Proteomics Clinical Applications

Supporting Information for Proteomics

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Uncovering the molecular networks in periodontitis

Supplemental Table 1. Genomics, Transcriptomics, Proteomics, Peptidomics and Metabolomics contribution for periodontitis diagnosis.
"+" and "-" symbols represent over- and underexpression, respectively.

Methodological Approach	Purpose	Sample	Findings	Ref.
Genomics				
PCR-RFLP	Investigation of cytokines genotypes to relate with periodontitis severity, including screening of 5 different gene positions: IL-1A (-889), IL-1B (-511), IL-1B (+3953), IL-1RN (intron 2) and TNFA (-308)	Blood from patients with mild or no periodontitis (n = 49), moderate (n = 42) and generalized severe periodontitis (n = 43)	In non-smokers patients, the occurrence of the combined genotype IL-1A (-889, allele 2) and IL-1B (+3953, allele 2) identified severe periodontitis	(*37*)
PCR-RFLP	Studying the association between IL-1B (+ 3954C/T) gene polymorphism and different clinical presentations of periodontitis in South Indian population	Oral epithelial cells from healthy subjects (n = 30), AP subjects (n = 30) and CP subjects (n = 30)	CP patients displayed statistically different ($p < 0.05$) percentages of T alleles (38%) when compared to control patients (19%) in the locus +3954 of IL-1B gene. Thus, IL-1B (+ 3954) polymorphism could be a risk factor for CP in this population	(*35*)
GWAS	Identifying possible *loci* associated with CP (screening of > 17 million SNPs and short insertions and deletions) in West Pomerania (Germany) population	4032 individuals from two cross-sectional independent studies: SHIP (n = 3365) and SHIP-TREND (n = 667)	None of the SNPs studied was significantly associated with CP	(*46*)
PCR-RFLP	Studying the association between four TNF-α gene polymorphisms (-1031T/C, -857C/T, -308G/A and -238G/A) and AP and CP in Chinese population	Blood from healthy subjects (n = 180), subjects with CP (n = 180) and subjects with AP (n = 180)	Two genotypes were associated with periodontitis in Chinese population TNF-α (-1031CC) genotype frequency was significantly higher ($p < 0.05$) in subjects with CP when compared to the healthy ones TNF-α (-308AA) genotype frequency was significantly higher ($p < 0.05$) in subjects with AP when compared to the healthy ones	(*34*)

TaqMan® PCR system	Analysis of genomic markers (SNPs) for severe periodontitis in Japanese population	Blood from healthy volunteers (n = 19) and from severe periodontitis patients (n = 22)	SNPs in GNRH1, PIK3R1, DPP4, FGL2 and CALCR can be genomic markers for severe periodontitis, once they were significantly different ($p < 0.01$) between healthy and diseased subjects	(*43*)
PCR-RFLP	Studying the association of 2 SNPs in COX-2 gene (-1195G/A and +8473C/T) with CP in North Indians	Blood from healthy subjects (n = 60) and from subjects with CP (n = 56)	Individual mutants SNPs differences were not statistically different ($p > 0.05$), though haplotype AT (-1195A/+8473T) was significantly associated with increased risk for CP ($p = 0.0370$)	(*29*)
Tetra-Primer Amplification Refractory Mutation System-PCR	Investigation of the association between 2 SNPs (-1087G/A and -597C/A) in IL-10 gene promoter and GCP and LAP in Jordanian population	Blood from control subjects (n = 86), subjects with CP (n = 105) and subjects with AP (n = 85)	-1087GA and -1087AA genotypes frequencies were significantly higher ($p < 0.001$) in subjects with CP when compared to the healthy ones -597CA and -597AA genotypes frequencies were significantly higher ($p = 0.021$) in subjects with CP when compared to the healthy ones There were not observed significant differences in allele distribution between controls and AP patients	(*30*)
TaqMan® Allelic Discrimination Assays PCR-RFLP	Studying SNPs in pattern recognition receptors genes including TLR2 (G2408A), TLR4 (A896G), TLR9 (T1486C), TLR9 (T1237C) and CD14 (C260T) in periodontally healthy patients and CP patients (Caucasians)	Blood from healthy subjects (n = 77) and from patients with CP (n = 114)	CC, CT and TT genotypes of CD14 (C260T) were statistically different between the groups ($p = 0.04$), being CC genotype more frequent in subjects with CP CC homozygosity increases 2.49-fold the risk of disease ($p = 0.04$) No more significant genotype differences were found between the two Caucasian groups	(*31*)
Meta-analysis based on 11 case-control PCR-based studies	Investigation of MMP-1 1G-to-2G polymorphism and its association to periodontal disease	Genomic DNA from diseased subjects (n = 1447) and healthy subjects (n = 1710) Details of the 11 studies unknown	MMP-1 -1607 2G/2G genotype showed an increased risk for periodontitis when compared to MMP-1-1607 1G/1G genotype (1.45-fold risk; $p = 0.04$)	(*44*)

