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Reliability of microarray analysis for studying periodontitis: low consistency in 2 periodontitis cohort data sets from different platforms and an integrative meta-analysis

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ABSTRACT

Purpose: The aim of this study was to compare the characteristic expression patterns of advanced periodontitis in 2 cohort data sets analyzed using different microarray platforms, and to identify differentially expressed genes (DEGs) through a meta-analysis of both data sets.

Methods: Twenty-two patients for cohort 1 and 40 patients for cohort 2 were recruited with the same inclusion criteria. The 2 cohort groups were analyzed using different platforms: Illumina and Agilent. A meta-analysis was performed to increase reliability by removing statistical differences between platforms. An integrative meta-analysis based on an empirical Bayesian methodology (ComBat) was conducted. DEGs for the integrated data sets were identified using the *limma* package to adjust for age, sex, and platform and compared with the results for cohorts 1 and 2. Clustering and pathway analyses were also performed.

Results: This study detected 557 and 246 DEGs in cohorts 1 and 2, respectively, with 146 and 42 significantly enriched gene ontology (GO) terms. Overlapping between cohorts 1 and 2 was present in 59 DEGs and 18 GO terms. However, only 6 genes from the top 30 enriched DEGs overlapped, and there were no overlapping GO terms in the top 30 enriched pathways. The integrative meta-analysis detected 34 DEGs, of which 10 overlapped in all the integrated data sets of cohorts 1 and 2.

Conclusions: The characteristic expression pattern differed between periodontitis and the healthy periodontium, but the consistency between the data sets from different cohorts and metadata was too low to suggest specific biomarkers for identifying periodontitis.

Keywords: Meta-analysis; Microarray analysis; Periodontitis; Transcriptome

INTRODUCTION

The analysis of patients' transcriptome profiles can reveal molecular mechanisms underlying disease [1]. The pathogenesis of periodontal disease has been suggested to involve a host response against microbial alterations [2], and various attempts have been made to evaluate

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

gene expression from gingival/periodontal tissue in periodontal lesions, focusing on the host response regarding the initiation or progression of periodontitis [3-5]. Previous studies have found different transcriptome profiles in periodontitis patients and healthy subjects [3,5-8], and many researchers and clinicians have expected that analyses of gene expression would clarify the phenotype of periodontitis and its underlying mechanism. However, previous studies have produced inconsistent and unreproducible results, and there is currently no consensus about the specific features of host responses in periodontal disease.

Gene expression can be affected by the timing of periodontal disease processes and the type of periodontitis. In an experimental gingivitis model, it was found that *IL-1A*, *IL1B*, *IL8*, *RANTES*, and *CSF3* played crucial roles in the induction and resolution of gingivitis [5]. Generalized aggressive periodontitis lesions showed significant up-regulation of *TNFRSF17*, *FCRL5*, *MZB1*, and *DSCI*, and the authors suggested that these genes could serve as biomarkers of the periodontitis phenotype [7]. Other studies of generalized chronic periodontitis suggested that *ZNF823*, *XBPI*, *RORA*, *TFAP2C*, and *ZNF57* are specific master regulators in periodontal disease processes [9]. However, even though specific genes have been suggested for certain types of periodontal disease classified based on only their clinical features, the clinical diagnosis of periodontitis does not correspond to the transcriptome profile pattern in reverse [10].

These variations in results might be also due to the specific methodologies used (e.g., microarray or RNA sequencing) or the use of application platforms from different manufacturers. A study using spatial transcriptome analysis found that *IGLL5*, *SSR4*, *MZB1*, and *XBPI* played key roles in chronic inflammatory lesions [11]. Massive parallel sequencing revealed that interferon regulatory factor 4 and chemokine (C-C motif) ligand 18 were up-regulated in periodontitis-affected tissue [6]. In particular, the expansion of microarray techniques has led to the emergence of a large number of commercial microarray platforms, which has raised concerns about inter-platform consistency [12]. A cross-platform study of human tissue [13] found that the gene expression profile was substantially dependent on the microarray platform used, with the greatest similarity found for data analyzed using the same platform.

Meta-analyses of microarray data sets have been increasingly employed to integrate data across platforms to make the results of microarray analyses more consistent and powerful [14]. Meta-analyses are useful when merging multiple datasets with a limited sample size, thereby improving the statistical power. This method has recently been applied to identify differentially expressed genes (DEGs) in breast cancer [15], pancreatic ductal adenocarcinoma [16], and ovarian cancer [17].

The purpose of this study was to compare the characteristic expression patterns of familial aggregated, generalized advanced periodontitis in 2 cohort data sets analyzed using different microarray platforms, and to identify consistent DEGs by performing an integrative meta-analysis of these 2 different data sets.

