

# Gene Dysregulation in Peripheral Blood of Young Females With Temporomandibular Joint Osteoarthritis

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## Research article

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# Abstract

## Background

Early onset of the disease and female preponderance are the unique features of the temporomandibular joint (TMJ) osteoarthritis (OA). The immune modulation mechanisms related to etiology of OA from other joints such as knee or hip have been suggested, but the immune-associated pathophysiology of TMJ OA, especially in young females, has not been elucidated. The present study aimed to investigate the immune-related pathophysiology of TMJ OA by analyzing transcriptional profiles of peripheral blood mononuclear cells which identify the differentially expressed genes (DEGs) in young females with TMJ OA.

## Methods

RNA-sequencing (RNA-seq) was conducted on 24 young females with TMJ OA (mean age  $19.3 \pm 3.1$  years) (RNAOA) and 11 age and sex matched healthy control (mean age  $20.5 \pm 3.7$  years) (CON). RNA-seq datasets were analyzed to identify genes, pathways, and regulatory networks of those who were involved in the development of TMJ OA.

## Results

RNA-seq data analysis revealed 41 DEGs between RNAOA and CON. A total of 16 gene ontology (GO) terms including 3 molecular and 13 biological terms were annotated via the GO function of molecular function and biological process. Through ingenuity pathway analysis (IPA), 21 annotated categories of diseases and functions were identified. There were six hub genes which showed significant results in both GO enrichment analysis and IPA, namely HLA-C, HLA-F, CXCL8, IL11RA, IL13RA1, and FCGR3B.

## Conclusions

The young females with TMJ OA showed alterations of the gene related to immune function in the blood and some of the changes may reflect inflammation, auto-reactivity, and altered T cell functions.

# Introduction

Osteoarthritis (OA) is characterized by the degradation of components of the extracellular matrix within the articular cartilage and the simultaneous remodeling of the underlying subchondral bone, in association with low inflammatory changes [1]. The epidemiology of OA of the TMJ is different from OA of the hand, knee, or hip joint, the incidence of which is associated with aging. Previous reports have already described female preponderance and early onset of the disease, especially from the pubertal phases to the early 20 s [2, 3]. The pathophysiology of the TMJ OA is multifactorial and complex, which includes diverse etiological factors such as prolonged parafunctional habits, abnormal occlusal relationship, sustained masticatory muscle tension, and hormonal imbalance [4, 5]. Given the early onset of the condition, TMJ OA cannot be considered to be just a simple degenerative disease such as the OA

of other joints associated with the aging process, so other etiological factors could be assumed. Nevertheless, the clear pathophysiology of the TMJ OA, particularly in young patients has not yet been elucidated so far.

Many emerging evidences have demonstrated the role of immune modulation mechanisms in the development and progression of OA [6–13]. These processes involved immune-modulating agents, in both innate and adaptive compartment such as cytokines, chemokines, T cells, and B cells [6–13]. TMJ OA has been considered as a low inflammatory arthritic condition and mainly depends on inflammation and elevated levels of inflammatory mediators and cytokines in the TMJ synovial fluid [14–17]. Even though one report suggested the possibilities of involvement of systemic immune dysfunction in occurrence of TMJ OA [18], the influences of systemic immune function and composition of immune cells in peripheral blood on incidence and progression of TMJ OA have not been fully clarified.

RNA sequencing (RNA-seq) technology has been utilized as a powerful tool to discover potential molecular mechanisms or therapeutic targets in a variety of diseases [19]. However, to the best of the knowledge, there are yet no reported studies which adopted RNA-seq technology to the patients with TMJ OA. Therefore, the molecular and genetic background of TMJ OA remains obscure and clinical treatment effects for TMJ OA are limited. Understanding comprehensive molecular profiling of TMJ OA is an essential step in discovering new candidate target molecules that are potentially involved in the pathogenesis of TMJ OA. Hence, the aim of the present study was to investigate the role of the immune-related pathophysiology of TMJ OA by analyzing the transcriptional profiles of RNA from peripheral blood mononuclear cells (PBMC) which identify the differentially expressed genes (DEGs) of young females with TMJ OA.

## Materials And Methods

### Participants

In the present study, a total of 35 young females (mean age  $19.7 \pm 3.1$  years; age range 15–25 years) were enrolled. Twenty-four female patients ( $19.3 \pm 3.1$  years; age range 15–25 years) with TMJ OA on at least one side of the condyles were consequently recruited from those who attended the TMD·Orofacial Pain Clinic at a university hospital from January, 2019 to November, 2019 (RNAOA). Eleven young females (mean age  $20.5 \pm 3.7$  years; age range 15–25 years) without any signs of the TMD and/or TMJ OA who voluntarily participated in the present study served as control (CON). Patients with the following conditions were excluded from the study: history of head and neck trauma prior to at least 6 months prior to study entry; autoimmune diseases; craniofacial anomalies; and neurodegenerative disorder. All participants in the RNAOA did not show any sign of capsule or myofascial pain in the orofacial area for at least 3 months before enrollment; hence the effect of pain condition on RNA transcription profiles could be excluded. Clinical parameters such as the degree of maximum unassisted opening as well as the duration of TMD symptoms including TMJ noise and difficulties in opening and/or closing the mouth were evaluated. Participants were diagnosed following the Diagnostic Criteria for Temporomandibular

Disorder Axis I [20, 21]. All female participants in the RNAOA showed erosive osseous bony changes on computed tomography images indicating the area of discontinuation of the cortical lining and adjacent bone. The research protocol was approved by the Institutional Review Board of the University Hospital (AJIRB-MED-GEN-18-449). Informed consents were obtained from all participants.

## **RNA extraction from PBMC and RNA seq**

To minimize the effect of circadian variation and menstruation cycle, peripheral blood was collected from all participants between 9:00 a.m. and 11:00 a.m. during their mid-luteal phases. For each individual, 3 ml peripheral blood was put into a PAXgene Blood RNA tube vacutainer tube (Qiagen, New York, USA). PBMC from the venous blood from all participants were isolated using Ficoll-Paque PLUS (Sigma-Aldrich). Trizol reagent (Thermo Fisher Scientific Invitrogen Inc., MA, USA) was utilized to isolate the total RNA from PBMC. Genomic DNA contamination was removed using RNase-free DNase I. The amount of total RNA was measured using NanoDrop 2000 (Thermo Fisher Scientific Invitrogen Inc., MA, USA) and the integrity and quality of RNA samples were analyzed using an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Inc., CA, USA). All samples passed quality control RNA integrity analysis ( $RIN \geq 7$ ). cDNA libraries were constructed using the TruSeq Stranded Total RNA Sample Preparation Kit (Illumina Inc, USA) according to the manufacturer's protocol. To produce 100 bp paired-end reads, the total RNA was sequenced using the Illumina NovaSeq 6000 system (Macrogen, Seoul, Korea). After sequencing, the indexed samples were demultiplexed before the generation of FASTQ files for analysis and assessed by FastQC version 0.11.7.