PCR-based and PCR-RFLP	Exploring the association between NOS3 gene polymorphisms (+ 894 G/T and intron 4 VNTR) and different forms of periodontal disease in Turkish population	Blood collected from healthy volunteers (n = 50), subjects with gingivitis (n = 31) or with AP (n = 23) or with CP (n = 26)	In CP group, NOS3 +894TT genotype was significantly increased ($p < 0.05$). Besides, there was a significant association between PD and CAL with GG genotype ($p = 0.001$) in CP group and with GT and TT genotype ($p < 0.02$) in AP group VNTR polymorphisms did not shown any significant association with PD or CAL	(*32*)
PCR-SSPs	Exploring the association of 3 PECAM-1 gene polymorphisms (Ser563Asn, Leu125Val and Arg670Gly) and periodontitis	Blood from healthy subjects (n = 101), from patients with AP (n = 53) and with CP (n = 52)	The only statistically significant difference was found when comparing Ser653 homozygous and Asn563 homozygous genotypes from control and diseased subjects (the first more frequent in controls, $p = 0.02$, and the latter more frequent in periodontitis-affected individuals, $p = 0.02$) There was no significant differences found both in allele and genotype frequencies among the different clinical presentations of periodontitis	(*33*)
PCR-RFLP	Investigation of the association between TLR genes polymorphisms (TLR-2 +2408G/A and -16934A/T; TLR-9 -1486C/T, -1237C/T and +2848A/G) and CP in Czech population	Blood from healthy subjects (n = 259) and from patients with CP (n = 222)	There was not found any significant association of the studied polymorphisms with CP, though -1486T/-1237T/+2848A haplotype combination of TLR9 gene was significantly more frequent in periodontitis patients than in controls ($p < 0.00001$) and -1486T/-1237T/+2848G haplotype combination was significantly less frequent in periodontitis patients than in controls ($p = 0.05$)	(*27*)
GWAS	Identifying genetic risk factors for AP in German Population	1255 subjects in 2 stages: GWAS1: GAP subjects (n = 141) and controls (n = 500); GWAS2: LAP (n = 142) and controls (n = 472)	Only GLT6D1 gene showed a significant association with GAP in GWAS1 (1.67-fold risk; $p = 1.8 \times 10^{-4}$) and with LAP in GWAS2 (1.65-fold risk; $p = 3.1 \times 10^{-4}$)	(*45*)