MATERIALS AND METHODS

Study design

Two clinical cohort data sets of healthy patients and patients with periodontitis were prospectively enrolled from the Department of Periodontology, Yonsei University Dental

Hospital by applying the same inclusion and exclusion criteria. Cohort 1 comprised 22 patients (12 with generalized advanced periodontitis and 10 with a healthy periodontium) recruited from September 2014 to September 2015, while cohort 2 comprised 40 patients (20 in the periodontitis group and 20 healthy subjects) recruited from August 2016 to February 2018. The data for the first cohort partially overlap with data published elsewhere for other experimental purposes (submitted data).

The experimental protocols and informed consent forms were designed according to the Declaration of Helsinki (Tokyo version revisited in 2004) and Good Clinical Practice guidelines. Each investigation was approved by the Institutional Review Board for Clinical Research of Yonsei University Dental Hospital (approval No. 2-2014-0026 and 2-2016-0012). Each patient provided informed written consent. The manuscript was prepared following the guidelines of the Strengthening the Reporting of Observational studies in Epidemiology statement.

Inclusion and exclusion criteria

The same inclusion and exclusion criteria were applied when recruiting each of the 2 independent cohorts. Patients enrolled in the periodontitis group had generalized severe periodontal inflammation, as well as >50% of alveolar bone loss along with the dental root on radiography, with clinical features of a probing depth of >7 mm, clinical attachment loss of >5 mm, and easy bleeding on probing. Additionally, they had a familial aggregation of severe periodontitis. Based on the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, patients who could be diagnosed with generalized periodontitis in stages III and IV with grade C (rapid progression) were enrolled in the periodontitis group.

The patients in the healthy group required gingivectomy for crown-lengthening procedures in a region that had no signs of gingival/periodontal inflammation, no bleeding on probing, a probing depth of <3 mm, and no evidence of alveolar bone loss.

The following exclusion criteria were applied: current or history of smoking, currently pregnant or lactating, any systemic diseases that affect periodontal status or the periodontal healing process (e.g., diabetes and malignancy), and a history of taking drugs that affect periodontal status (e.g., calcium-channel blockers, phenytoin, and cyclosporine).

Gingival tissue collection

All patients received nonsurgical periodontal treatment, scaling, and root planing at 2–4 weeks before collecting gingival tissue. In the periodontitis group, gingival tissues were harvested during flap surgery around teeth showing a probing depth of >7 mm and bleeding on probing. Samples were obtained with a size of 3 mm and included the marginal gingiva, connective tissue, and periodontal pocket. Healthy gingival tissues were taken from sites with a probing depth of <3 mm and no bleeding on probing. All samples were immediately stored in RNAlater solution (RNAlater Stabilization Solution AM7020, Life Technologies, Carlsbad, CA, USA) at room temperature for 24 hours, and then subsequently at -80°C.

RNA isolation

Total RNA was isolated from the gingival tissues of each patient using TRIzol LS Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA was isolated from saliva using the Oragene RNA purification protocol with the Qiagen RNeasy Micro kit (Qiagen, Valencia, CA, USA).

Quality and quantity of RNA

Quality and quantity control tests were applied to the isolated total RNA samples before the microarray analysis was performed. The RNA concentration (in nanograms per microliter) and purity (absorbance ratios at 260 nm/280 nm and 260 nm/230 nm) were determined using a spectrophotometer (ND-1000, NanoDrop, Wilmington, DE, USA). The quality of the RNA was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) based on the RNA integrity number (RIN). The criteria for quality control were an RIN >7, an absorbance ratio at 260 nm/280 nm >1.7, and an absorbance ratio at 260 nm/230 nm >1.0.

Microarray analysis

The 2 cohort data sets were analyzed using the different microarray platforms as follows:

Cohort 1: total RNA was amplified and purified using the TargetAmp-Nano Labeling Kit for the Illumina Expression BeadChip device (EPICENTRE, Madison, WI, USA) to yield biotinylated complementary RNA (cRNA) according to the manufacturer's instructions. Total RNA (100 ng) was reverse-transcribed to complementary DNA (cDNA) using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, transcribed *in vitro*, and labeled with biotin-NTP. After purification, the cRNA was quantified using a spectrophotometer (ND-1000, NanoDrop). Biotinylated cRNAs were prepared from 0.55 µg of total RNA using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin, TX, USA). Labeled cRNA samples (750 ng) were hybridized to individual Human HT-12 (version 4.0) Expression BeadChip devices for 17 hours at 58°C according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The array signals were detected using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead-array manual. Arrays were scanned using an Illumina bead-array confocal scanner.