Using HISAT2 version 2.1.0 with the best score matches reported for each read, all libraries were aligned to hg19 assembly of human genome. The mapped reads were assembled using STRING Tie version 1.3.4d. Inter gene expression comparisons were based on calculated fragments per kilobase of transcript per million (FPKM) mapped reads.

## **Differential expression analysis of RNA-seq data**

The expression level was normalized by calculating FPKM mapped reads. For DEG analysis, the value of  $\log_2$  (fold change) were calculated. Fold change values were calculated by dividing FPKMs from RNAOA by those from CON. The DEGs with an adjusted  $P \leq 0.05$  and  $\log_2$  (fold changes)  $\leq -1$  or  $\geq 1$  were determined. Using heatmap function, the hierarchical clustering of the expression profiles of detected DEGs was conducted.

## **Bioinformatics analysis**

Using online tool STRING analysis (<http://string-db.org>, version 11.0), gene ontology (GO) pathway enrichment analysis was conducted to compare gene transcription patterns between RNAOA and CON and assess the functional association between encoded proteins. False discovery rate (FDR) adjusted  $P$  values were calculated for each enriched biological pathway and the threshold was set to an adjusted  $P < 0.01$ . Gene set enrichment analysis (GSEA, version 4.0.3) was used to compare gene dysregulation patterns in RNAOA with those in CON.

The threshold was set to an unadjusted  $P < 0.05$  to fully explore the results by ingenuity pathway analysis (IPA; Ingenuity System Inc., Redwood City, CA, USA). Using IPA, biological processes, canonical pathways, and networks of analysis were analyzed. An enrichment score measures the overlap of observed and predicted gene sets. A z-score assesses the match of observed and predicted regulation patterns, serving as a predictor for the activation state of the identified regulator molecule.

## Quantitative real time polymerase chain reaction validation

To validate the differential expression, gene expression levels were examined using quantitative real time polymerase chain reaction (qRT-PCR). qRT-PCR reactions were conducted via the Step ONE Plus (ABI, Life Technologies, CA, USA) using SYBR premix EX Taq II (Applied Biosystems, Foster City, CA, USA), and cDNA was synthesized using 1  $\mu$ g of mRNA. The gene expression results were obtained using the formula  $2^{-(\Delta Ct)}$ , and the fold change was calculated by the formula  $2^{-(\Delta\Delta Ct)}$ . The qRT-PCR values were normalized using the average of the expression of the reference gene, GAPDH. Finally, the averaged fold ratios from the reference housekeeping gene were used as the relative mRNA level. Each experiment was conducted in triplicate. Two reactions, one without template and one without reverse transcriptase were also performed.

## Statistical analysis

The differences in demographic features and TMD characteristics between CON and RNAOA were evaluated using Mann-Whitney U tests. A t-test identified significantly changed gene transcripts between RNAOA and CON. The gene expressions were defined as fold changes. Statistical analysis was achieved by setting the change in gene expression threshold to an unadjusted  $P < 0.01$  for encoded protein functional network analysis (FNA). The threshold was set to an unadjusted  $P < 0.05$  to fully explore the results by IPA. To compare gene expression levels obtained by qRT-PCR between RNA OA and CON, Mann-Whitney U tests were utilized. The level of significance was set as  $P < 0.05$ .

## Results

### Demographic features and TMD symptoms in participants

The differences in age ( $P = 0.268$ ) and body mass index ( $P = 0.740$ ) did not show statistical significance between CON and RNAOA. Participants in RNAOA showed  $16.4 \pm 28.0$  months of TMD symptom durations and the amount of maximum unassisted opening showed a significant difference between CON and RNAOA ( $P = 0.036$ ) (Table 1).

Table 1  
Demographic features and TMD characteristics of participants

	CON (n = 11)	RNAOA (n = 24)	<i>P</i> value
Age	23.2 ± 11.4	19.3 ± 3.1	0.268
BMI	20.6 ± 2.2	20.5 ± 2.7	0.740
Duration of TMD symptoms (months)	0	16.4 ± 28.0	< 0.001**
Maximum unassisted opening (mm)	47.5 ± 4.7	42.8 ± 5.7	0.036*
Descriptive values are shown as mean ± SD.			
Data obtained from Mann-Whitney U tests.			
* <i>P</i> < 0.05, ** <i>P</i> < 0.001 by Mann-Whitney U tests.			

## Gene dysregulation in peripheral blood of patients with TMJ OA

There were 27685 transcripts in total, and transcripts with an FPKM value less than 5 were excluded, leaving 12788 transcripts to be analyzed. After excluding the following FPKM baseline gene, 440 genes were differentially expressed in the RNAOA compared with those in the CON ( $|\text{fold change}| > 1$ ,  $P < 0.05$ ). A total of 41 genes were expressed significantly differentially between RNAOA and CON with 17 genes upregulated and 24 down regulated in the RNAOA ( $\log_2(\text{fold change}) \geq 1$  or  $\leq -1$ ,  $P < 0.05$ ) (Table 2). A heatmap using hierarchical clustering analysis showing the expression levels of each of these genes per individual are provided (Fig. 1). The genes with the highest fold change in RNAOA include FGFR2, EREG, WTH3DI, CXCL8, and LINC02458 and those with the lowest fold change were SYNGR1, CYP27A1, IL11RA, MAVS, and MIR941-2 (Table 2).