16S rRNA genes 454 pyrosequencing	Comparing subgingival bacterial communities between healthy subjects and subjects with CP	Subgingival plaques from healthy controls (n = 29) and from subjects with CP (n = 29)	123 species were identified in disease, and 53 in health *Spirochaetes*, *Synergistetes*, *Bacteroidetes*, *Clostridia*, *Negativicutes* and *Erysipelotrichia* were disease-associated and *Proteobacteria* and the class *Bacilli* were health-associated	(*80*)
16S rRNA microarrays	Characterize subgingival microbiota in African-American children with LAP	Subgingival plaques from healthy and diseased sites of children with LAP (n = 31), from healthy sites from healthy siblings (n = 11) and from healthy sites from controls (n = 9)	*Aggregatibacter actinomycetemcomitans* and *Filifactor alocis* were more prevalent in diseased sites in LAP ($p < 0.03$) *Aggregatibacter actinomycetemcomitans*, *Filifactor alocis*, *Tannerella sp*., *Solobacterium moorei*, *Parvimonas micra*, *Capnocytophaga sp*. were more common in LAP ($p < 0.01$)	(*79*)
Transcriptomics				
qRT-PCR	Comparing S100A2 mRNA expression between healthy subjects, and subjects with chronic gingivitis, moderate periodontitis and severe periodontitis	Gingival tissues from healthy subjects (n = 14), patients with chronic gingivitis (n = 13), with moderate (n = 48) and severe periodontitis (n = 27)	Subjects with moderate or severe periodontitis displayed significantly higher levels of S100A2 mRNA, S100A8 mRNA and S100A9 mRNA ($p < 0.05$) than healthy subjects	(*36*)
qRT-PCR	Comparing the expression of TLR5, TLR7, TLR9, TLR2, TLR4, IFN-α1 mRNA between gingivitis and periodontitis	Gingival tissues collected from patients with gingivitis (n = 27) and from patients with moderate to advanced CP (n = 59)	TLR2, TLR4, TLR7 and TLR9 transcripts were significantly higher in periodontitis patients than in gingivitis patients ($p < 0.01$) IFN-α1 transcript was also significantly higher in periodontitis patients than in gingivitis patients ($p = 0.033$)	(*28*)

cDNA synthesis (RNA reverse transcription) and Human Genome Arrays	Gene expression profiling in healthy and diseased gingival tissues	Gingival tissue samples from healthy sites (n = 64) and from diseased sites (n = 183) from patients with periodontitis	12744 probe sets were differentially expressed among healthy and diseased tissue samples ($p < 9.15 \times 10^{-7}$), 5295 of those were up-regulated and 7449 were down-regulated Further gene ontology analysis revealed 61 gene groups differentially expressed ($p < 0.05$), including apoptosis, antimicrobial humoral response, antigen presentation, regulation of metabolism, signal transduction and angiogenesis	(*41*)
Gene expression microarray Cluster analysis	Contrasting gene expression profiles of neutrophils between patients with CP and matched healthy subjects	PMN isolated from blood and oral rinse obtained from healthy subjects (n = 4) and from patients with GCP (n = 4)	In healthy subjects, 588 genes were differentially expressed between blood PMN and oral PMN ($p < 0.05$), while in ill subjects, 3593 genes were differentially expressed between blood PMN and oral PMN ($p < 0.05$) Healthy subjects showed up-regulation of several signaling pathways: NOD-like receptor, cell adhesion molecules and IL-17, while ill subjects showed up-regulation of other signaling pathways: TLR, caspase cascade, apoptosis ($p < 0.05$)	(*42*)
RNA sequencing Cluster analysis	Gene expression profiling to compare healthy and diseased gingival sites in the same patient	Gingival tissues samples from healthy and periodontitis-affected sites in patients with periodontitis (n = 10)	453 differently expressed genes ($p < 0.01$), were observed in periodontitis-affected gingiva, with 381 up-regulated and 72 down-regulated genes Immune and inflammatory responsive genes displayed the most distinct pattern ($p < 0.05$) Transcripts of inflammatory mediators (IL-1β, IL-6, IL-8, TNF-α, RANTES and MCP-1) were highly expressed in periodontitis-affected gingiva	(*40*)

