontology groups differentially expressed between aggressive periodontitis group and healthy group can be viewed in Supplementary table 4. Further, pathway analysis identified 4 differentially expressed pathways: antigen processing and presentation, natural killer cell mediated cytotoxicity, graft-versus-host disease, and FC gamma R-mediated phagocytosis (table 4).

Comparison II – Chronic periodontitis group and Healthy group

Comparing chronic periodontitis group and healthy group transcriptomes analysis revealed that 10 probes sets were up-regulated and 33 transcripts were down-regulated. Fold-changes in expression ranged between 4.8 and 2.03 for the 10 probes sets with increased expression, and between -10.17 and -2.40 for the top 15 probes sets with decreased expression (table 5). Genes encoding proteins which mediate the presentation antigens - CD1b molecule (CD1B) and major histocompatibility complex, class II, DQ alpha 1 (HLA-DQA1), and genes related to

response to external stimulus and inflammation, such as kallikrein B plasma (Fletcher factor) 1 (KLKB1), and neuropeptide Y (NPY), were detected as up-regulated. Lipopolysaccharide binding protein (LBP), selectin E (SELE), Toll-like receptor 8 (TLR8), integrin-binding sialoprotein (IBSP) major structural of the bone matrix, and ubiquitously transcribed tetratricopeptide repeat gene (UTY) involved in oxireductase activity, were found to be down-regulated.

Gene ontology analysis identified 3 up-regulated ontological groups: regulation of proteolysis, antigen processing and presentation, and regulation of multicellular organismal process. In contrast, 24 ontological groups, including defense response, immune system processes, and response to wounding, were found to be down-regulated (table 6 for top GOs). The complete list of gene ontology groups differentially expressed between chronic periodontitis group and healthy group can be viewed in Supplementary table 5. Pathway analysis reveals that cell adhesion molecules pathway was differentially expressed.

Comparison III – Chronic periodontitis group and Aggressive periodontitis group

Comparison III showed that 74 probes sets were higher expressed and 94 were lower expressed in chronic periodontitis group compared to aggressive periodontitis group. Fold-changes in expression ranged between 7.27 and 3.05 for the top 15 probes sets, with increased expression, and between -4.3 and -2.90 for the top 15 probes sets with decreased expression (table 7). Genes for epithelial components and differentiation, including psoriasis susceptibility 1 (PSORS1C1), caspase 14 (CASP14), and genes involved in lymphocyte development, endothelial cell growth and migration (early growth response 3 - EGR3) were found to be higher expressed. On the other hand, genes encoding interleukin-6 (IL6), interleukin-1β (IL1B), selectin E (SELE), and toll-like receptor 8 (TLR8) were detected as lower expressed genes.

Gene ontology analysis identified 19 differentially higher expressed groups, including epidermis and ectoderm development, tissue development, phospholipid catabolic process, and leukotriene metabolic process. In contrast, 56 groups were lower expressed, including immune response, immune system process, inflammatory response (table 8 for top GOs). The complete list of gene ontology groups differentially expressed between chronic periodontitis group and aggressive periodontitis group can be viewed in Supplementary table 4 Further, pathway analysis identified 6 differentially expressed pathways, including arachidonic acid metabolism, Fc gamma R-mediated phagocytosis and Toll-like receptor signaling pathway (table 4).

Discussion

The present study describe the gene expression profiles of healthy masticatory mucosa in subjects with a past history of chronic periodontitis and generalized aggressive periodontitis using microarray analyses, including comparisons, gene ontology and pathway analysis. The primary aim of this study was assess and identify differences in gene expression between chronic and aggressive periodontitis. Thereby, we enrolled subjects who have not experienced destructive periodontitis, as demonstrated by clinical periodontal data and intraoral radiographs, to control molecular events without disease revelance, once transcriptomes of healthy gingival tissues of individuals who have not experienced periodontitis may be different those of subjects with periodontitis (Demmer *et al.*, 2008; Becker *et al.*, 2012). Another important point is the employment of healthy masticatory mucosa for microarray analysis, instead healthy gingival tissue adjacent to teeth, aiming eliminates the influence of the subgingival microbiota in gene expression of gingival tissues (Papapanou *et al.*, 2009).

Our findings demonstrate significant differences in gene expression between healthy masticatory mucosa of subjects in aggressive periodontitis group

versus healthy group, chronic periodontitis in comparison to healthy group, and, especially between chronic periodontitis group and aggressive periodontitis group. This report does not include an in-depth discussion of specific differentially regulated pathways in groups assessed but rather provides examples, for a few genes with altered expression that could be associated to disease development and progression, which underscore the usefulness of the expression data.

Comparing gene expression in aggressive periodontitis group to healthy group, through the top genes, gene ontology groups and pathway analysis, natural killer cell related genes (killer cell immunoglobulin-like receptors and killer cell lectin-like receptors) were the major genes up-regulated. Natural killer (NK) cells are cytotoxic lineage of lymphocytes that can mediate lysis of certain tumor cells and virus-infected cells without previous activation. They can also regulate specific humoral and cell-mediated immunity. Recent studies have demonstrated the relationship between natural killer cells and inflamed gingival tissues, and aggressive periodontitis (Krämer *et al*., 2013; Muthukuru, 2012). In a recent study Krämer *et al*. (2013) reported significant induction of the NK cell mediated cytotoxicity, mainly by overexpression of CD2-like receptor activating cytotoxic cells (CRACC), in aggressive periodontitis lesions when compared to tissues from chronic periodontitis lesions. Jönsson *et al*. (2011) reported that multiple genes associated with natural killer (NK) cell function, including killer cell lectin-like receptors, were differentially regulated during induction of experimental gingivitis. They are of particular interest as NK cells represent a link between a bacterially induced immune response and an auto-immune component that has been suggested to play a role in the pathobiology of periodontitis (Yamazaki *et al*., 2001).

Gene ontology enrichment analyses among down-regulated genes in aggressive periodontitis group vs. healthy group demonstrated that most of these genes were involved in neural process. Interestingly, Offenbacher *et al*. (2009) reported that genes and pathways associated with transmission of nerve impulses,

nerve development, and maturation were moderately up-regulated in induction phase of experimental gingivitis. These genes were linked broadly to epithelial tissues, vasculature, and wound healing, representing a novel and unanticipated finding for gingivitis (Offenbacher *et al*., 2009). On the other hand, the activation of neural pathways does not appear to represent a dominant transcriptome pathway in periodontitis (Demmer *et al*., 2008). Despite these contradictory results, our work suggests that the role of neural process in tissue homeostasis, and inflammation, requires future investigation for clarify its role on periodontal disease.

Genes involved in proliferation, differentiation and cornification of keratinocytes were down-regulated in aggressive periodontitis, both in comparison 1 as comparison 3 (up-regulated in chronic group or, alternatively, least expressed in aggressive group). Recent microarray studies of disease gingival tissues have also reported decreased gene expression of epithelial components. Desmocollin 1, member of cadherin superfamily of Ca^{2+} dependent cell adhesion molecules, were found to be least expressed in diseased gingival tissue, and in refractory periodontitis (Kim *et al*., 2006; Demmer *et al*., 2008). Similarly Abe *et al.* (2011) found that genes for epithelial constructive and cytoskeletal proteins, including keratins, epiplakins and dermokines, were found to be down-regulated in periodontitis-affected gingival tissues.

In comparison II genes with biological function in proteolysis and regulation of multicellular organismal process, among them kallikrein B 1 and neuropeptide Y, were found as up-regulated. Kallikrein is glycoprotein that participates in the surface-dependent activation of blood coagulation, fibrinolysis, kinin generation and inflammation. It was involved in the inflammatory response to *Porphyromonas gingivalis* triggering bradykinin production (Rapala-Kozik *et al.*, 2011). Bradykinin are powerful proinflammatory mediators, and they have been implicated in the pathogenesis of periodontitis due to their potential to strongly upregulate bone resorption (Brechter *et al*., 2008).

An unexpected result was overexpression in chronic periodontitis of gene encoding neuropeptide Y (NPY). There is some evidence that NPY is involved in pathogenesis of periodontal disease, with inhibitory effects on immune mechanisms (Del Rey *et al*., 1981), and protective bone metabolism (Haug & Heyeraas, 2006). Indeed Lundy *et al*. (2009) found higher levels of NPY in healthy sites compared to periodontitis sites. On the other hand, severe or prolonged neurogenic inflammation may result in the inflammatory response mediating injury rather than facilitating repair, as normally occurs (Lundy & Linden, 2004). Moreover, NPY is involved in a shift in the ratio of T-helper 1 to T-helper 2 cells (Bedoui *et al*., 2003), which is a characteristic of chronic periodontitis.