Table 2  
Transcripts with altered gene expression in the RNAOA compared to CON

Gene Symbol	Gene Name	Log <sub>2</sub> (Fold Change)	P value
FGFR2	fibroblast growth factor receptor 2	1.51	0.020
EREG	epiregulin	1.33	0.029
WTH3DI	RAB6C-like	1.21	0.027
CXCL8	C-X-C motif chemokine ligand 8	1.19	0.048
LINC02458	long intergenic non-protein coding RNA 2458	1.18	0.041
SNORD133	small nucleolar RNA, C/D box 133	1.13	0.032
FCER1A	Fc fragment of IgE receptor 1a	1.13	0.036
MS4A3	membrane spanning 4-domains A3	1.12	0.042
RPL21	ribosomal protein L21	1.10	0.012
ITGB8	integrin subunit beta 8	1.08	0.004
GPR82	G protein-coupled receptor 82	1.08	0.049
LOC105377267	uncharacterized LOC105377267	1.07	0.024
SNORA1	small nucleolar RNA, H/ACA box 1	1.05	0.025
SNORA104	small nucleolar RNA, H/ACA box 104	1.05	0.020
MS4A2	membrane spanning 4-domains A2	1.04	0.017
HRH4	histamine receptor H4	1.03	0.029
ACAT2	acetyl-CoA acetyltransferase 2	1.03	0.039
GAS5-AS1	GAS5 antisense RNA 1	1.02	0.032
DOCK1	dedicator of cytokinesis 1	1.01	0.020
CA8	carbonic anhydrase 8	1.01	0.020
IRAK1BP1	interleukin 1 receptor associated kinase 1 binding protein 1	1.01	0.002
CPA3	carboxypeptidase A3	1.01	0.018
GTSF1	gametocyte specific factor 1	1.01	0.049
ZNF257	zinc finger protein 257	1.00	0.031
HLA-F	major histocompatibility complex, class I, F	-1.00	0.014
HSPA6	heat shock protein family A (Hsp70) member 6	-1.01	0.003

Gene Symbol	Gene Name	Log <sub>2</sub> (Fold Change)	P value
NCF1C	neutrophil cytosolic factor 1C pseudogene	-1.01	0.047
HLA-C	major histocompatibility complex, class I, C	-1.02	0.022
IL13RA1	interleukin 13 receptor subunit alpha 1	-1.02	0.034
TNFRSF8	TNF receptor superfamily member 8	-1.02	0.026
FCGR3B	Fc fragment of IgG receptor IIIb	-1.03	0.050
TMEM176B	transmembrane protein 176B	-1.03	0.047
MIR941-3	microRNA 941-3	-1.03	0.019
TNFRSF25	TNF receptor superfamily member 25	-1.03	0.039
CCHCR1	coiled-coil alpha-helical rod protein 1	-1.04	0.042
UTS2	urotensin 2	-1.04	0.043
SYNGR1	synaptogyrin 1	-1.06	0.030
CYP27A1	cytochrome P450 family 27 subfamily A member 1	-1.11	0.007
IL11RA	interleukin 11 receptor subunit alpha	-1.11	0.044
MAVS	mitochondrial antiviral signaling protein	-1.14	0.029
MIR941-2	microRNA 941-2	-1.46	0.031

## Functional network analysis

The FNA of proteins encoded by differentially expressed gene transcripts ( $n = 41$ ,  $P < 0.05$ ) were assessed using the STRING portal (<http://string-db.org>, version 11.0). A total of 16 GO terms ( $P < 0.01$ ) including 3 molecular and 13 biological terms were annotated by the GO function of molecular function and biological process (Fig. 2). Four clusters of strong functional associations with significant levels of network enrichment ( $P = 0.000821$ ) were observed: 1) HLA-C, HLA-F, TNFRSF25, CCHCR1, FCER1A, MS4A2, and CPA3, 2) FGFR2, EREG, CXCL8, HRH4, and 3) UTS2, FCGR3B and TNFRSF8, and 4) IL11RA and IL13RA1 (Fig. 3).

## Pathway analysis using IPA

IPA was carried out on the transcriptome dataset with significance set at  $P < 0.05$  and  $|\log_2(\text{fold change})| > 1$ . IPA identified 20 annotated categories of diseases and functions that were significantly upregulated ( $z\text{-score} > 2.0$ ) and included a total of 13 of the DEGs from the study. The 5 top canonical pathways were associated with role of natural killer cells, communication between innate and adaptive immune cells, and T cell signaling (Table 3). Nine genes were involved in five top canonical pathways

namely, FCGR3B, HLA-C, HLA-F, HSPA-6, CXCL8, ITGB8, FCER1A, IL11RA, and IL13RA1. Hence, these pathways reinforce significant immune and inflammatory dysregulation occurring in the pathogenesis of TMJ OA.

Table 3  
Annotated categories of diseases and functions from IPA

<b>Ingenuity canonical pathways</b>	<b>Z-Score</b>	<b>Molecules</b>
Natural Killer Cell Signaling	3.63	FCGR3B, HLA-C, HLA-F, HSPA6
Communication between Innate and Adaptive Immune Cells	3.36	CXCL8, HLA-C, HLA-F
Virus Entry via Endocytic Pathways	3.23	HLA-C, HLA-F, ITGB8
White Adipose Tissue Browning Pathway	2.99	FCER1A, FGFR2, MS4A2
STAT3 Pathway	2.93	FGFR2, IL11RA, IL13RA1
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	2.9	HLA-C, HLA-F
Antigen Presentation Pathway	2.78	HLA-C, HLA-F
Tec Kinase Signaling	2.69	FCER1A, MS4A2, TNFRSF25
Graft-versus-Host Disease Signaling	2.61	HLA-C, HLA-F
Autoimmune Thyroid Disease Signaling	2.59	HLA-C, HLA-F
Role of NFAT in Regulation of the Immune Response	2.57	FCER1A, FCGR3B, MS4A2
Dendritic Cell Maturation	2.56	FCGR3B, HLA-C, HLA-F
Systemic Lupus Erythematosus Signaling	2.28	FCGR3B, HLA-C, HLA-F
Cardiac Hypertrophy Signaling (Enhanced)	2.19	CXCL8, FGFR2, IL11RA, IL13RA1
Allograft Rejection Signaling	2.11	HLA-C, HLA-F
CTLA4 Signaling in Cytotoxic T Lymphocytes	2.08	HLA-C, HLA-F
Crosstalk between Dendritic Cells and Natural Killer Cells	2.08	HLA-C, HLA-F
Protein Ubiquitination Pathway	2.07	HLA-C, HLA-F, HSPA6
OX40 Signaling Pathway	2.07	HLA-C, HLA-F
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	2.04	DOCK1, FCGR3B

## qRT-PCR

The gene expression patterns of 6 hub genes which showed significant results in both GO enrichment analysis and IPA including HLA-C, HLA-F, CXCL8, IL11RA, IL13RA1, and FCGR3B transcripts were tested via qRT-PCR. There were significant differences in HLA-C ( $P=0.030$ ), CXCL8 ( $P=0.022$ ), and IL11RA transcripts ( $P=0.018$ ) (Fig. 4).