Microarrays qRT-PCR	Identifying specific gene expression profiles and biological pathways in periodontitis-affected gingival tissues	Gingival tissue samples from healthy and periodontitis-affected sites in patients with severe CP (n = 3)	In periodontitis-affected gingival tissues, 28 genes were differently expressed in contrast to healthy tissues. From those, 15 were up-regulated and 13 were down-regulated (> 2-fold, $p < 0.05$) Pathway analysis revealed 15 up-regulated pathways (including leukocyte transendothelial migration) and 5 down-regulated pathways (including cell communication) in affected tissues ($p < 0.05$)		(*39*)
Proteomics					
Label-free nanoUPLC-MS/MS	Screening proteome differences between healthy subjects and patients with CP	SWS from healthy subjects (n = 20) and diseased patients (n = 20)	20 differently expressed proteins (> 1.5-fold; $p < 0.05$) Plastin-2, profilin-1, neutrophil collagenase, α-2-macroglobulin, complement C3, lactotransferrin and MMP-9 confirmed as potential markers	+	(*53*)
nanoLC-ESI-MS/MS SDS-PAGE-nanoLC-ESI-MS/MS	Collecting full GCF proteome from periodontally healthy subjects	GCF from healthy subjects (n = 9)	199 identified proteins ($p < 0.1$) with several sets of macromolecules more specific to GCF than to serum: early inflammation, immune response, proteins involved in defending from and killing bacteria, proteinases, modulators of cytokines and extracellular matrix constituents		(*54*)
Enzymatic assays Zymography	Studying enzymatic activities and specificity of WS proteases in periodontal health and disease	SWS supernatant from healthy subjects (n = 23) and patients with moderate to severe periodontitis (n = 25)	Subjects belonging to periodontal disease group showed a stronger gelatinolytic/collagenolytic activity ($p < 0.001$), although histatin 5 and Z-RGYR-MCA (synthetic substrate to test trypsin-like activities) proteolysis was not statistically different	+	(*105*)
2-DE-HPLC-ESI-MS/MS	Comparing proteomic profile of UWS between healthy subjects and others with GAP	UWS from healthy subjects (n = 5) and from patients with GAP (n = 5)	11 differently expressed proteins ($p < 0.05$) in GAP: serum albumin, Ig γ2 chain C region, Ig α2 chain C region, vitamin D-binding protein, salivary α-amylase and zinc-α2 glycoprotein overexpressed lactotransferrin, elongation factor 2, 14-3-3 sigma, PLUNC2 precursor and CA 6 underexpressed	+ -	(*55*)

2-DE-MALDI-TOF 2-DE-LC-MS/MS	Identifying differences in salivary proteome in active periodontitis after treatment	SWS from subjects with generalized periodontitis (n = 9)	The level of 15 proteins changed significantly ($p < 0.05$) after the treatment, remarkably the proteins belonging to S100 family were increased (S100 A8, A9 and A6)	+	(*56*)
2-DE-HPLC-MS/MS HPLC-MS/MS Western Blotting	Comparing GCF proteome with supragingival saliva proteome in periodontally healthy and diseased subjects	GCF and WS from healthy subjects (n = 5) and subjects with mild (n = 3), moderate (n = 3) and severe periodontitis (n = 5)	327 proteins identified in GCF, 8 of which were distinctly expressed in GCF ($p < 0.05$), including haptoglobin, superoxide dismutase 1, ALB protein, apolipoprotein A-I, and dermcidin Subjects with severe periodontitis displayed higher expression of superoxide dismutase 1 ($p < 0.0131$) and dermcidin ($p < 0.0162$) than the healthy ones	 +	(*57*)
Label-free Quantitative LC-MS^{E} (nanoHPLC-Q-TOF)	Investigating GCF exudatome from healthy and periodontally diseased sites	GCF from healthy subjects (n = 5) and from GAP patients (n = 5)	154 retrieved proteins (with ≥ 2 unique peptide hits) Periodontitis samples have shown different levels of some proteins: increased expression of microbial proteins, L-plastin and decreased expression of annexin-1, neutrophil defensins, cystatin B and IgG	 + -	(*58*)
2-DE-MALDI-TOF/TOF nanoLC-ESI-Q-TOF	Comparing protein profiles of UWS between healthy subjects and others with CP	UWS from healthy subjects (n = 10) and from CP patients (n = 10)	CP patients showed differently expressed proteins: increased expression of Ig heavy chain V-III region, serum albumin, hemoglobin, α-amylase and decreased expression of Cystatin-SN precursor (Cystatin-1)	+ -	(*59*)
SDS-PAGE-nanoLC-Q-TOF	Studying GCF proteome from healthy gingival crevice and from periodontal pocket	GCF from a healthy subject (n = 1) and two subjects with periodontitis (n = 2)	104 proteins detected present in both periodontally healthy and diseased sites, 64 proteins detected only in healthy sites and 63 proteins detected only in periodontitis sites ($p < 0.05$) Some of the proteins were identified for the first time: ceruloplasmin, glycogen phosphorylase, glutathione S-transferase, phosphoglycerate mutase, proriasin, S100A11 and resistin		(*60*)