Cohort 2: RNA labeling and hybridization were performed using the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (version 6.5, 2010, Agilent Technologies). Total RNA (100 ng) from each sample was linearly amplified and labeled with Cy3-dCTP. The labeled cRNAs were purified by using an RNAsasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (in units of picomoles of Cy3 per microgram of cRNA) were measured using the NanoDrop ND-1000 device (NanoDrop). Each labeled cRNA (600 ng) was fragmented by adding 5 µL of 10× blocking agent and 1 µL of 25× fragmentation buffer, and then heated at 60°C for 30 minutes. Finally, 25 µL of 2× GE hybridization buffer was added to dilute the labeled cRNA. Hybridization solution (40 µL) was dispensed into the gasket slide and assembled in Agilent SurePrint G3 Human GE 8X60K microarrays (version 3, Agilent Technologies). The slides were incubated for 17 hours at 65°C in an Agilent hybridization oven, and then washed at room temperature using the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (version 6.5, 2010, Agilent Technologies). The hybridized array was immediately scanned with an Agilent Microarray Scanner D (Agilent Technologies).

Raw data preparation and statistical analysis

Raw data were extracted using the software provided by each manufacturer: Illumina GenomeStudio (version 2011.1) Gene Expression Module (version 1.9.0) for cohort 1, and Agilent Feature Extraction Software (version 11.0.1.1) for cohort 2. The measured intensities of the array probes were transformed by taking the logarithms of values and normalizing them into quartiles. Statistical significance of the expression data was determined based on

the relative fold change and the independent *t*-test, in which the null hypothesis was that no difference existed among the 2 groups in a single cohort. The cutoff criterion for probes was a change of at least 2-fold, with a *P*value of <0.05.

Unsupervised hierarchical clustering analysis was performed using complete linkage and a Euclidean distance metric with the expression profile of DEGs. We used the DAVID analysis with gene ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes databases, based on the Fisher exact test with *P*values corrected by the false discovery rate. All data analysis and visualization of DEGs were conducted using R software (version 3.1.2).

Integrative meta-analysis

The Illumina expression data (cohort 1) were preprocessed by applying quantile normalization using the R package lumi [18]. Agilent expression data (cohort 2) were preprocessed by quantile normalization using the limma software package. The probes for each data set were collapsed into genes using the MaxMean method, which involves choosing the probe set with the maximum average signal across the samples. Data from both platforms were integrated using empirical Bayesian frameworks (ComBat) [19]. The ComBat method integrates data from multiple genes with similar expression distributions in each data set to estimate the average and variance in each gene [20]. From the integrated data sets, the genes most likely to be differentially expressed in periodontitis patients versus controls were selected by analyzing the gene expression microarray data with the limma software package. The workflow of the meta-analysis is shown in Figure 1.

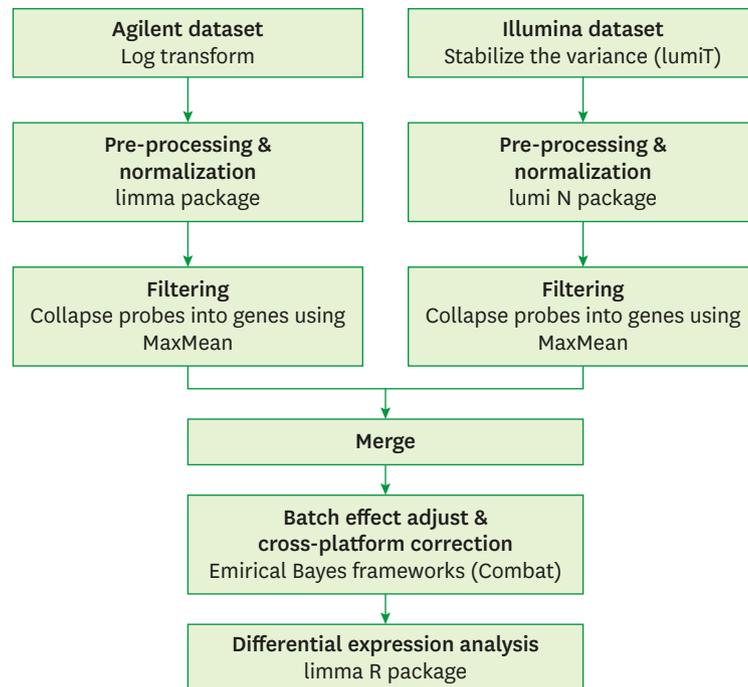


Figure 1. Workflow of the integrative meta-analysis for integrating Illumina and Agilent expression data.

RESULTS

Patient characteristics

Table 1 lists the characteristics of cohorts 1 and 2. Cohort 1 consisted of 6 men and 6 women, with a mean age of 42.2 years (range, 30–50 years). Based on the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, 8 and 4 of the patients were diagnosed with periodontitis in stages 3 and 4, respectively. The healthy group consisted of 7 men and 3 women, with a mean age of 43.7 years (range, 20–71 years).