## Discussion

Early onset of diseases, especially during pubertal phases and early 20 s is the unique feature of TMJ OA [2]. The progression of TMJ OA may accompany compromised masticatory function and altered craniofacial morphology, which could affect an individual's quality of life [22–24]. However, to the date, exact pathophysiology of TMJ OA particularly in young patients and clear molecular mechanisms of the development of TMJ OA have not yet been elucidated, so far. Immune dysfunction has been considered as one of the main etiological factors which may result in bony destruction in patients with OA from other joints [6–9, 12, 13], but studies which clearly elucidate the immunological background of TMJ OA especially in young patients are sparse. RNA-seq is an effective tool for clarifying molecular mechanisms in genetic levels and could provide the novel therapeutic targets involved in a certain condition [19]. To the best of the knowledge, no study ever attempted to reveal the immune related etiology of TMJ OA using RNA-seq technology. Consequently, the aim of the present study was to investigate the role of immune dysfunction by analyzing transcriptional profiles of PBMC and search for new therapeutic targets for the management of TMJ OA in young patients.

Declined acquired immune responses accompanied with increased auto-reactivity have been detected in the elderly [25] and relationships in this altered innate immune function, T cell and B cell responses, and cartilage and bone degradation have been reported [26, 27]. Aforementioned results from IPA demonstrated the associations among various autoimmune disorders, including autoimmune thyroid disease and systemic lupus erythematosus (SLE), and occurrence of TMJ OA. Previous studies already mentioned the high prevalence of temporomandibular disorders in patients with autoimmune thyroid disorders or SLE [28–30]. Even though severe condylar resorption in patients with rheumatoid arthritis or juvenile idiopathic arthritis have been detected [31], few reports ever mentioned the bony changes of condyles in other autoimmune diseases such as autoimmune thyroid disorders or SLE. Because patients with autoimmune disorders were excluded from the present study, clear associations between autoimmune thyroid disorders or SLE and TMJ OA could not be clarified. Even though, previous studies which dealt with the associations between innate immunity and OA progression focused on increased auto-reactivity related with aging process, the role of altered innate immune function in bony destruction of TMJ condyles even in young females could be assumed through this study.

Accumulating evidence suggested the involvement of inflammatory and immune responses in the pathogenesis of OA [32]. The results from the present study demonstrated the increased expression levels of chemokine(C-X-C motif) ligand 8 (CXCL8), the involvement of signal transducers and activators of transcription 3 (STAT3) pathway, and cytokine mediated immune response in the development of TMJ OA in young females. Interleukin-8/CXCL8 (IL-8/CXCL8) has been found to be an attractant for

neutrophils and a population of lymphocytes [33]. The increased level of IL-8 in synovial fluid from the knee OA and TMD patients with disc displacement have been reported [34–37], but the mechanisms of IL-8 in subchondral bone changes in TMJ have not been clearly revealed. One study has reported the elevated serum levels of CXCL8 and expression levels of STAT3 in knee OA patients [38]. This study suggested that CXCL8 may inactivate the mitosis of chondrocytes and further aggravate OA progression and indirectly induce the Janus kinase (JAK)/STAT3 signaling in chondrocytes. Although the specific molecular mechanisms of CXCL8 and STAT3 could not be revealed in the present results, the associations between CXCL8, STAT3 signaling, and subchondral bony destruction of TMJ condyles in young females could be suspected.

The peripheral blood of OA patients have been analyzed and revealed that patients with OA have shown altered levels of CD8 + T cells and more cytotoxic profiles in comparison with healthy controls [11, 39–41]. One animal study suggested the molecular mechanisms of the role of CD8 + T cells on the OA process that CD8 + T cells were activated once OA had been initiated and cartilage degeneration occurred more slowly in CD8 + T cell knockout mice than in wild-type [42]. The results from this study also showed the relationships between the occurrence of TMJ OA and T cell activity through increased expression levels of genes related to Tec kinase signaling, nuclear factor of activated T cells (NFAT) regulation, and OX40 signaling. Furthermore, cytotoxic T lymphocyte mediated apoptosis and CTLA4 signaling pathway which have associations with cytotoxic T cell function, seem to have roles in the incidence of TMJ OA. A previous study has focused on the role of T helper cell in synovial fluid on the subchondral bony changes in TMJ OA patients [16], but no study ever attempted to clarify altered cytotoxic T cell activities in blood in patients with TMJ OA.

To the best of our knowledge, the present study is the first study which attempted to reveal the genetic and molecular background of TMJ OA in young patients using RNA-seq technology. Several previous reports which dealt with the systemic immune dysfunction and the development of OA have focused on the role of the aging process and the senescence of immune cells. In addition, most of the studies which analyzed the relationships between levels of the immune-modulating agents in blood and OA dealt with weight bearing axial joints such as the knee and hip not with peripheral joints. Aforementioned results showed that alteration systemic immune function could have a role in the development of arthritic conditions even in young patients with OA from small peripheral joints such as TMJ. However, the present study still has several limitations, first of all, RNA-seq was conducted using only PBMC, not using the synovial tissue. Secondly, only female participants were included. Even though previous studies confirmed the female preponderance of TMJ OA, the absence of male participants would limit the understanding of the genetic etiology of the TMJ OA. Thirdly, owing to cross-sectional characteristics of the study, the information regarding the changes in the transcriptome profiles accordance with the progression or recovery of TMJ OA could not be provided. Future prospective RNA-seq studies with large samples including both male and female participants, using both PBMC and synovial tissue would be required.

## Conclusion

In conclusion, in the present study, young female TMJ OA patients showed alterations in the blood immune cell expression and some of the changes may reflect inflammation, auto-reactivity, and altered T cell functions. For proper management and successful treatment of TMJ OA in young females, future research regarding immune-modulation based therapy would be warranted.