Lipopolysaccharide binding protein (LBP), selectin E, and Toll-like receptor 8 (TLR-8) encoding genes overlapped with the most gene ontology groups down-regulated in chronic periodontitis group compared to healthy group. Interestingly, selectin E and TLR-8 also were down-regulated in comparison III. The protein encoded by lipopolysaccharide binding protein (LPB) gene is involved in the acute-phase immunologic response to gram-negative bacterial infections, possibly to prevent exaggerated responses to LPS (Thompson & Kitchens, 2006). Studies have indicated that LBP may be involved in the initiation and development of periodontal disease (Wang & Ohura, 2002). Indeed, Ren *et al*. (2009) reported that LBP suppressed interleukin-1β (IL-1β) expression induced by LPS in human gingival fibroblasts. However, there is evidence that depending on the concentrations of LBP and can be both enabling and inhibiting cellular response to LPS, with higher concentrations of LPB inhibiting pro-inflammatory cytokine production (TNF-α) (Amura *et al*., 1997). Therefore, lower expression of LBP gene in chronic periodontitis patients may impair the immune-inflammatory response to periodontopathogens, contributing to initiation and progression of periodontal disease.

Other gene lesser expressed in chronic periodontitis was toll-like receptor 8 (TLR-8). Toll-like receptors plays a fundamental role in pathogen

recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. TLR-8, as TLR-3, TLR-7, and TLR-9, recognizes viral and/or bacterial nucleic acid (Mahanonda & Pichyangkul, 2007). There is only one study that evaluated the expression and distribution of TLR-8 in periodontitis, by immunohistochemistry, that reported that TLR-8 was found to be expressed by epithelial cells at similar frequency in healthy and in periodontitis, while in the connective tissue percentage of TLR-8 was significantly higher in periodontal gingival samples than healthy tissue (Beklen *et al*., 2008). More studies are needed to better understanding of the role of TLR-8 in the pathogenesis of periodontal disease. May be speculate that a lower expression in this receptor could contribute to development of periodontal disease, for change innate immunity to periodontopathogens.

Additionally, the microarray analysis displays a distinct gene expression profile between chronic periodontitis and aggressive periodontitis. Interestingly, immune system process, response to wounding, cell adhesion, and biological adhesion gene ontology group was down-regulated in chronic periodontitis group both in this comparison as compared to healthy group. Similarly, in aggressive periodontitis immune system process and immune response was up-regulated in this comparison (least expressed in chronic periodontitis, or alternatively, most expressed in aggressive periodontitis) and in comparison I (aggressive periodontitis group and healthy group). These findings highlight the importance of these groups in chronic and aggressive periodontitis, further corroborating the idea that the chronic and aggressive periodontitis may have distinct pathobiology.

Interestingly pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) were most expressed in aggressive periodontitis than chronic periodontitis. Similar to our report, several studies have demonstrated that generalized aggressive periodontitis subjects appear to have a hyper-inflammatory response that may increase host susceptibility to tissue destruction. Duarte *et al.*

(2010) reported elevated serum levels of TNF-α in subjects with generalized aggressive periodontitis compared to periodontal healthy subjects at 6 months post-therapy. Moreover, Bastos *et al*. (2009) evaluated TNF-α and interleukin-4 (IL-4) levels in GCF from generalized aggressive periodontitis and healthy subjects (control sites); they reported that healthy and diseased sites from aggressive periodontitis presented high levels of pro-inflammatory cytokine TNF-α when compared to healthy subjects, while the concentration of anti-inflammatory cytokine IL-4 was lower in aggressive periodontitis than healthy subjects.

We believe that these genes play an important role in the tissue homeostasis and/or periodontal disease susceptibility. However, it should be pointed out that the results of this study are limited, because there were relatively low number of specimens, which warrants caution in interpretation and generalizability. Therefore the presented gene expression data need to be subjected to confirmatory steps, such polymerase chain reaction (PCR) methods for confirmation on the mRNA level, and by proteomic analyses.

In conclusion, we demonstrate differences in gene expression profile of healthy masticatory mucosa in chronic periodontitis, and particularly in aggressive periodontitis, when compared among them and with healthy group, whose could be associated to differences in the pathogenesis between them.

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Table 1. Patient characteristics.

Characteristics	Aggressive	Chronic	Healthy	p-value
Age (mean±sd)	32.25 ± 5.85 A	51.75 ± 3.30 B	46.25 ± 11.44 AB	0.0153
FMPI (mean±sd)	23.10 ± 6.53 A	17.05 ± 8.42 A	19.36 ± 5.38 A	0.2696
FMBS (mean±sd)	24.83 ± 9.05 A	23.53 ± 7.69 A	19.39 ± 2.31 A	0.5428
PPD (mean±sd)	2.35 ± 0.05 A	2.28 ± 0.27 A	2.13 ± 0.23 A	0.3504
CAL (mean±sd)	5.44 ± 1.04 A	5.44 ± 0.90 A	5.15 ± 0.72 A	0.8729

Diferente letters (capital for intergroup analysis) indicate statistical difference by one-way ANOVA / Tukey test

Sd – Standard Deviation; FMPI – Full Mouth Plaque Index; FMBS – Full Mouth Bleeding Index; PPD – Probing Pocket Depth; CAL – Clinical Attachment Level.

Table 2. Top differentially expressed genes in aggressive periodontitis group relative to healthy group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
SLC35F3	solute carrier family 35, member F3	6.10	0.005
LOC554223	hypothetical LOC554223	3.57	0.038
MUC16	mucin 16, cell surface associated	3.52	0.029
KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	3.22	0.016
KIR3DL1	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1	3.14	0.012
KLRC1	killer cell lectin-like receptor subfamily C, member 1	3.08	0.029
KIR2DS5	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5	3.07	0.015
KIR2DL3	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 3	2.90	0.022
EDN2	endothelin 2	2.80	0.027
KIR2DL5A	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 5A	2.76	0.018
KLRC4	killer cell lectin-like receptor subfamily C, member 4	2.75	0.017
KIR2DS2	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 2	2.70	0.012
KLRC3	killer cell lectin-like receptor subfamily C, member 3	2.67	0.011
SH2D1B	SH2 domain containing 1B	2.64	0.005
N/A	Homo sapiens cDNA FLJ46698 fis, clone TRACH3013684.	2.62	0.031
Down-Regulated			
EGR3	early growth response 3	-6.36	0.002
ZFY	zinc finger protein, Y-linked	-6.33	0.036
CYorf15A	chromosome Y open reading frame 15A	-5.25	0.043
LOC284116	hypothetical protein LOC284116	-4.89	0.001
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	-4.81	0.003
CYorf15B	chromosome Y open reading frame 15B	-4.30	0.031
NLGN4Y	neuroligin 4, Y-linked	-3.98	0.014
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	-3.90	0.001
CASP14	caspase 14, apoptosis-related cysteine peptidase	-3.87	0.032
ECHDC3	enoyl Coenzyme A hydratase domain containing 3	-3.71	0.011
RPS4Y1	ribosomal protein S4, Y-linked 1	-3.46	0.050
HAL	histidine ammonia-lyase	-3.42	0.019
SLITRK5	SLIT and NTRK-like family, member 5	-3.29	0.025
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	-3.16	0.026
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	-3.10	0.016

FC – Fold Change - describes the ratio of mean expression levels in aggressive periodontitis groups over the mean expression levels in healthy group. Multiple probe sets map to a single gene.

Table 3. Top Gene Ontology Groups differentially expressed in aggressive periodontitis group relative to healthy group.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
Cellular defense response	GO:0006968	2,3E-7	6	12,8
Response to stimulus	GO:0050896	3,3E-5	20	42,6
Defense response	GO:0006952	5,2E-5	9	19,1
Immune response	GO:0006955	1,2E-4	9	19,1
Immune system process	GO:0002376	2,7E-4	10	21,3
Response to stress	GO:0006950	3,1E-2	9	19,1
Natural killer cell activation	GO:0030101	4,7E-2	2	4,3
Down-Regulated				
Neurotransmitter transport	GO:0006836	2,2E-5	7	5,6
Secretion	GO:0046903	1,1E-3	9	7,2
Nervous system development	GO:0007399	1,1E-3	18	14,4
Developmental process	GO:0032502	2,8E-3	35	28,0
Generation of a signal involved in cell-cell signaling	GO:0003001	2,8E-3	5	4,0
Transmission of nerve impulse	GO:0019226	2,8E-3	9	7,2
Secretion by cell	GO:0032940	3,0E-3	7	5,6
Synaptic transmission	GO:0007268	4,4E-3	8	6,4
Multicellular organismal development	GO:0007275	4,5E-3	32	25,6
Anatomical structure development	GO:0048856	5,3E-3	29	23,2

Count – gene involved in the GO term.