Cohort 2 consisted of 7 women and 13 men with a mean age of 46.7 years (range, 38–61 years), of whom 18 and 2 patients had periodontitis in stages 3 and 4, respectively. The healthy group consisted of 3 men and 17 women with a mean age of 40.7 years (range, 24–62 years).

Hierarchical clustering

Both cohort groups were divided into 2 groups based on the results of hierarchical clustering (Figure 2). In the results for both cohorts, each clustered group matched the grouping by clinical phenotype (periodontitis and healthy groups). However, the results for cohort 1,

Table 1. Demographics of the study participants

| Characteristics | Cohort 1 (Illumina) | Cohort 2 (Agilent) |
|---------------------|---------------------|--------------------|
| No. of patients | 12 | 20 |
| Age (yr) | 42.2 (30–50) | 46.7 (38–61) |
| Sex | | |
| Male | 6 (50) | 7 (35) |
| Female | 6 (50) | 13 (65) |
| Periodontitis stage | | |
| I | 0 | 0 |
| II | 0 | 0 |
| III | 8 (67) | 18 (60) |
| IV | 4 (33) | 2 (40) |

Data are number (%) or mean (range) values.

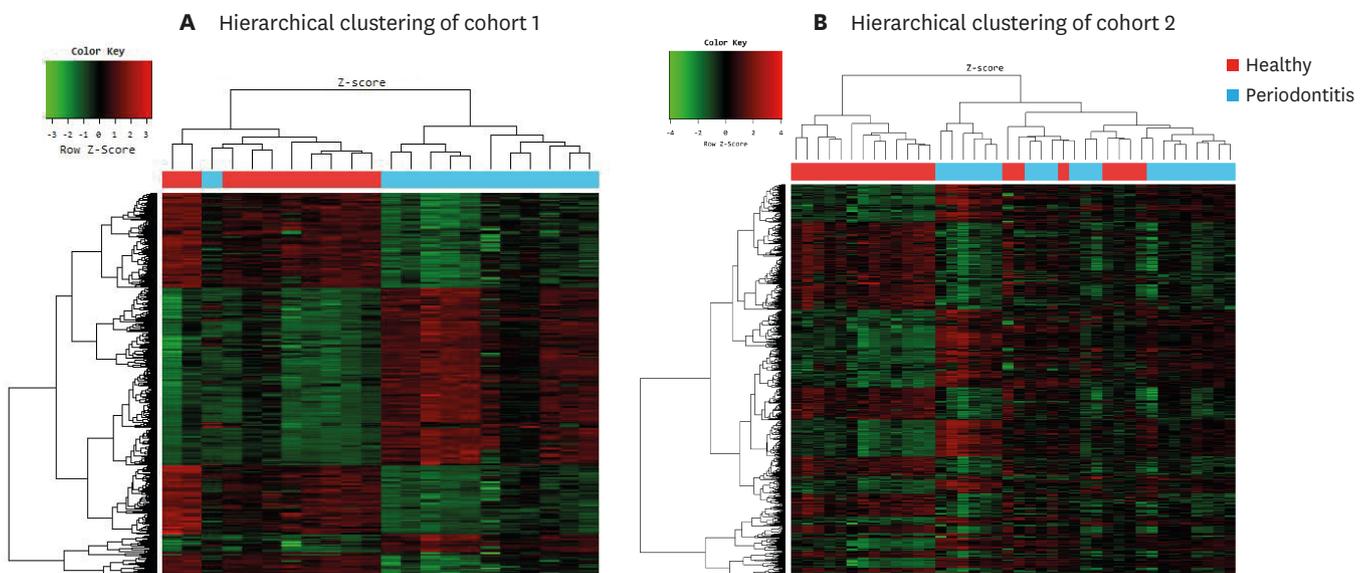


Figure 2. Hierarchical clustering and heatmap for (A) cohort 1 and (B) cohort 2.

a sample from the periodontitis group was exceptionally clustered with the healthy group. In the result for cohort 2, 7 samples from the control group were clustered with the periodontitis group.

Differential gene expression profiling

The 557 DEGs detected in cohort 1 comprised 286 that were up-regulated and 271 that were down-regulated, while the 246 DEGs detected in cohort 2 comprised 192 up-regulated and 54 down-regulated genes. Overlapping between the 2 cohorts was present in 59 DEGs, corresponding to 10.5% and 24% of the results for cohorts 1 and 2, respectively (Figure 3). The overlapping DEGs are listed in detail in Supplementary Table 1. The top 30 up- and down-regulated genes, which represented the characteristic molecular signature of each cohort, showed 6 overlapping genes (*CD79A*, *KRT3*, *KRT10*, *CALML5*, *ASPRV1*, and *SLURP1*), which was more limited than the results for the entire list (Table 2).