## Abbreviations

DEG

Differentially expressed gene; FDR:False discovery rate; FNA:Functional network analysis; FPKM:Fragments per kilobase of transcript per million; IPA:Ingenuity pathway analysis; OA:Osteoarthritis; PBMC:Peripheral blood mononuclear cells; RNA-seq:RNA sequencing; SLE:Systemic lupus erythematosus; TMJ:Temporomandibular joint

## Declarations

### Ethics approval and consent to participants

The research protocol was approved by the Institutional Review Board of the University Hospital (AJIRB-MED-GEN-18-449). Informed consents were obtained from all participants.

### Consent for publication

Not applicable

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The author certify that no conflicts of interests were involved in this paper.

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## Author contribution

A single author Jeong-Hyun Kang contributed to the obtaining of funding, conception and design of the article, collection of data, analysis of data, interpretation of data, and construction of manuscript.

## References

1. Zarb GA, Carlsson GE. Temporomandibular disorders: osteoarthritis. *J Orofac Pain*. 1999; 13: 295-306.
2. Kim K, Wojczynska A, Lee JY. The incidence of osteoarthritic change on computed tomography of Korean temporomandibular disorder patients diagnosed by RDC/TMD; a retrospective study. *Acta Odontol Scand*. 2016; 74: 337-342.
3. Manfredini D, Piccotti F, Ferronato G, Guarda-Nardini L. Age peaks of different RDC/TMD diagnoses in a patient population. *J Dent*. 2010; 38: 392-399.
4. Tanaka E, Detamore MS, Mercuri LG. Degenerative disorders of the temporomandibular joint: etiology, diagnosis, and treatment. *J Dent Res*. 2008; 87: 296-307.
5. Wang XD, Zhang JN, Gan YH, Zhou YH. Current understanding of pathogenesis and treatment of TMJ osteoarthritis. *J Dent Res*. 2015; 94: 666-673.
6. Haseeb A, Haqqi TM. Immunopathogenesis of osteoarthritis. *Clin Immunol*. 2013; 146: 185-196.
7. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011; 7: 33-42.
8. Li YS, Luo W, Zhu SA, Lei GH. T Cells in Osteoarthritis: Alterations and Beyond. *Front Immunol*. 2017; 8: 356.
9. Lopes EBP, Filiberti A, Husain SA, Humphrey MB. Immune Contributions to Osteoarthritis. *Curr Osteoporos Rep*. 2017; 15: 593-600.
10. Lurati A, Laria A, Gatti A, Brando B, Scarpellini M. Different T cells' distribution and activation degree of Th17 CD4+ cells in peripheral blood in patients with osteoarthritis, rheumatoid arthritis, and healthy donors: preliminary results of the MAGENTA CLICAO study. *Open Access Rheumatol*. 2015; 7: 63-68.
11. Ponchel F, Burska AN, Hensor EM, Raja R, Campbell M, Emery P, et al. Changes in peripheral blood immune cell composition in osteoarthritis. *Osteoarthritis Cartilage*. 2015; 23: 1870-1878.
12. Weber A, Chan PMB, Wen C. Do immune cells lead the way in subchondral bone disturbance in osteoarthritis? *Prog Biophys Mol Biol*. 2019; 148: 21-31.

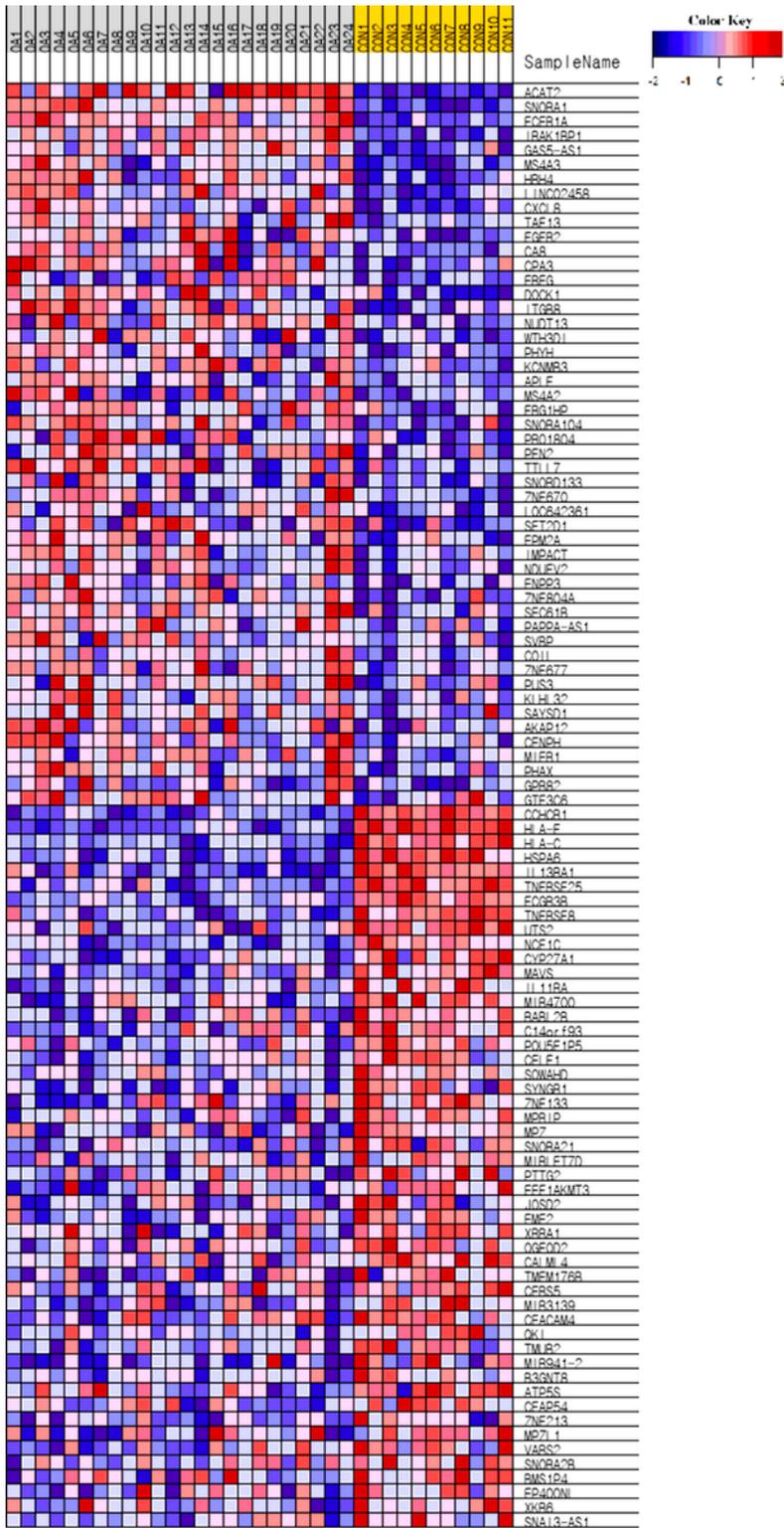
13. Woodell-May JE, Sommerfeld SD. Role of Inflammation and the Immune System in the Progression of Osteoarthritis. *J Orthop Res.* 2020; 38: 253-257.
14. Kacena MA, Merrel GA, Konda SR, Wilson KM, Xi Y, Horowitz MC. Inflammation and bony changes at the temporomandibular joint. *Cells Tissues Organs.* 2001; 169: 257-264.
15. Kellesarian SV, Al-Kheraif AA, Vohra F, Ghanem A, Malmstrom H, Romanos GE, et al. Cytokine profile in the synovial fluid of patients with temporomandibular joint disorders: A systematic review. *Cytokine.* 2016; 77: 98-106.
16. Monasterio G, Castillo F, Rojas L, Cafferata EA, Alvarez C, Carvajal P, et al. Th1/Th17/Th22 immune response and their association with joint pain, imagenological bone loss, RANKL expression and osteoclast activity in temporomandibular joint osteoarthritis: A preliminary report. *J Oral Rehabil.* 2018; 45: 589-597.
17. Xiong H, Li W, Li J, Fang W, Ke J, Li B, et al. Elevated leptin levels in temporomandibular joint osteoarthritis promote proinflammatory cytokine IL-6 expression in synovial fibroblasts. *J Oral Pathol Med.* 2019; 48: 251-259.
18. Nishioka M, Ioi H, Matsumoto R, Goto TK, Nakata S, Nakasima A, et al. TMJ osteoarthritis/osteoarthrosis and immune system factors in a Japanese sample. *Angle Orthod.* 2008; 78: 793-798.
19. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet.* 2009; 10: 57-63.
20. Ahmad M, Hollender L, Anderson Q, Kartha K, Ohrbach R, Truelove EL, et al. Research diagnostic criteria for temporomandibular disorders (RDC/TMD): development of image analysis criteria and examiner reliability for image analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 107: 844-860.
21. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network\* and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache.* 2014; 28: 6-27.
22. Bertram S, Moriggl A, Neunteufel N, Rudisch A, Emshoff R. Lateral cephalometric analysis of mandibular morphology: discrimination among subjects with and without temporomandibular joint disk displacement and osteoarthrosis. *J Oral Rehabil.* 2012; 39: 93-99.
23. Bertram S, Moriggl A, Rudisch A, Emshoff R. Structural characteristics of bilateral temporomandibular joint disc displacement without reduction and osteoarthrosis are important