% regulated – percentage of genes involved in given term (number of genes involved in given term divided by the total number of input gene).

Table 4. Differentially expressed pathways, determined by pathway analysis

Pathways	p-value	Counts
Comparison I - Aggressive periodontitis vs Healthy		
Antigen processing and presentation	2,3E-13	15
Natural killer cell mediated cytotoxicity	2,4E-8	13
Graft-versus-host disease	2,3E-7	8
Fc gamma R-mediated phagocytosis	9,7E-2	4
Comparison II - Chronic periodontitis vs Healthy		
Cell adhesion molecules (CAMs)	5,6E-2	3
Comparison III - Chronic vs Aggressive periodontitis		
Hematopoietic cell lineage	1,4E-3	6
Fc gamma R-mediated phagocytosis	1,4E-2	5
Arachidonic acid metabolism	1,7E-2	4
Cell adhesion molecules (CAMs)	4,0E-2	5
ECM-receptor interaction	4,9E-2	4
Toll-like receptor signaling pathway	7,6E-2	4

Counts – gene counts included in the Kyoto Encyclopedia of Genes and Genomes pathway.

Table 5. Top differentially expressed genes in chronic periodontitis group relative to healthy group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
HS3ST4	heparan sulfate (glucosamine) 3-O-sulfotransferase 4	4.80	0.019
PDK4	pyruvate dehydrogenase kinase, isozyme 4	3.46	0.046
KLKB1	kallikrein B, plasma (Fletcher factor) 1	2.55	0.050
CD1B	CD1b molecule	2.43	0.005
HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	2.33	0.027
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	2.23	0.029
NPTX2	neuronal pentraxin II	2.12	0.011
NPTX2	neuronal pentraxin II	2.12	0.027
NPY	neuropeptide Y	2.03	0.040
C1D	nuclear DNA-binding protein	2.03	0.031
Down-Regulated			
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	-10.17	0.050
IBSP	integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	-5.76	0.048
CYorf15A	chromosome Y open reading frame 15A	-5.39	0.041
CYorf14	chromosome Y open reading frame 14	-4.63	0.043
DDX43	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	-4.50	0.012
CYorf15B	chromosome Y open reading frame 15B	-4.46	0.028
NLGN4Y	neuroligin 4, Y-linked	-4.11	0.013
GLT1D1	glycosyltransferase 1 domain containing 1	-3.10	0.007
SELE	selectin E (endothelial adhesion molecule 1)	-2.87	0.049
LILRA1	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 1	-2.68	0.016
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	-2.68	0.046
ZNF533	zinc finger protein 533	-2.61	0.041
NGFB	nerve growth factor, beta polypeptide	-2.54	0.034
FCN1	ficolin (collagen/fibrinogen domain containing) 1	-2.52	0.030
N/A	Homo sapiens cDNA FLJ14320 fis, clone PLACE3000455.	-2.40	0.016

FC – Fold Change - describes the ratio of mean expression levels in chronic periodontitis groups over the mean expression levels in healthy group. Multiple probe sets map to a single gene.

Table 6. Top Gene Ontology Groups differentially expressed in chronic periodontitis group relative to healthy group.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
Regulation of proteolysis	GO:0030162	3,0E-2	2	22,2
Antigen processing and presentation	GO:0019882	4,6E-2	2	22,2
Regulation of response to external stimulus	GO:0032101	8,7E-2	2	22,2
Down-Regulated				
Defense response	GO:0006952	1,4E-3	6	20,0
Immune system process	GO:0002376	2,0E-3	7	23,3
Multi-organism process	GO:0051704	2,2E-3	6	20,0
Cell adhesion	GO:0007155	2,5E-3	6	20,0
Biological adhesion	GO:0022610	2,5E-3	6	20,0
Response to lipopolysaccharide	GO:0032496	5,2E-3	3	10,0
Response to wounding	GO:0009611	5,9E-3	5	16,7
Response to stress	GO:0006950	6,5E-3	8	26,7
Response to molecule of bacterial origin	GO:0002237	6,5E-3	3	10,0
Response to other organism	GO:0051707	7,6E-3	4	13,3

Count – gene involved in the GO term.

% regulated – percentage of genes involved in given term (number of genes involved in given term divided by the total number of input gene).

Table 7. Top differentially expressed genes in chronic periodontitis group relative to aggressive periodontitis group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
PSORS1C1	psoriasis susceptibility 1 candidate 1	7.27	0.009
EGR3	early growth response 3	6.29	0.002
ECHDC3	enoyl Coenzyme A hydratase domain containing 3	4.51	0.004
CASP14	caspase 14, apoptosis-related cysteine peptidase	4.16	0.026
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	4.09	0.001
LOC284116	hypothetical protein LOC284116	3.87	0.003
CCDC85B	coiled-coil domain containing 85B	3.82	0.028
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	3.64	0.008
FLJ16165	FLJ16165 protein	3.64	0.025
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	3.54	0.010
HAL	histidine ammonia-lyase	3.54	0.016
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	3.46	0.018
KLK9	kallikrein 9	3.41	0.002
PNPLA1	patatin-like phospholipase domain containing 1	3.21	0.046
SPRR4	small proline rich protein 4	3.05	0.045
Down-Regulated			
SLC35F3	solute carrier family 35, member F3	-4.3	0.042
SELE	selectin E (endothelial adhesion molecule 1)	-3.9	0.039
IL6	interleukin 6 (interferon, beta 2)	-3.8	0.030
LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	-3.7	0.014
FPRL1	formyl peptide receptor-like 1	-3.5	0.013
LOC554223	hypothetical LOC554223	-3.5	0.034
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	-3.4	0.002
LOC440607	Fc-gamma receptor I B2	-3.3	0.004
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	-3.3	0.009
CCL23	chemokine (C-C motif) ligand 23	-3	0.034
LYSMD1	LysM, putative peptidoglycan-binding, domain containing 1	-3	0.008
PSCA	prostate stem cell antigen	-3	0.046
N/A	Homo sapiens cDNA FLJ14320 fis, clone PLACE3000455.	-3	0.004
KLRF1	killer cell lectin-like receptor subfamily F, member 1	-2.9	0.012
ELOVL2	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	-2.9	0.048

FC – Fold Change - describes the ratio of mean expression levels in chronic periodontitis groups over the mean expression levels in aggressive periodontitis group. Multiple probe sets map to a single gene.

Table 8. Top Gene Ontology Groups differentially expressed in chronic periodontitis group relative to aggressive periodontitis.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
Phospholipid catabolic process	GO:0009395	3,4E-3	3	4,3
Leukotriene metabolic process	GO:0006691	3,4E-3	3	4,3
Cellular alkene metabolic process	GO:0043449	3,7E-3	3	4,3
Lipid catabolic process	GO:0016042	4,9E-3	5	7,1
Epidermis development	GO:0008544	6,1E-3	5	7,1
Ectoderm development	GO:0007398	7,9E-3	5	7,1
Icosanoid metabolic process	GO:0006690	1,5E-2	3	4,3
Unsaturated fatty acid metabolic process	GO:0033559	1,7E-2	3	4,3
Cellular lipid metabolic process	GO:0044255	1,7E-2	7	10,0
Ion transport	GO:0006811	3,1E-2	8	11,4
Down-Regulated				
Immune response	GO:0006955	5,0E-6	14	16,7
Immune system process	GO:0002376	1,3E-5	16	19,0
Cell adhesion	GO:0007155	3,1E-5	13	15,5
Biological adhesion	GO:0022610	3,2E-5	13	15,5
Inflammatory response	GO:0006954	6,5E-5	9	10,7
Response to wounding	GO:0009611	7,1E-5	11	13,1
Defense response	GO:0006952	2,4E-4	11	13,1
Locomotion	GO:0040011	4,5E-4	9	10,7
Cell-cell adhesion	GO:0016337	1,1E-3	7	8,3
Response to external stimulus	GO:0009605	1,4E-3	12	14,3

Count – gene involved in the GO term.