The integrative meta-analysis identified 34 genes as being differentially expressed, with a difference of at least 1.5-fold between the groups (Figure 4): 11 were up-regulated and 23 were down-regulated. The meta-analysis findings were also compared with those obtained by individual analyses in both data sets to evaluate the reproducibility and consistency across the microarray data sets. This analysis revealed that 29 genes were shared with the results for cohort 1 and 12 overlapped with the results for cohort 2. Ten of the genes were consistently identified by the 3 analysis methods (Table 3), while 3 genes (*BFSP2*, *ACY3*, and *CRYBB1*) were identified as DEGs in the integrative meta-analysis, but not in the individual analyses.

Pathway analysis

In cohorts 1 and 2, 146 and 42 GO terms were enriched, respectively. In cohort 1, the most-enriched pathway was related to epithelial differentiation and keratinization, followed by the immune response pathway. In cohort 2, the cellular process pathway was the most-enriched

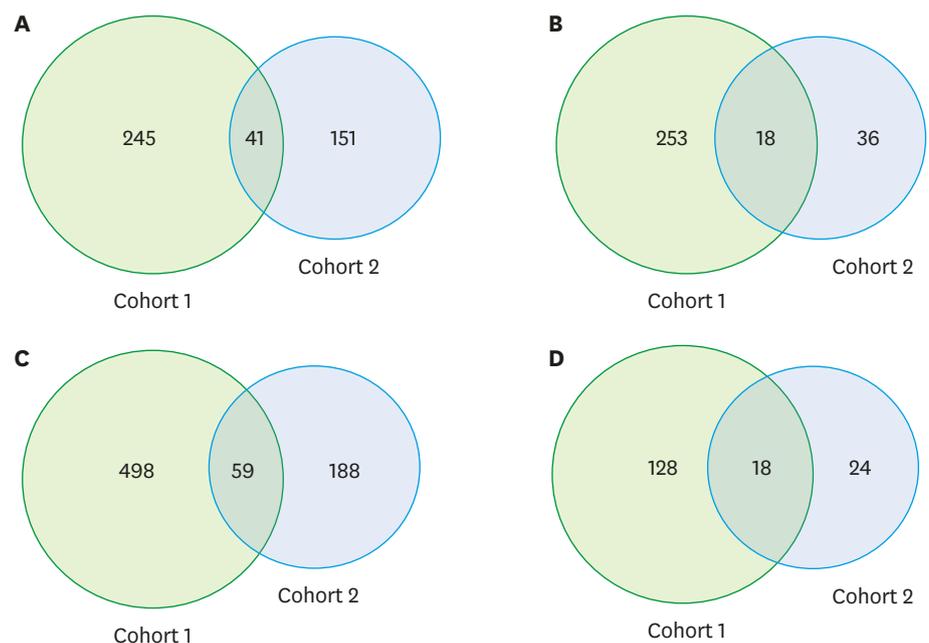


Figure 3. Venn diagrams of (A) up-regulated genes, (B) down-regulated genes, (C) all of the differentially expressed genes, and (D) gene ontology terms detected in cohorts 1 and 2.