- determinants of horizontal mandibular and vertical ramus deficiency: a magnetic resonance imaging study. *J Oral Maxillofac Surg.* 2011; 69: 1898-1904.
24. Kang JH, Yang IH, Hyun HK, Lee JY. Dental and skeletal maturation in female adolescents with temporomandibular joint osteoarthritis. *J Oral Rehabil.* 2017; 44: 879-888.
  25. Tomer Y, Shoenfeld Y. Ageing and autoantibodies. *Autoimmunity.* 1988; 1: 141-149.
  26. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. *Ann Rheum Dis.* 2001; 60: 619-626.
  27. Pazar B, Ea HK, Narayan S, Kolly L, Bagnoud N, Chobaz V, et al. Basic calcium phosphate crystals induce monocyte/macrophage IL-1beta secretion through the NLRP3 inflammasome in vitro. *J Immunol.* 2011; 186: 2495-2502.
  28. Grozdinska A, Hofmann E, Schmid M, Hirschfelder U. Prevalence of temporomandibular disorders in patients with Hashimoto thyroiditis. *J Orofac Orthop.* 2018; 79: 277-288.
  29. Song HS, Shin JS, Lee J, Lee YJ, Kim MR, Cho JH, et al. Association between temporomandibular disorders, chronic diseases, and ophthalmologic and otolaryngologic disorders in Korean adults: A cross-sectional study. *PLoS One.* 2018; 13: e0191336.
  30. Aliko A, Ciancaglini R, Alushi A, Tafaj A, Ruci D. Temporomandibular joint involvement in rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis. *Int J Oral Maxillofac Surg.* 2011; 40: 704-709.
  31. Dolwick FM. *Arthritis and allied conditions—a textbook of rheumatology.* Philadelphia, Lippincott Williams & Wilkins. 2001.
  32. Creamer P, Hochberg MC. Osteoarthritis. *Lancet.* 1997; 350: 503-508.
  33. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest.* 1989; 84: 1045-1049.
  34. Koh SM, Chan CK, Teo SH, Singh S, Merican A, Ng WM, et al. Elevated plasma and synovial fluid interleukin-8 and interleukin-18 may be associated with the pathogenesis of knee osteoarthritis. *Knee.* 2020; 27: 26-35.
  35. Pierzchala AW, Kusz DJ, Hajduk G. CXCL8 and CCL5 expression in synovial fluid and blood serum in patients with osteoarthritis of the knee. *Arch Immunol Ther Exp (Warsz).* 2011; 59: 151-155.
  36. Matsumoto K, Honda K, Ohshima M, Yamaguchi Y, Nakajima I, Micke P, et al. Cytokine profile in synovial fluid from patients with internal derangement of the temporomandibular joint: a preliminary

study. *Dentomaxillofac Radiol.* 2006; 35: 432-441.