% regulated – percentage of genes involved in given term (number of genes involved in given term divided by the total number of input gene).

Supplementary table 1. Differentially expressed genes in aggressive periodontitis group relative to healthy group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
SLC35F3	solute carrier family 35, member F3	6.10	0.005
LOC554223	hypothetical LOC554223	3.57	0.038
MUC16	mucin 16, cell surface associated	3.52	0.029
KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	3.22	0.016
KIR3DL1	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1	3.14	0.012
KLRC1	killer cell lectin-like receptor subfamily C, member 1	3.08	0.029
KIR2DS5	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5	3.07	0.015
KIR2DL3	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 3	2.90	0.022
EDN2	endothelin 2	2.80	0.027
KIR2DL5A	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 5A	2.76	0.018
KLRC4	killer cell lectin-like receptor subfamily C, member 4	2.75	0.017
KIR2DS2	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 2	2.70	0.012
KLRC3	killer cell lectin-like receptor subfamily C, member 3	2.67	0.011
SH2D1B	SH2 domain containing 1B	2.64	0.005
N/A	Homo sapiens cDNA FLJ46698 fis, clone TRACH3013684.	2.62	0.031
PLGLA1	plasminogen-like A1	2.62	0.045
KIR2DL1	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 1	2.62	0.020
KLRC2	killer cell lectin-like receptor subfamily C, member 2	2.59	0.021
LOC653489	similar to Ran-binding protein 2 (RanBP2) (Nuclear pore complex protein Nup358) (Nucleoporin Nup358) (358 kDa nucleoporin) (P270)	2.59	0.041
KIR2DS4	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 4	2.57	0.010
RGPD4	RANBP2-like and GRIP domain containing 4	2.56	0.049
TXLNB	taxilin beta	2.52	0.015
KIR3DL2	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2	2.52	0.031
KLHL4	kelch-like 4 (Drosophila)	2.48	0.003
N/A	Homo sapiens clone KIR2DS2v2 killer-cell Ig-like receptor mRNA, complete cds.	2.45	0.007
LQK1	LQK1 hypothetical protein short isoform	2.44	0.031
CLDN8	claudin 8	2.42	0.022
KIR2DS1	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 1	2.36	0.010
KIR2DL2	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 2	2.34	0.014
IL18RAP	interleukin 18 receptor accessory protein	2.34	0.033
LOC646299	hypothetical protein LOC646299	2.33	0.002
C12orf54	chromosome 12 open reading frame 54	2.31	0.001
KIR3DL3	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 3	2.31	0.020
INSL3	insulin-like 3 (Leydig cell)	2.31	0.029
C13orf18	chromosome 13 open reading frame 18	2.30	0.035

HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	2.30	0.050
FLJ26175	FLJ26175 protein	2.29	0.004
WDR49	WD repeat domain 49	2.29	0.010
KIR2DS3	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 3	2.27	0.001
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	2.25	0.023
N/A	Homo sapiens clone FLB7723 PRO2055 mRNA, complete cds.	2.21	0.030
FHAD1	forkhead-associated (FHA) phosphopeptide binding domain 1	2.17	0.007
LOC440895	similar to LIM and senescent cell antigen-like domains 3	2.17	0.029
PENK	proenkephalin	2.14	0.035
PDE4D	phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, Drosophila)	2.13	0.024
C2orf27	chromosome 2 open reading frame 27	2.13	0.029
C1D	nuclear DNA-binding protein	2.13	0.028
KLRD1	killer cell lectin-like receptor subfamily D, member 1	2.10	0.026
OR1N2	olfactory receptor, family 1, subfamily N, member 2	2.09	0.022
LOC644246	hypothetical protein LOC644246	2.09	0.039
NPTX2	neuronal pentraxin II	2.08	0.028
FPR1	formyl peptide receptor 1	2.07	0.016
NNMT	nicotinamide N-methyltransferase	2.07	0.039
NTRK2	neurotrophic tyrosine kinase, receptor, type 2	2.06	0.013
AADACL1	arylacetamide deacetylase-like 1	2.04	0.015
TTLL9	tubulin tyrosine ligase-like family, member 9	2.03	0.009
Down-Regulated			
EGR3	early growth response 3	-6.36	0.002
ZFY	zinc finger protein, Y-linked	-6.33	0.036
CYorf15A	chromosome Y open reading frame 15A	-5.25	0.043
LOC284116	hypothetical protein LOC284116	-4.89	0.001
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	-4.81	0.003
CYorf15B	chromosome Y open reading frame 15B	-4.30	0.031
NLGN4Y	neuroligin 4, Y-linked	-3.98	0.014
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	-3.90	0.001
CASP14	caspase 14, apoptosis-related cysteine peptidase	-3.87	0.032
ECHDC3	enoyl Coenzyme A hydratase domain containing 3	-3.71	0.011
RPS4Y1	ribosomal protein S4, Y-linked 1	-3.46	0.050
HAL	histidine ammonia-lyase	-3.42	0.019
SLITRK5	SLIT and NTRK-like family, member 5	-3.29	0.025
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	-3.16	0.026
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	-3.10	0.016
SIM2	single-minded homolog 2 (Drosophila)	-3.05	0.043
EDN3	endothelin 3	-3.02	0.044

KLK9	kallikrein 9	-2.89	0.005
SNRP70	small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen)	-2.88	0.004
HRH3	histamine receptor H3	-2.84	0.017
N/A	Homo sapiens cDNA clone MGC:88155 IMAGE:30417356, complete cds.	-2.84	0.043
LOC644193	similar to Y51B11A.1	-2.84	0.008
LOC441161	hypothetical LOC441161	-2.83	0.007
DLX2	distal-less homeobox 2	-2.80	0.007
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	-2.79	0.039
KIAA1545	KIAA1545 protein	-2.74	0.006
FAM83G	family with sequence similarity 83, member G	-2.73	0.002
N/A	Homo sapiens cDNA FLJ44636 fis, clone BRACE2025452.	-2.73	0.005
PAX8	paired box gene 8	-2.72	0.003
JMJD2B	jumonji domain containing 2B	-2.69	0.003
COMP	cartilage oligomeric matrix protein	-2.65	0.038
NTNG1	netrin G1	-2.64	0.022
FLJ45455	FLJ45455 protein	-2.64	0.015
LOC283174	hypothetical protein LOC283174	-2.63	0.011
SERPINB7	serpin peptidase inhibitor, clade B (ovalbumin), member 7	-2.63	0.025
TRPV3	transient receptor potential cation channel, subfamily V, member 3	-2.62	0.020
FBS1	fibrosin 1	-2.59	0.009
RP13-297E16.1	DNA segment on chromosome X and Y (unique) 155 expressed sequence, isoform 1	-2.57	0.006
PLA2G4E	phospholipase A2, group IVE	-2.56	0.010
DKK2	dickkopf homolog 2 (Xenopus laevis)	-2.54	0.005
SLC6A11	solute carrier family 6 (neurotransmitter transporter, GABA), member 11	-2.51	0.005
C1orf170	chromosome 1 open reading frame 170	-2.51	0.029
IQSEC1	IQ motif and Sec7 domain 1	-2.50	0.009
PSG2	pregnancy specific beta-1-glycoprotein 2	-2.50	0.043
CBX4	chromobox homolog 4 (Pc class homolog, Drosophila)	-2.49	0.003
LOC339240	keratin pseudogene	-2.48	0.017
CALML5	calmodulin-like 5	-2.48	0.009
SEMA7A	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)	-2.47	0.002
FLJ90166	hypothetical protein FLJ90166	-2.47	0.033
POU2F3	POU domain, class 2, transcription factor 3	-2.47	0.002
SERPINB12	serpin peptidase inhibitor, clade B (ovalbumin), member 12	-2.46	0.028
RIMS3	regulating synaptic membrane exocytosis 3	-2.45	0.034
LOC284837	hypothetical protein LOC284837	-2.43	0.001
EGR3	early growth response 3	-2.42	0.010
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	-2.40	0.001
N/A	Homo sapiens cDNA FLJ43768 fis, clone TESTI2049422.	-2.39	0.002
CENTB5	centaurin, beta 5	-2.39	0.001

ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	-2.39	0.010
CYP26B1	cytochrome P450, family 26, subfamily B, polypeptide 1	-2.34	0.007
C1orf34	chromosome 1 open reading frame 34	-2.34	0.024
PSG9	pregnancy specific beta-1-glycoprotein 9	-2.34	0.049
PVRL1	poliovirus receptor-related 1 (herpesvirus entry mediator C	-2.33	0.040
TCEB3C	transcription elongation factor B polypeptide 3C (elongin A3)	-2.33	0.002
IRX6	iroquois homeobox protein 6	-2.33	0.042
N/A	Homo sapiens sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6C, mRNA (cDNA clone MGC:138675 IMAGE:40036286), complete cds.	-2.31	0.002
BAI1	brain-specific angiogenesis inhibitor 1	-2.30	0.012
RP5-1119A7.4	hypothetical protein FLJ23322	-2.29	0.001
DNM1	dynamin 1	-2.29	0.000
ZBTB7B	zinc finger and BTB domain containing 7B	-2.26	0.008
LOC654433	hypothetical LOC654433	-2.26	0.042
FLJ13137	hypothetical gene supported by AK125122	-2.25	0.011
CRMP1	collapsin response mediator protein 1	-2.24	0.032
PPFIA3	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 3	-2.24	0.006
DEGS2	degenerative spermatocyte homolog 2, lipid desaturase (Drosophila)	-2.23	0.049
LOC646182	similar to XG glycoprotein precursor (Protein PBDX)	-2.23	0.004
SOX14	SRY (sex determining region Y)-box 14	-2.23	0.001
ADRB1	adrenergic, beta-1-, receptor	-2.23	0.007
SEMA4C	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	-2.23	0.005
USP2	ubiquitin specific peptidase 2	-2.22	0.019
VANGL2	vang-like 2 (van gogh, Drosophila)	-2.22	0.002
B4GALNT3	beta-1,4-N-acetyl-galactosaminyl transferase 3	-2.22	0.022
TBX1	T-box 1	-2.22	0.016
WDR72	WD repeat domain 72	-2.21	0.016
D2HGDH	D-2-hydroxyglutarate dehydrogenase	-2.21	0.009
TNKS1BP1	tankyrase 1 binding protein 1, 182kDa	-2.20	0.007
KLHDC3	kelch domain containing 3	-2.20	0.001
SCAMP5	secretory carrier membrane protein 5	-2.20	0.025
OR2W3	olfactory receptor, family 2, subfamily W, member 3	-2.19	0.037
XYLT1	xylosyltransferase I	-2.19	0.009
CIB2	calcium and integrin binding family member 2	-2.19	0.001
LOC401356	similar to CG31151-PB, isoform B	-2.18	0.005
FLJ39501	cytochrome P450, family 2, subfamily E, polypeptide 2 homolog	-2.17	0.006
ARHGAP23	Rho GTPase activating protein 23	-2.15	0.035
KCNC3	potassium voltage-gated channel, Shaw-related subfamily, member 3	-2.15	0.008

ICAM5	intercellular adhesion molecule 5, telencephalin	-2.15	0.006
CLN8	ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)	-2.14	0.028
PTGS1	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	-2.14	0.003
SEPT5	septin 5	-2.14	0.005
KIAA0310	KIAA0310	-2.14	0.015
N/A	Homo sapiens cDNA FLJ36021 fis, clone TESTI2016568.	-2.13	0.012
PLA2G4F	phospholipase A2, group IVF	-2.13	0.036
MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	-2.12	0.046
MGC12760	hypothetical protein MGC12760	-2.11	0.042
PLEKHG5	pleckstrin homology domain containing, family G (with RhoGef domain) member 5	-2.09	0.009
LOC402275	similar to Limbic system-associated membrane protein precursor (LSAMP)	-2.09	0.008
KCNJ9	potassium inwardly-rectifying channel, subfamily J, member 9	-2.09	0.015
PTBP1	polypyrimidine tract binding protein 1	-2.08	0.003
FADS2	fatty acid desaturase 2	-2.08	0.037
ALOXE3	arachidonate lipoxygenase 3	-2.08	0.007
DDX12	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 12 (CHL1-like helicase homolog, S. cerevisiae)	-2.07	0.005
C14orf121	chromosome 14 open reading frame 121	-2.07	0.026
DMRTA2	DMRT-like family A2	-2.07	0.030
FLJ14186	hypothetical gene supported by AK024248	-2.07	0.000
KIRREL	kin of IRRE like (Drosophila)	-2.06	0.031
PCDH21	protocadherin 21	-2.06	0.021
FLJ90231	hypothetical protein FLJ90231	-2.06	0.044
KCNK5	potassium channel, subfamily K, member 5	-2.05	0.037
NRBP2	nuclear receptor binding protein 2	-2.05	0.001
BCL2L2	BCL2-like 2	-2.05	0.000
CBLN1	cerebellin 1 precursor	-2.05	0.010
NFIX	nuclear factor I/X (CCAAT-binding transcription factor)	-2.04	0.011
LOC643757	hypothetical protein LOC643757	-2.04	0.013
SLC35E2	solute carrier family 35, member E2	-2.04	0.011
SDC3	syndecan 3 (N-syndecan)	-2.03	0.008
EPN1	epsin 1	-2.02	0.018
CLSTN2	calsyntenin 2	-2.02	0.029
LOC55908	hepatocellular carcinoma-associated gene TD26	-2.02	0.005
OTOP1	otopetrin 1	-2.02	0.043
WDR62	WD repeat domain 62	-2.02	0.028
CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit	-2.01	0.007
TNK2	tyrosine kinase, non-receptor, 2	-2.01	0.011
FCHSD1	FCH and double SH3 domains 1	-2.01	0.012
LOC402382	similar to collagen, type I, alpha 2	-2.01	0.002
PLEKHH3	pleckstrin homology domain containing, family H (with MyTH4 domain) member 3	-2.01	0.014

TCF7	transcription factor 7 (T-cell specific, HMG-box)	-2.00	0.004
TMEM16H	transmembrane protein 16H	-2.00	0.001

Supplementary table 2. Differentially expressed genes in chronic periodontitis group relative to healthy group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
HS3ST4	heparan sulfate (glucosamine) 3-O-sulfotransferase 4	4.80	0.019
PDK4	pyruvate dehydrogenase kinase, isozyme 4	3.46	0.046
KLKB1	kallikrein B, plasma (Fletcher factor) 1	2.55	0.050
CD1B	CD1b molecule	2.43	0.005
HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	2.33	0.027
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	2.23	0.029
NPTX2	neuronal pentraxin II	2.12	0.011
NPY	neuropeptide Y	2.03	0.040
C1D	nuclear DNA-binding protein	2.03	0.031
Down-Regulated			
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	-10.17	0.050
IBSP	integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	-5.76	0.048
CYorf15A	chromosome Y open reading frame 15A	-5.39	0.041
CYorf14	chromosome Y open reading frame 14	-4.63	0.043
DDX43	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	-4.50	0.012
CYorf15B	chromosome Y open reading frame 15B	-4.46	0.028
NLGN4Y	neuroligin 4, Y-linked	-4.11	0.013
GLT1D1	glycosyltransferase 1 domain containing 1	-3.10	0.007
SELE	selectin E (endothelial adhesion molecule 1)	-2.87	0.049
LILRA1	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 1	-2.68	0.016
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	-2.68	0.046
ZNF533	zinc finger protein 533	-2.61	0.041
NGFB	nerve growth factor, beta polypeptide	-2.54	0.034
FCN1	ficolin (collagen/fibrinogen domain containing) 1	-2.52	0.030
N/A	Homo sapiens cDNA FLJ14320 fis, clone PLACE3000455.	-2.40	0.016
EMR1	egf-like module containing, mucin-like, hormone receptor-like 1	-2.38	0.013
LBP	lipopolysaccharide binding protein	-2.35	0.048
OR2W3	olfactory receptor, family 2, subfamily W, member 3	-2.35	0.025
ADRB1	adrenergic, beta-1-, receptor	-2.29	0.006
EIF2A	eukaryotic translation initiation factor 2A, 65kDa	-2.27	0.032
WDR72	WD repeat domain 72	-2.26	0.014
NFE2	nuclear factor (erythroid-derived 2), 45kDa	-2.25	0.020
LOC654433	hypothetical LOC654433	-2.22	0.045
LOC643331	similar to Kinase suppressor of ras-1 (Kinase suppressor of ras) (mKSR1) (Hb protein)	-2.20	0.031
LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains),	-2.20	0.015

	member 2		
N/A	Homo sapiens mRNA	-2.19	0.036
LOC645402	similar to deubiquitinating enzyme 3	-2.18	0.038
KIAA1383	KIAA1383	-2.18	0.004
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	-2.18	0.028
NLGN4X	neuroligin 4, X-linked	-2.10	0.029
PAX1	paired box gene 1	-2.07	0.023
TLR8	toll-like receptor 8	-2.04	0.097
DMRTA2	DMRT-like family A2	-2.00	0.037

Supplementary table 3. Differentially expressed genes in chronic periodontitis group relative to aggressive periodontitis group.