Table 2. Top 30 up- and down-regulated genes in cohorts 1 and 2

| Cohort 1 | | | | Cohort 2 | | | |
|--------------|----------|----------------|----------|----------------|-----------|----------------|-----------|
| Up-regulated | | Down-regulated | | Up-regulated | | Down-regulated | |
| Gene ID | FC | Gene ID | FC | Gene ID | FC | Gene ID | FC |
| CD79A | 10.70203 | LOR | -22.2158 | SMR3A | 11.436173 | KRT3 | -4.565210 |
| IGLL3 | 9.832818 | KRT1 | -20.3013 | MGC16025 | 9.058753 | RNA5-8S5 | -3.816495 |
| JSRP1 | 9.049904 | C6orf15 | -13.0641 | lnc-C15orf57-2 | 7.738360 | CALML5 | -3.778477 |
| LOC649923 | 9.017490 | KRT2 | -12.6161 | GDF15 | 6.481650 | ASPRV1 | -3.761501 |
| CAMK1G | 8.994684 | ASPRV1 | -11.4212 | lnc-C14orf79-2 | 6.210376 | LOC101928824 | -3.609794 |
| MMP7 | 8.756478 | LCE2B | -10.5204 | NYX | 6.190459 | SPRR2G | -3.230397 |
| LOC652694 | 8.456885 | CDSN | -9.91216 | RAPH1 | 5.761884 | CXCL10 | -3.082538 |
| FAM46C | 7.700154 | RPTN | -9.89320 | lnc-GLB1L2-3 | 5.712509 | RALGDS | -3.025441 |
| MGC29506 | 7.587524 | LCE2D | -9.44505 | MSH3 | 5.647456 | DEFB4A | -3.023950 |
| LOC652102 | 7.534289 | KRT76 | -9.24682 | lnc-UCHL3-5 | 5.457625 | TPPP3 | -2.999892 |
| LOC647506 | 7.441141 | KPRP | -8.82649 | lnc-ZKSCAN1-1 | 5.277875 | KRT10 | -2.841696 |
| MMP7 | 7.277230 | LCE2A | -8.64623 | lnc-TAF1C-1 | 4.837153 | VSIG8 | -2.772933 |
| LOC651751 | 7.071169 | LCE2C | -8.54430 | LOC574538 | 4.650281 | CXCL10 | -2.772913 |
| LOC647450 | 6.982818 | LCE3A | -8.19021 | LSP1 | 4.641915 | PMEL | -2.759982 |
| TNFRSF17 | 6.807763 | KRT3 | -7.28640 | LYG2 | 4.567214 | NEFM | -2.719225 |
| LOC652493 | 6.444931 | FLG | -6.58204 | TBC1D3L | 4.466769 | lnc-CCDC117-1 | -2.657902 |
| CRKRS | 6.290947 | LCE3D | -6.56352 | COL27A1 | 4.239985 | CXCL9 | -2.606975 |
| RGS1 | 6.109839 | LY6G6C | -6.46794 | KRT73 | 4.160282 | SLURP1 | -2.579585 |
| RNF126P1 | 6.106207 | LCE3E | -6.43703 | lnc-BTRC-2 | 4.070695 | IFI44L | -2.561653 |
| LOC642113 | 5.927212 | SLURP1 | -5.69952 | TNRC18 | 4.007028 | KRTDAP | -2.532567 |
| CD27 | 5.752022 | PLA2G4D | -5.57919 | BCL2L15 | 3.985797 | WISP2 | -2.484823 |
| FER1L4 | 5.735650 | CALML5 | -5.57193 | CD79A | 3.956875 | SH3GL3 | -2.409029 |
| POU2AF1 | 5.724181 | LOC730833 | -5.49426 | lnc-NBPF3-4 | 3.929255 | ARL9 | -2.383946 |
| CD19 | 5.617619 | LOC653499 | -5.34391 | lnc-MFAP4-1 | 3.852914 | DSC2 | -2.372728 |
| LAX1 | 5.516125 | AADA2L2 | -5.24062 | lnc-CS-1 | 3.793785 | PSMD10 | -2.361956 |
| SEL1L3 | 5.469453 | KRT10 | -5.19668 | lnc-FAM131B-1 | 3.760248 | lnc-PLTP-1 | -2.354330 |
| FCRLA | 5.355335 | LOC441052 | -4.89137 | lnc-COX19-2 | 3.644528 | PCNXL4 | -2.339869 |
| ASB16 | 5.056340 | LOC645869 | -4.88024 | LOC101927577 | 3.608607 | JAKMIP3 | -2.271844 |
| ST6GAL1 | 4.999486 | DSG1 | -4.69387 | C4B | 3.525520 | LINC01197 | -2.251761 |
| LOC649210 | 4.985094 | LGALS7B | -4.68949 | IGLL1 | 3.517933 | CYP2C9 | -2.204945 |

Gray shading indicates genes that overlapped between the top 30 lists for cohorts 1 and 2. FC: fold change.

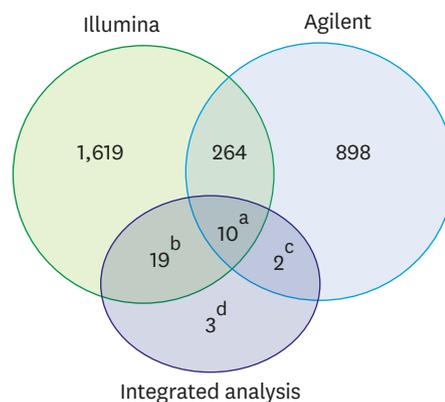


Figure 4. Comparison of cohorts 1 and 2 using an integrative meta-analysis. (a) Overlapping genes in the 3 analysis methods: Illumina, Agilent, and integrative meta-analysis (Table 4). (b) The remaining DEGs in individual Illumina analyses and the integrative meta-analysis. (c) The remaining DEGs in individual Agilent analyses and the integrative meta-analysis. (d) Genes that were differentially expressed in the integrative meta-analysis, but not in individual analyses. DEG: differentially expressed gene.

term, followed by other intracellular pathways. A comparison of all GO terms detected in each cohort revealed that 18 GO terms overlapped (d in Figure 4). In the top 30 enriched