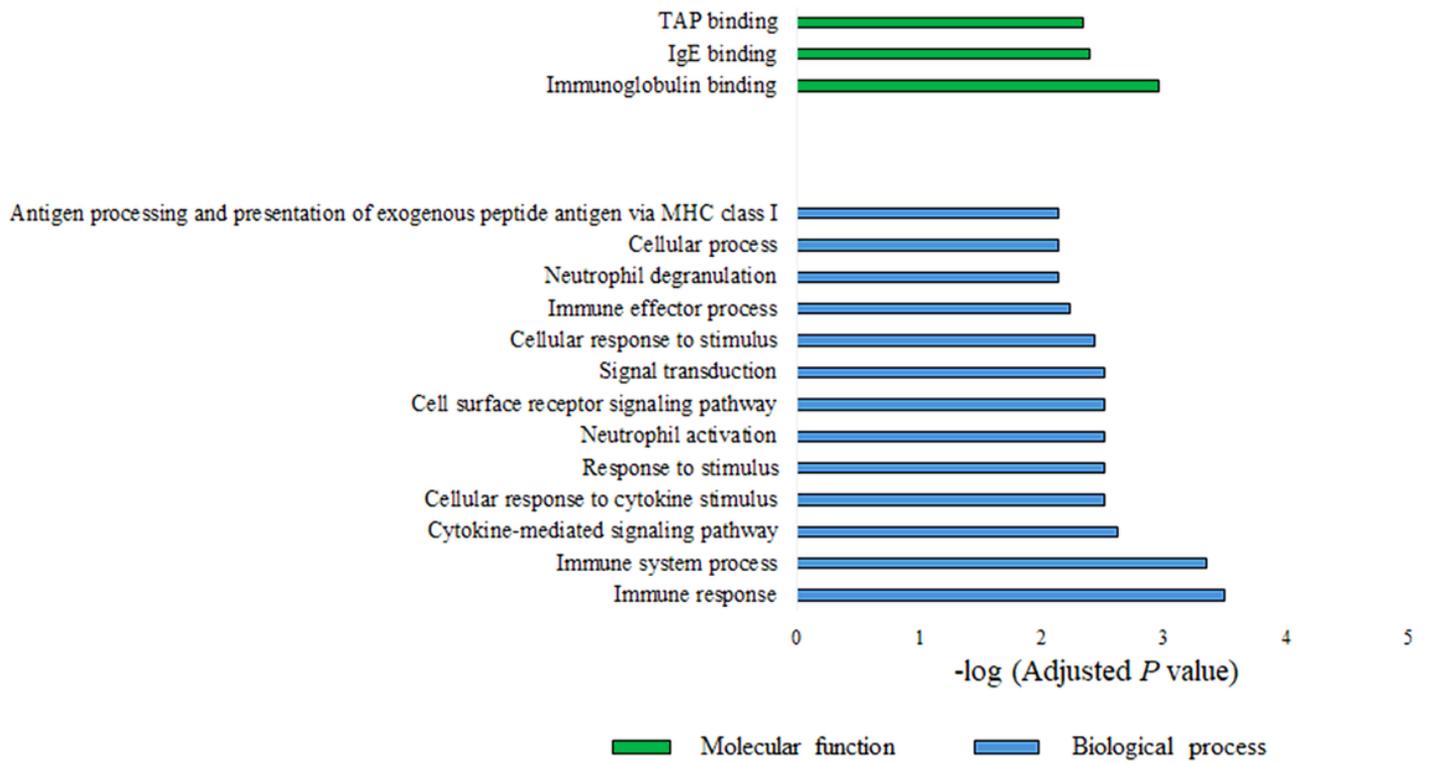
37. Sato J, Segami N, Nishimura M, Yoshitake Y, Kaneyama K, Kitagawa Y. Expression of interleukin 8 in synovial tissues in patients with internal derangement of the temporomandibular joint and its relationship with clinical variables. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007; 103: 467-474.
38. Yang P, Tan J, Yuan Z, Meng G, Bi L, Liu J. Expression profile of cytokines and chemokines in osteoarthritis patients: Proinflammatory roles for CXCL8 and CXCL11 to chondrocytes. *Int Immunopharmacol.* 2016; 40: 16-23.
39. Kuryliszyn-Moskal A. Comparison of blood and synovial fluid lymphocyte subsets in rheumatoid arthritis and osteoarthritis. *Clin Rheumatol.* 1995; 14: 43-50.
40. Apinun J, Sengprasert P, Yuktanandana P, Ngarmukos S, Tanavalee A, Reantragoon R. Immune Mediators in Osteoarthritis: Infrapatellar Fat Pad-Infiltrating CD8+ T Cells Are Increased in Osteoarthritic Patients with Higher Clinical Radiographic Grading. *Int J Rheumatol.* 2016; 2016: 9525724.
41. Sae-Jung T, Sengprasert P, Apinun J, Ngarmukos S, Yuktanandana P, Tanavalee A, et al. Functional and T Cell Receptor Repertoire Analyses of Peripheral Blood and Infrapatellar Fat Pad T Cells in Knee Osteoarthritis. *J Rheumatol.* 2019; 46: 309-317.
42. Hsieh JL, Shiau AL, Lee CH, Yang SJ, Lee BO, Jou IM, et al. CD8+ T cell-induced expression of tissue inhibitor of metalloproteinases-1 exacerbated osteoarthritis. *Int J Mol Sci.* 2013; 14: 19951-19970.