Gene Symbol	Gene Name	FC	p
Up-Regulated			
PSORS1C1	psoriasis susceptibility 1 candidate 1	7.27	0.009
EGR3	early growth response 3	6.29	0.002
ECHDC3	enoyl Coenzyme A hydratase domain containing 3	4.51	0.004
CASP14	caspase 14, apoptosis-related cysteine peptidase	4.16	0.026
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12	4.09	0.001
LOC284116	hypothetical protein LOC284116	3.87	0.003
CCDC85B	coiled-coil domain containing 85B	3.82	0.028
ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	3.64	0.008
FLJ16165	FLJ16165 protein	3.64	0.025
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	3.54	0.010
HAL	histidine ammonia-lyase	3.54	0.016
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	3.46	0.018
KLK9	kallikrein 9	3.41	0.002
PNPLA1	patatin-like phospholipase domain containing 1	3.21	0.046
SPRR4	small proline rich protein 4	3.05	0.045
SERPINB12	serpin peptidase inhibitor, clade B (ovalbumin), member 12	2.9	0.012
CYP4F11	cytochrome P450, family 4, subfamily F, polypeptide 11	2.9	0.049
USP2	ubiquitin specific peptidase 2	2.85	0.005
HKDC1	hexokinase domain containing 1	2.83	0.003
SLC15A1	solute carrier family 15 (oligopeptide transporter), member 1	2.79	0.038
ABCG4	ATP-binding cassette, sub-family G (WHITE), member 4	2.73	0.017
IRX6	iroquois homeobox protein 6	2.7	0.021
DLGAP1	discs, large (Drosophila) homolog-associated protein 1	2.68	0.038
SEMA7A	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)	2.63	0.001
PLA2G4E	phospholipase A2, group IVE	2.59	0.009
LOC339240	keratin pseudogene	2.55	0.015
DKK2	dickkopf homolog 2 (Xenopus laevis)	2.5	0.005
COMP	cartilage oligomeric matrix protein	2.49	0.049
EEF1A2	eukaryotic translation elongation factor 1 alpha 2	2.48	0.012
MAT1A	methionine adenosyltransferase I, alpha	2.46	0.040
DLX2	distal-less homeobox 2	2.42	0.040
FLJ10781	hypothetical protein FLJ10781	2.41	0.034
MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	2.4	0.036
SLC6A11	solute carrier family 6 (neurotransmitter transporter, GABA), member 11	2.39	0.007
KIF1A	kinesin family member 1A	2.38	0.006

LOC339240	keratin pseudogene	2.35	0.015
SCTR	secretin receptor	2.35	0.009
ARSE	arylsulfatase E (chondrodysplasia punctata 1)	2.32	0.039
LOC644003	similar to Mucin-2 precursor (Intestinal mucin 2)	2.31	0.029
RIMS3	regulating synaptic membrane exocytosis 3	2.31	0.045
ALOX12B	arachidonate 12-lipoxygenase, 12R type	2.31	0.029
SLC6A17	solute carrier family 6, member 17	2.29	0.042
BTNL9	butyrophilin-like 9	2.27	0.024
CES4	carboxylesterase 4-like	2.27	0.016
CALML5	calmodulin-like 5	2.26	0.016
SP6	Sp6 transcription factor	2.26	0.007
LOC645438	similar to CG7874-PA	2.24	0.023
AGC1	aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	2.22	0.047
RP3-402H5.2	similar to dJ402H5.2 (novel protein similar to worm and fly proteins)	2.2	0.006
ALOXE3	arachidonate lipoxygenase 3	2.2	0.005
LOC644193	similar to Y51B11A.1	2.16	0.036
C1orf34	chromosome 1 open reading frame 34	2.15	0.038
FIS	FIS	2.14	0.029
CHRFAM7A	CHRNA7 (cholinergic receptor, nicotinic, alpha 7, exons 5-10) and FAM7A (family with sequence similarity 7A, exons A-E) fusion	2.12	0.042
FAM83G	family with sequence similarity 83, member G	2.12	0.011
SCNN1G	sodium channel, nonvoltage-gated 1, gamma	2.11	0.012
FLJ39501	cytochrome P450, family 2, subfamily E, polypeptide 2 homolog	2.1	0.004
SERPINA5	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	2.1	0.041
FCHSD1	FCH and double SH3 domains 1	2.09	0.008
FLJ30934	hypothetical protein FLJ30934	2.09	0.042
LCE3B	late cornified envelope 3B	2.09	0.049
PLA2G2F	phospholipase A2, group IIF	2.09	0.009
MFAP3L	microfibrillar-associated protein 3-like	2.08	0.030
CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)	2.08	0.017
TPO	thyroid peroxidase	2.06	0.043
N/A	Homo sapiens cDNA FLJ44636 fis, clone BRACE2025452.	2.06	0.030
CYP4F2	cytochrome P450, family 4, subfamily F, polypeptide 2	2.06	0.000
XCR1	chemokine (C motif) receptor 1	2.06	0.043
SLC26A9	solute carrier family 26, member 9	2.05	0.006
N/A	Homo sapiens megakaryocyte-enhanced gene transcript 1 protein (MEGT1) mRNA, complete cds.	2.04	0.043
LY6H	lymphocyte antigen 6 complex, locus H	2.03	0.037
HIF3A	hypoxia inducible factor 3, alpha subunit	2.02	0.037
TAGLN3	transgelin 3	2.01	0.003

TRPV3	transient receptor potential cation channel, subfamily V, member 3	2.01	0.017
Down-Regulated			
SLC35F3	solute carrier family 35, member F3	-4.3	0.042
SELE	selectin E (endothelial adhesion molecule 1)	-3.9	0.039
IL6	interleukin 6 (interferon, beta 2)	-3.8	0.030
LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	-3.7	0.014
FPRL1	formyl peptide receptor-like 1	-3.5	0.013
LOC554223	hypothetical LOC554223	-3.5	0.034
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	-3.4	0.002
LOC440607	Fc-gamma receptor I B2	-3.3	0.004
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	-3.3	0.009
CCL23	chemokine (C-C motif) ligand 23	-3	0.034
LYSMD1	LysM, putative peptidoglycan-binding, domain containing 1	-3	0.008
PSCA	prostate stem cell antigen	-3	0.046
N/A	Homo sapiens cDNA FLJ14320 fis, clone PLACE3000455.	-3	0.004
KLRF1	killer cell lectin-like receptor subfamily F, member 1	-2.9	0.012
ELOVL2	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	-2.9	0.048
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif, 3	-2.9	0.034
TCTEX1D1	Tctex1 domain containing 1	-2.9	0.043
APLN	apelin, AGTRL1 ligand	-2.9	0.017
SH2D1B	SH2 domain containing 1B	-2.8	0.023
AQP9	aquaporin 9	-2.8	0.048
RGPD4	RANBP2-like and GRIP domain containing 4	-2.8	0.003
LOC642681	hypothetical protein LOC642681	-2.8	0.025
CR1L	complement component (3b/4b) receptor 1-like	-2.7	0.037
TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	-2.7	0.020
TLR7	toll-like receptor 7	-2.7	0.007
GLT1D1	glycosyltransferase 1 domain containing 1	-2.7	0.014
SIGLEC7	sialic acid binding Ig-like lectin 7	-2.6	0.017
LPPR4	plasticity related gene 1	-2.6	0.027
HS6ST2	heparan sulfate 6-O-sulfotransferase 2	-2.6	0.037
ZNF229	zinc finger protein 229	-2.5	0.022
CD300LF	CD300 molecule-like family member f	-2.5	0.022
ARHGAP20	Rho GTPase activating protein 20	-2.5	0.050
CSTL1	cystatin-like 1	-2.5	0.024
EMR1	egf-like module containing, mucin-like, hormone receptor-like 1	-2.5	0.010
LOC653127	similar to GTPase activating Rap/RanGAP domain-like 4	-2.5	0.014
N/A	Homo sapiens cDNA FLJ38999 fis, clone NT2RI2021625.	-2.5	0.020
CNDP1	carnosine dipeptidase 1 (metallopeptidase M20 family)	-2.4	0.015