Table 3. Overlapping genes in the 3 analysis methods: Illumina, Agilent, and integrative meta-analysis

| Gene | Gene description | Fold change | Adjusted P value |
|---------------|---|-------------|------------------|
| <i>CXCL14</i> | C-X-C motif chemokine ligand 14 | 1.50272 | 6.61E-10 |
| <i>NEFM</i> | Neurofilament medium | 1.53014 | 3.30E-10 |
| <i>TPTE2</i> | Transmembrane phosphoinositide 3-phosphatase and tensin homolog 2 | -1.61476 | 1.15E-10 |
| <i>MME</i> | Membrane metalloendopeptidase | -1.48587 | 6.25E-10 |
| <i>PDE9A</i> | Phosphodiesterase 9A | -1.52411 | 4.98E-10 |
| <i>CD27</i> | CD27 molecule | -1.52025 | 6.57E-10 |
| <i>ASB16</i> | Ankyrin repeat and SOCS box containing 16 | -1.57525 | 2.78E-10 |
| <i>CFI</i> | Complement factor I | -1.50476 | 8.60E-10 |
| <i>CD79A</i> | CD79a molecule | -1.73224 | 6.34E-12 |
| <i>BEX5</i> | Brain expressed x-linked 5 | -1.52092 | 6.86E-10 |

pathways (Table 4), which represent the functional change pattern in a diseased state, the results were irrelevant, with no overlapping pathways.

DISCUSSION

The aims of this study were to compare the characteristic transcriptomic patterns for periodontitis and to identify consistent DEGs in 2 cohorts using different microarray platforms. This study applied a comparative analysis and integrative meta-analysis to

Table 4. Top 30 enriched pathways in cohorts 1 and 2

| Cohort 1 | | | Cohort 2 | | |
|------------|--|----------|------------|-------------------------------------|----------|
| GO ID | Term | P value | GO ID | Term | P value |
| GO:0007398 | Ectoderm development | 1.71E-19 | GO:0044699 | Single-organism process | 1.29E-96 |
| GO:0008544 | Epidermis development | 9.03E-19 | GO:0009987 | Cellular process | 1.53E-95 |
| GO:0009913 | Epidermal cell differentiation | 4.27E-15 | GO:0005623 | Cell | 7.34E-93 |
| GO:0030216 | Keratinocyte differentiation | 1.00E-14 | GO:0044464 | Cell part | 2.87E-92 |
| GO:0006955 | Immune response | 1.46E-12 | GO:0044763 | Single-organism cellular process | 4.67E-86 |
| GO:0031424 | Keratinization | 5.23E-12 | GO:0005488 | Binding | 3.16E-74 |
| GO:0030855 | Epithelial cell differentiation | 2.02E-10 | GO:0005622 | Intracellular | 1.89E-70 |
| GO:0005576 | Extracellular region | 4.23E-09 | GO:0043226 | Organelle | 1.27E-66 |
| GO:0030057 | Desmosome | 3.66E-08 | GO:0044424 | Intracellular part | 1.95E-66 |
| GO:0005615 | Extracellular space | 3.12E-07 | GO:0065007 | Biological regulation | 3.55E-66 |
| GO:0060429 | Epithelium development | 4.02E-07 | GO:0050789 | Regulation of biological process | 7.62E-65 |
| GO:0043296 | Apical junction complex | 7.37E-07 | GO:0050896 | Response to stimulus | 4.74E-64 |
| GO:0009611 | Response to wounding | 7.66E-07 | GO:0043227 | Membrane-bounded organelle | 2.95E-61 |
| GO:0016327 | Apicolateral plasma membrane | 1.07E-06 | GO:0005737 | Cytoplasm | 2.05E-60 |
| GO:0006952 | Defense response | 1.47E-06 | GO:0008152 | Metabolic process | 7.32E-57 |
| GO:0001533 | Cornified envelope | 1.66E-06 | GO:0016020 | Membrane | 8.92E-56 |
| GO:0044421 | Extracellular region part | 2.02E-06 | GO:0050794 | Regulation of cellular process | 2.29E-55 |
| GO:0007155 | Cell adhesion | 3.11E-05 | GO:0005515 | Protein binding | 9.76E-54 |
| GO:0022610 | Biological adhesion | 3.11E-05 | GO:0051716 | Cellular response to stimulus | 4.69E-52 |
| GO:0002684 | Positive regulation of immune system process | 4.03E-05 | GO:0044425 | Membrane part | 6.58E-50 |
| GO:0006954 | Inflammatory response | 4.34E-05 | GO:0044444 | Cytoplasmic part | 1.4E-49 |
| GO:0001775 | Cell activation | 5.79E-05 | GO:0007154 | Cell communication | 8.47E-49 |
| GO:0003823 | Antigen binding | 9.09E-05 | GO:0043229 | Intracellular organelle | 7.12E-46 |
| GO:0016477 | Cell migration | 9.90E-05 | GO:0044700 | Single organism signaling | 4.15E-45 |
| GO:0012505 | Endomembrane system | 2.13E-04 | GO:0023052 | Signaling | 4.75E-45 |
| GO:0016337 | Cell-cell adhesion | 2.84E-04 | GO:0071704 | Organic-substance metabolic process | 1.34E-43 |
| GO:0005911 | Cell-cell junction | 3.43E-04 | GO:0031224 | Intrinsic component of membrane | 3.02E-43 |
| GO:0048584 | Positive regulation of response to stimulus | 3.49E-04 | GO:0007165 | Signal transduction | 8.41E-43 |
| GO:0044432 | Endoplasmic reticulum part | 3.82E-04 | GO:0016021 | Integral component of membrane | 1.2E-41 |
| GO:0048870 | Cell motility | 4.07E-04 | GO:0044237 | Cellular metabolic process | 3.78E-40 |