SELDI-TOF-MS MALDI-TOF-MS/MS Orbitrap MS	Comparing salivary protein/peptide profile between obese patients with or without periodontitis	SWS from healthy subjects (n = 19), from obese subjects with periodontitis (n = 13) and from obese subjects without periodontitis (n = 25)	Obese patients with periodontitis showed lower mean intensity of α-defensin (36.47 ± 19.84 μA) than obese patients without periodontitis (43.44 ± 30.34 μA) ($p < 0.05$) Besides obese patients showed higher mean intensity of α-defensin (40.99 ± 30.34 μA) than healthy controls (27.1± 23.98 μA) ($p < 0.05$)		(*61*)
EIA	Studying the validity of 4 molecules (MIP-1α, OPG, β-CTX and ICTP) as biomarkers for alveolar bone remodeling in CP subjects	UWS from healthy subjects (n = 40) and from patients with generalized moderate to severe CP	MIP-1α was found in higher levels in periodontitis patients than in healthy subjects (18-fold; $p < 0.0001$) The other molecular markers either fell below of detection limit or were not differently expressed with statistic significance	+	(*106*)
2-DE-LC-ESI-MS/MS	Searching for salivary proteins related to periodontitis in T2DM patients	UWS from periodontally healthy T2DM patients (n = 10) and from periodontally diseased T2DM patients (n = 15)	T2DM patients with periodontitis displayed significantly different levels of some proteins ($p < 0.012$): PLS-2, LEI, Ig J chain pIgR, Arp 3, CA 6, IL-1Ra	+ -	(*63*)
Peptidomics					
WCX fractionation-MALDI-TOF nanoLC-ESI-MS/MS	Comparing peptide mass fingerprints of orthodontic patients with or without periodontitis	UWS from orthodontic patients without periodontitis (n = 8), orthodontic patients with periodontitis (n = 8) and patients with periodontitis (n = 8)	Orthodontic patients with periodontitis displayed significantly different levels ($p < 0.05$) of certain peptides when comparing with orthodontic patients without periodontitis: an increased profile of ITIH4 Isoform 2 of inter-α-trypsin inhibitor heavy chain H4 precursor and SERPINA1were found and a decreased profile of ACTB Actin, cytoplasmic 1; FGA Isoform1 of fibrinogen α chain precursor (m/z = 2621.9 and m/z = 3154.4); 280-kDa protein; F2 Prothrombin precursor; WWCE Isoform 1 of vWF C and EGF domain-containing protein precursor; m/z = 5435.2 (unknown) were identified	+ -	(*62*)

Metabolomics					
NMR	Exploring saliva metabolic profile and finding an association with periodontitis	WS from healthy subjects (n = 22), patients with gingivitis (n = 3), with LCP (n = 4), with GCP (n = 21) or with GAP (n = 2)	Patients with GCP displayed a distinctive saliva metabolic profile when with healthy subjects ($p < 0.05$): increased profile of acetate, γ-aminobutyrate, n-butyrate, succinate, trimethylamine, propionate, phenylalanine and valine and decreased profile of pyruvate and N-acetyl groups	+ -	(*98*)