C9orf26	chromosome 9 open reading frame 26 (NF-HEV)	-2.4	0.019
LOC148203	hypothetical protein LOC148203	-2.4	0.010
GARNL4	GTPase activating Rap/RanGAP domain-like 4	-2.4	0.015
CAPS2	calcyphosine 2	-2.4	0.013
EIF2A	eukaryotic translation initiation factor 2A, 65kDa	-2.4	0.024
LILRA1	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 1	-2.4	0.030
NRCAM	neuronal cell adhesion molecule	-2.4	0.050
TLR8	toll-like receptor 8	-2.4	0.050
ELOVL3	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	-2.4	0.018
LOC653489	similar to Ran-binding protein 2 (RanBP2) (Nuclear pore complex protein Nup358) (Nucleoporin Nup358) (358 kDa nucleoporin) (P270)	-2.3	0.011
FLJ13391	hypothetical protein FLJ13391	-2.3	0.050
ZNF718	zinc finger protein 718	-2.3	0.018
ALPK2	alpha-kinase 2	-2.3	0.027
LEMD1	LEM domain containing 1	-2.3	0.000
FLJ44054	hypothetical protein LOC643364	-2.3	0.027
OLR1	oxidised low density lipoprotein (lectin-like) receptor 1	-2.3	0.044
DNM3	dynamin 3	-2.2	0.014
FLJ45743	hypothetical protein LOC642484	-2.2	0.028
FLJ42461	FLJ42461 protein	-2.2	0.007
HGF	hepatocyte growth factor (hepapoietin A	-2.2	0.002
C12orf36	chromosome 12 open reading frame 36	-2.2	0.036
GDF9	growth differentiation factor 9	-2.2	0.039
KIAA1383	KIAA1383	-2.2	0.004
KLHL4	kelch-like 4 (Drosophila)	-2.2	0.007
CLDN23	claudin 23	-2.2	0.033
ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	-2.2	0.023
NFE2	nuclear factor (erythroid-derived 2), 45kDa	-2.2	0.025
FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	-2.2	0.047
ACVR1C	activin A receptor, type IC	-2.1	0.037
NNMT	nicotinamide N-methyltransferase	-2.1	0.022
LOC387647	hypothetical gene supported by BC014163	-2.1	0.004
LAMA1	laminin, alpha 1	-2.1	0.007
SDS	serine dehydratase	-2.1	0.045
FLJ46875	hypothetical LOC440918	-2.1	0.048
ZNF542	zinc finger protein 542	-2.1	0.014
PDE4D	phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, Drosophila)	-2.1	0.021
CPNE8	copine VIII	-2.1	0.050
IL1B	interleukin 1, beta	-2.1	0.048
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	-2.1	0.033

PCDH17	protocadherin 17	-2.1	0.041
LOC653043	similar to cell recognition molecule CASPR3	-2.1	0.020
LOC345079	similar to RIKEN cDNA 5730467H21	-2.1	0.020
N/A	Homo sapiens mRNA similar to LOC166173 (cDNA clone MGC:34832 IMAGE:5203730), complete cds.	-2.1	0.005
FNDC3B	fibronectin type III domain containing 3B	-2.1	0.038
GCKR	glucokinase (hexokinase 4) regulator	-2.1	0.035
SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	-2	0.001
LOC643331	similar to Kinase suppressor of ras-1 (Kinase suppressor of ras) (mKSR1) (Hb protein)	-2	0.046
C4orf18	chromosome 4 open reading frame 18	-2	0.031
SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	-2	0.014
CERKL	ceramide kinase-like	-2	0.028
SEMA6B	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6B	-2	0.001
LOC643194	hypothetical protein LOC643194	-2	0.043
DKFZP566N034	hypothetical protein DKFZp566N034	-2	0.033
LOC646373	hypothetical protein LOC646373	-2	0.024
CLDN22	claudin 22	-2	0.016
LOC643355	hypothetical protein LOC643355	-2	0.049
CREB5	cAMP responsive element binding protein 5	-2	0.037

Supplementary table 4. Gene Ontology Groups differentially expressed in aggressive periodontitis group relative to healthy group.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
Cellular defense response	GO:0006968	2,3E-7	6	12,8
Response to stimulus	GO:0050896	3,3E-5	20	42,6
Defense response	GO:0006952	5,2E-5	9	19,1
Immune response	GO:0006955	1,2E-4	9	19,1
Immune system process	GO:0002376	2,7E-4	10	21,3
Response to stress	GO:0006950	3,1E-2	9	19,1
Natural killer cell activation	GO:0030101	4,7E-2	2	4,3
Down-Regulated				
neurotransmitter transport	GO:0006836	2,2E-5	7	5,6
secretion	GO:0046903	1,1E-3	9	7,2
nervous system development	GO:0007399	1,1E-3	18	14,4
developmental process	GO:0032502	2,8E-3	35	28,0
generation of a signal involved in cell-cell signaling	GO:0003001	2,8E-3	5	4,0
transmission of nerve impulse	GO:0019226	2,8E-3	9	7,2
secretion by cell	GO:0032940	3,0E-3	7	5,6
synaptic transmission	GO:0007268	4,4E-3	8	6,4
multicellular organismal development	GO:0007275	4,5E-3	32	25,6
anatomical structure development	GO:0048856	5,3E-3	29	23,2
system development	GO:0048731	6,8E-3	27	21,6
multicellular organismal process	GO:0032501	8,2E-3	42	33,6
phospholipid catabolic process	GO:0009395	9,9E-3	3	2,4
neuron differentiation	GO:0030182	1,1E-2	9	7,2
regulation of neurotransmitter levels	GO:0001505	1,1E-2	4	3,2
cellular developmental process	GO:0048869	1,1E-2	21	16,8
fatty acid biosynthetic process	GO:0006633	1,7E-2	4	3,2
cell-cell signaling	GO:0007267	2,2E-2	10	8,0
neurotransmitter secretion	GO:0007269	2,3E-2	3	2,4
unsaturated fatty acid biosynthetic process	GO:0006636	2,3E-2	3	2,4
potassium ion transport	GO:0006813	2,4E-2	5	4,0
peripheral nervous system development	GO:0007422	2,5E-2	3	2,4
system process	GO:0003008	2,7E-2	18	14,4
cell projection morphogenesis	GO:0048858	2,7E-2	6	4,8
localization	GO:0051179	2,7E-2	30	24,0

cellular lipid metabolic process	GO:0044255	2,8E-2	9	7,2
cell differentiation	GO:0030154	2,9E-2	19	15,2
regulation of blood pressure	GO:0008217	3,2E-2	4	3,2
cell part morphogenesis	GO:0032990	3,2E-2	6	4,8
cell morphogenesis	GO:0000902	3,6E-2	7	5,6
ion transport	GO:0006811	3,8E-2	11	8,8
generation of neurons	GO:0048699	3,8E-2	9	7,2
tissue development	GO:0009888	3,9E-2	10	8,0
blood circulation	GO:0008015	3,9E-2	5	4,0
circulatory system process	GO:0003013	3,9E-2	5	4,0
regulation of norepinephrine secretion	GO:0014061	4,1E-2	2	1,6
metal ion transport	GO:0030001	4,1E-2	8	6,4
axonogenesis	GO:0007409	4,4E-2	5	4,0
cell communication	GO:0007154	4,6E-2	11	8,8
unsaturated fatty acid metabolic process	GO:0033559	4,8E-2	3	2,4
organ development	GO:0048513	4,8E-2	19	15,2
transport	GO:0006810	5,0E-2	26	20,8
regulation of secretion	GO:0051046	5,0E-2	5	4,0

Supplementary table 5. Gene Ontology Groups differentially expressed in chronic periodontitis group relative to healthy group.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
Regulation of proteolysis	GO:0030162	3,0E-2	2	22,2
Antigen processing and presentation	GO:0019882	4,6E-2	2	22,2
Regulation of response to external stimulus	GO:0032101	8,7E-2	2	22,2
Down-Regulated				
defense response		1,4E-3	6	20,0
immune system process		2,0E-3	7	23,3
multi-organism process		2,2E-3	6	20,0
cell adhesion		2,5E-3	6	20,0
biological adhesion		2,5E-3	6	20,0
response to lipopolysaccharide		5,2E-3	3	10,0
response to wounding		5,9E-3	5	16,7
response to stress		6,5E-3	8	26,7
response to molecule of bacterial origin		6,5E-3	3	10,0
response to other organism		7,6E-3	4	13,3
positive regulation of metabolic process		8,0E-3	6	20,0
opsonization		8,5E-3	2	6,7
inflammatory response		1,0E-2	4	13,3
cell surface receptor linked signal transduction		1,1E-2	8	26,7
response to stimulus		1,3E-2	11	36,7
immune response		1,5E-2	5	16,7
immune effector process		1,5E-2	3	10,0
cellular component organization		1,6E-2	9	30,0
response to biotic stimulus		1,6E-2	4	13,3
regulation of defense response		1,7E-2	3	10,0
regulation of interleukin-8 production		2,1E-2	2	6,7
detection of biotic stimulus		2,5E-2	2	6,7
response to bacterium		3,0E-2	3	10,0
response to external stimulus		3,7E-2	5	16,7

Supplementary table 6. Gene Ontology Groups differentially expressed in chronic periodontitis group relative to aggressive periodontitis.