None of the top 30 enriched pathways overlapped between cohorts 1 and 2.

GO: gene ontology.

data sets obtained using different microarray platforms to analyze 2 periodontitis cohorts recruited using the same criteria. The characteristic gene expression pattern of gingival tissue differed between the periodontitis patients and healthy controls, in terms of the clustering hierarchy of the heatmap from both cohorts and the integrated data sets. There were 59 common DEGs in the 2 cohorts and 10 common DEGs in both the 2 cohorts and the integrated data sets. However, the inconsistency between the data sets at both the DEG and biological pathway levels hindered the identification of specific biomarkers for periodontitis.

Poor consistency was shown in this study, by the results for the 2 data sets and by the meta-analysis results, which made it difficult to suggest biomarker genes that were significantly and consistently associated with the pathogenesis of periodontitis, as shown by overlapping DEGs. The present data sets were compared with DEG lists from previous studies to evaluate the poor consistency in gene expression patterns. The DEG list obtained by Demmer et al. [21] overlaps by only 12% with cohort 1 and by 10% with cohort 2, while that of Becker et al. [22] overlaps by 26% with cohort 1 and only by 1 gene with cohort 2. Of the 81 potential biomarkers presented in an experimental gingivitis study [5], only 14 and 2 genes overlap with cohorts 1 and 2, respectively. Data sets from 5 previous periodontitis studies [5,6,21-23] were also compared, and poor consistency was also observed. No genes commonly detected in all 5 studies, only 9 genes were common to 4 of the data sets, and 46 genes were common to 3 of the data sets. Of 13 biomarkers for periodontitis identified using spatial transcriptome techniques [11], 5 genes were detected in cohort 1, 6 genes were detected in cohort 2, and 1 gene was detected in the integrated data sets. Comparing the findings of the 5 previous studies with these biomarkers reveals that fewer than 3 common genes were detected in each study. Previous studies also have shown minimal overlap of DEGs in microarray analyses, as well as in the 3 data sets of the present study. It was difficult to find a reasonable consensus related to the microarray data for periodontitis, including the data sets in the present and previous studies.

The diversity of these results could be partly due to a lack of clarity about the current diagnostic criteria of periodontitis and the complexity of the underlying pathogenesis. A new classification of periodontitis was released in 2017 [24] following the American Academy of Periodontology classification in 1999. Both classification systems are based on only clinical features and risk factors, rather than the pathological background. In contemporary periodontology, viewpoints on the etiology of periodontitis have evolved from the keystone pathogen hypothesis to the concept that it is more important to consider the environment, host genetics, and host response [25]. Due to the complexity of periodontitis, there are conflicting findings on the pathology and clinical presentation of the disease, such as its severity [26,27]. Therefore, these current systems can include patients with various pathological backgrounds and phases in the same disease category. Since microarray analysis can detect gene expression in lesions, the lack of consistency in the gene expression data for these cohorts could be due to the heterogeneity of host responses in periodontitis lesions that are classified into the same category using the current diagnosis systems.

This study confirmed the presence of specific gene expression patterns in the gingival tissue of periodontitis patients, as has also been reported previously. However, a limitation is that the number of samples in the 2 cohorts is too small to statistically confirm the consistency (or lack thereof) of the findings. In addition, there are limitations due to the current diagnosis system of periodontitis and differences between the microarray platform. Microarray data should be conservatively interpreted when attempting to identify specific

biomarkers for diagnosing or identifying the etiology of periodontitis, due to the poor reliability and consistency found in the expression patterns from various datasets.

SUPPLEMENTARY MATERIAL

Supplementary Table 1

The overlapping differentially expressed genes between cohorts 1 and 2

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