## Figures



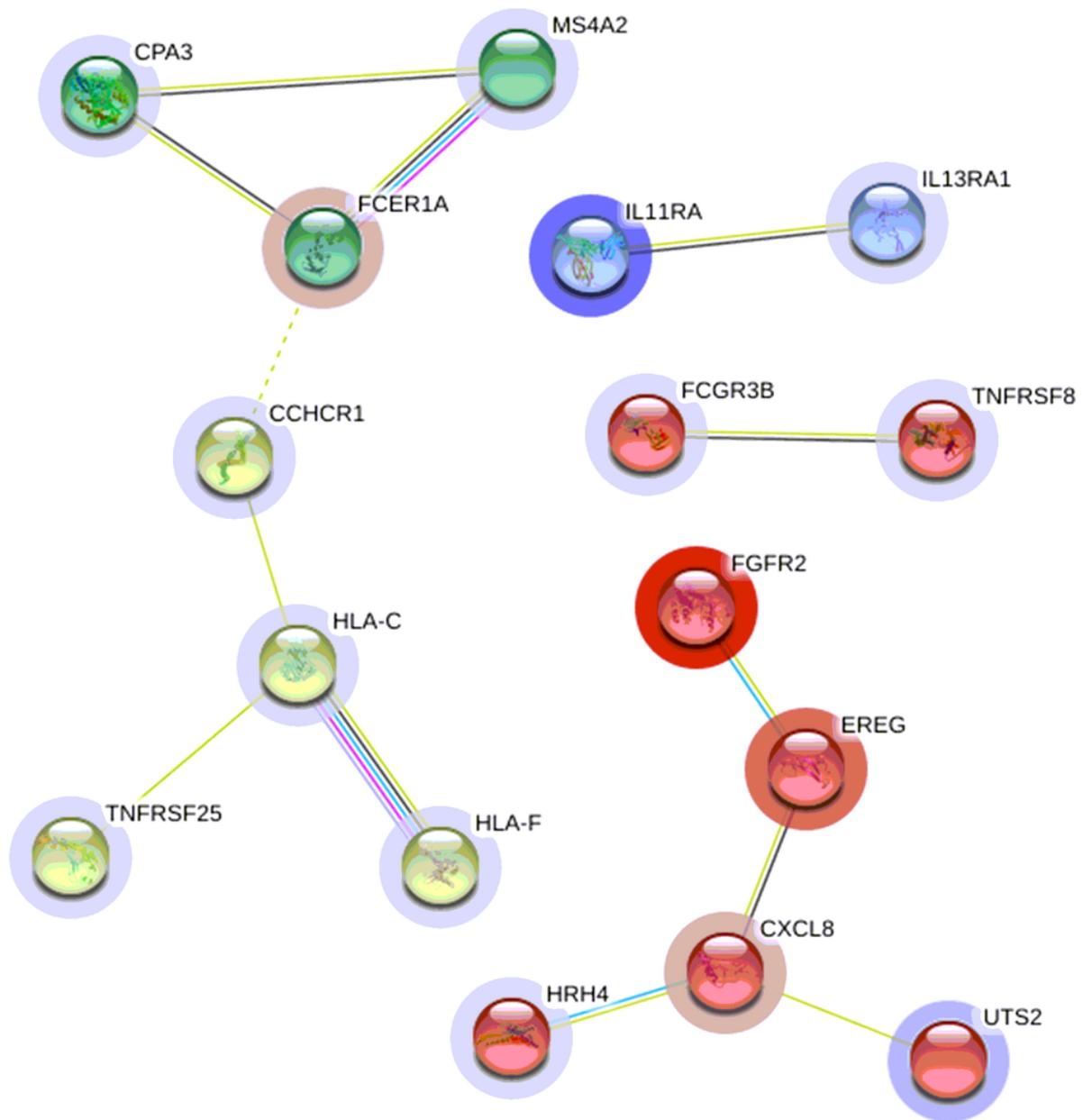
**Figure 1**

Heatmap demonstrating the expression patterns of DEGs between RNAOA and CON. The color key at the top indicates relative gene expression. Red and blue colors represent higher and lower gene expression levels, respectively.



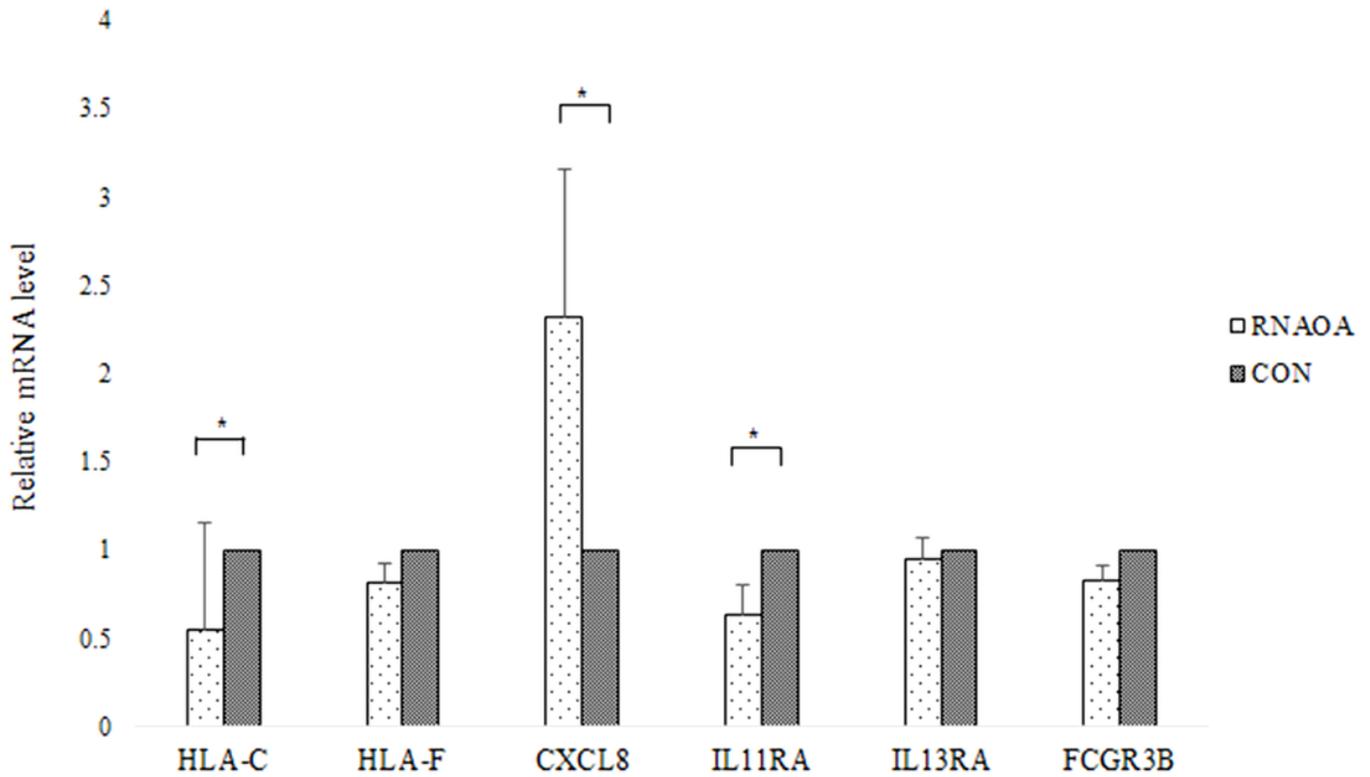
**Figure 2**

GO pathway enrichment analysis of dysregulated genes in peripheral blood of young females with TMJ OA. A total of 16 GO terms ( $P < 0.01$ ) including 3 molecular and 13 biological terms were annotated using the GO function of molecular function and biological process.



**Figure 3**

Four clusters of strong functional associations with significant levels of network enrichment.



**Figure 4**

Validation of RNA-seq results using qRT-PCR. Normalized fold expressions of mRNA are shown. Error bar represents mean  $\pm$  standard error. Mann-Whitney U tests were used to compare gene expression levels (\* P < 0.05).