Group Name	ID	p-value	Count	% regulated
Up-Regulated				
phospholipid catabolic process	GO:0009395	3,4E-3	3	4,3
leukotriene metabolic process	GO:0006691	3,4E-3	3	4,3
cellular alkene metabolic process	GO:0043449	3,7E-3	3	4,3
lipid catabolic process	GO:0016042	4,9E-3	5	7,1
epidermis development	GO:0008544	6,1E-3	5	7,1
ectoderm development	GO:0007398	7,9E-3	5	7,1
icosanoid metabolic process	GO:0006690	1,5E-2	3	4,3
unsaturated fatty acid metabolic process	GO:0033559	1,7E-2	3	4,3
cellular lipid metabolic process	GO:0044255	1,7E-2	7	10,0
ion transport	GO:0006811	3,1E-2	8	11,4
cellular lipid catabolic process	GO:0044242	3,7E-2	3	4,3
metal ion transport	GO:0030001	3,7E-2	6	8,6
phospholipid metabolic process	GO:0006644	4,0E-2	4	5,7
lipid metabolic process	GO:0006629	4,1E-2	8	11,4
neurotransmitter transport	GO:0006836	4,3E-2	3	4,3
organophosphate metabolic process	GO:0019637	4,5E-2	4	5,7
transport	GO:0006810	4,6E-2	17	24,3
tissue development	GO:0009888	4,7E-2	7	10,0
establishment of localization	GO:0051234	5,0E-2	17	24,3
Down-Regulated				
immune response	GO:0006955	5,0E-6	14	16,7
immune system process	GO:0002376	1,3E-5	16	19,0
cell adhesion	GO:0007155	3,1E-5	13	15,5
biological adhesion	GO:0022610	3,2E-5	13	15,5
inflammatory response	GO:0006954	6,5E-5	9	10,7
response to wounding	GO:0009611	7,1E-5	11	13,1
defense response	GO:0006952	2,4E-4	11	13,1
locomotion	GO:0040011	4,5E-4	9	10,7
cell-cell adhesion	GO:0016337	1,1E-3	7	8,3
response to external stimulus	GO:0009605	1,4E-3	12	14,3
positive regulation of interleukin-6 production	GO:0032755	2,9E-3	3	3,6
regulation of defense response	GO:0031347	3,2E-3	5	6,0
defense response to virus	GO:0051607	3,2E-3	3	3,6
positive regulation of defense response	GO:0031349	3,7E-3	4	4,8
regulation of cytokine biosynthetic process	GO:0042035	3,8E-3	4	4,8
cell motion	GO:0006928	3,9E-3	8	9,5

taxis	GO:0042330	4,7E-3	5	6,0
chemotaxis	GO:0006935	4,7E-3	5	6,0
regulation of response to stress	GO:0080134	6,1E-3	6	7,1
leukocyte adhesion	GO:0007159	6,3E-3	3	3,6
cell migration	GO:0016477	6,3E-3	6	7,1
cell motility	GO:0048870	9,7E-3	6	7,1
localization of cell	GO:0051674	9,7E-3	6	7,1
regulation of interleukin-6 production	GO:0032675	1,0E-2	3	3,6
positive regulation of interferon-alpha biosynthetic process	GO:0045356	1,7E-2	2	2,4
cell morphogenesis	GO:0000902	1,7E-2	6	7,1
positive regulation of cytokine biosynthetic process	GO:0042108	1,8E-2	3	3,6
positive regulation of response to stimulus	GO:0048584	1,8E-2	5	6,0
response to chemical stimulus	GO:0042221	1,8E-2	12	14,3
cell morphogenesis involved in differentiation	GO:0000904	2,0E-2	5	6,0
positive regulation of interferon-beta biosynthetic process	GO:0045357	2,1E-2	2	2,4
regulation of interferon-beta biosynthetic process	GO:0045354	2,1E-2	2	2,4
regulation of interferon-alpha biosynthetic process	GO:0045354	2,1E-2	2	2,4
response to stress	GO:0006950	2,3E-2	14	16,7
leukocyte migration	GO:0050900	2,4E-2	3	3,6
cellular component morphogenesis	GO:0032989	2,6E-2	6	7,1
response to stimulus	GO:0050896	2,7E-2	23	27,4
organic acid biosynthetic process	GO:0016053	2,8E-2	4	4,8
carboxylic acid biosynthetic process	GO:0046394	2,8E-2	4	4,8
locomotory behavior	GO:0007626	2,9E-2	5	6,0
positive regulation of interleukin-8 biosynthetic process	GO:0045416	2,9E-2	2	2,4
regulation of interferon-alpha production	GO:0032647	3,4E-2	2	2,4
response to other organism	GO:0051707	3,5E-2	5	6,0
cytokine-mediated signaling pathway	GO:0019221	3,6E-2	3	3,6
positive regulation of molecular function	GO:0044093	3,8E-2	7	8,3
regulation of interleukin-8 biosynthetic process	GO:0045414	3,8E-2	2	2,4
regulation of vascular endothelial growth factor production	GO:0010574	3,8E-2	2	2,4
regulation of inflammatory response	GO:0050727	4,1E-2	3	3,6
regulation of cytokine production	GO:0001817	4,2E-2	4	4,8
regulation of chemokine biosynthetic process	GO:0051239	4,2E-2	2	2,4
regulation of multicellular organismal process	GO:0051239	4,4E-2	9	10,7
cell surface receptor linked signal transduction	GO:0007166	4,5E-2	14	16,7
positive regulation of interferon-gamma biosynthetic process	GO:0045078	4,6E-2	2	2,4
regulation of response to stimulus	GO:0048583	4,7E-2	6	7,1
activation of MAPK activity	GO:0000187	4,7E-2	3	3,6
Behavior	GO:0007610	4,9E-2	6	7,1

Conclusão

Dentro dos limites do presente estudo, pode-se concluir que:

i) A grande quantidade de dados gerados através das tecnologias ômicas (genômica, transcriptômica, proteômica e metabolômica) permite a identificação de novas moléculas e vias metabólicas envolvidas no equilíbrio entre saúde e doença periodontal, aumentando a compreensão da patogênese das periodontites. Consequentemente, elas podem ser aplicadas futuramente no diagnóstico das periodontites, através da identificação de um ou mais biomarcadores para detecção de doença ativa, predizer sua futura progressão, e avaliar a resposta ao tratamento periodontal. Melhorando assim o manejo clínico dos pacientes através de cuidados personalizados, ao permitir detecção e intervenção precoce.

ii) A análise do microarray e a mineração de dados demonstraram que a periodontite crônica, e especialmente a periodontite agressiva apresentam diferenças no perfil de expressão gênica tecidual entre si e em comparação com indivíduos sem histórico de periodontite. Indicando que essas doenças podem apresentar diferentes vias e mecanismos de destruição periodontal.

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COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Implantes dentais osseointegrados em pacientes com histórico de periodontite agressiva e crônica. Avaliação clínica, microbiológica e imunoenzimática"**, protocolo nº 017/2010, dos pesquisadores Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati e Tiago Taiete, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 30/10/2010.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Osseointegrated implants in patients with history of aggressive and chronic periodontitis. Clinical, microbiological and immunoenzymatic analysis"**, register number 017/2010, of Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati and Tiago Taiete, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 10/30/2010.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.