PCR: Polymerase Chain Reaction; **IL**: interleukin; **IL-1RN**: interleukin-1 receptor antagonist gene; **TNFA**: tumor necrosis factor alpha gene; **RFLP**: Restriction Fragment Length Polymorphism; **AP**: Aggressive Periodontitis; **CP**: Chronic Periodontitis; **GWAS**: Genome wide-association study; **SNP**: single nucleotide polymorphism; **SHIP**: Study of Health In Pomerania; **SHIP-TREND**: (population-based cohort study); **TNF-α**: tumor necrosis factor alpha; **GNRH1**: gonadotropin-releasing hormone 1 gene; **PIK3R1**: phosphatidylinositol 3-kinase regulatory 1 gene; **DPP4**: dipeptidylpeptidase 4 gene; **FGL2**: fibrinogen-like 2 gene; **CALCR**: calcitonin receptor gene; **COX-2**: cyclooxygenase-2 gene; **GCP**: Generalized Chronic Periodontitis; **LAP**: Localized Aggressive Periodontitis; **TLR**: Toll-like receptor; **CD**: cluster of differentiation; **MMP**: matrix metalloproteinase; **NOS3**: endothelial nitric oxide synthase; **VNTR**: variable number of tandem repeats; **PD**: probing depth; **CAL**: clinical attachment level; **PCR-SSPs**: Polymerase Chain Reaction with Sequence-Specific Primers; **PECAM-1**: Platelet Endothelial Cell Adhesion Molecule-1; **GAP**: Generalized Aggressive Periodontitis; **GTL6D1**: glycosyltransferase 6 domain containing 1; **rRNA**: ribosomal RNA; **qRT-PCR**: Quantitative Real Time PCR; **mRNA**: messenger RNA; **IFN-α1**: interferon-α1; **cDNA**: complementary DNA; **PMN**: polymorphonuclear leukocytes; **NOD**: nucleotide-binding oligomerization domain; **RANTES**: Regulated upon Activation, Normal T-cell Expressed, and Secretes; **MCP-1**: Monocyte Chemotactic Protein-1; **UPLC**: Ultra Performance Liquid Chromatography; **MS**: Mass Spectrometry; **LC**: Liquid Chromatography; **SWS**: Stimulated Whole Saliva; **ESI**: Electrospray Ionization; **SDS-PAGE**: Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis; **GCF**: Gingival Crevicular Fluid; **WS**: Whole Saliva; **Z-RGYR-MCA**: histatin-derived synthetic analog benzyloxycarbonyl-Arg-Gly-Tyr-Arg-methyl cumaryl amide; **2-DE**: Two-dimensional gel Electrophoresis; **HPLC**: High Performance Liquid Chromatography; **UWS**: Unstimulated Whole Saliva; **Ig**: Immunoglobulin; **PLUNC2**: short Palate, Lung and Nasal epithelium Carcinoma-associated protein 2; **CA**: carbonic anhydrase; **MALDI-TOF**: Matrix-Assisted Laser Desorption Ionization-Time-Of-Flight; **Q-TOF**: Quadrupole-Time-Of-Flight; **SELDI**: Surface-Enhanced Laser Desorption/Ionization; **EIA**: Enzyme Immunosorbent Assays; **MIP-1α**: Macrophage Inflammatory Protein-1α; **OPG**: Osteoprotegerin; **β-CTX**: C-terminal type-1 collagen telopeptide; **ICTP**: pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; **T2DM**: Type 2 Diabetes Mellitus; **PLS-2**: Plastin-2; **LEI**: Leukocyte Elastase Inhibitor; **pIgR**: polymeric Immunoglobulin Receptor; **Arp**: Actin-related protein; **IL-1Ra**: interleukin-1 receptor antagonist; **WCX**: Weak Cation exchange; **vWF**: von Willebrand Factor; **NMR**: Nuclear Magnetic Ressonance; **LCP**: Localized Chronic Periodontitis