Genome-wide profiling of lncRNA and mRNA expression in CRSwNP

MINGLEI LIU[1*], PING GUO[1*], JUN AN[1], CHAO GUO[2], FENGXIANG LU[1] and YANHUA LEI[1]

Departments of [1]Otolaryngology, Head and Neck Surgery, and [2]Anesthesiology, The First People's Hospital of Jining, Jining, Shandong 272000, P.R. China

Received January 31, 2018; Accepted October 3, 2018

DOI: 10.3892/mmr.2019.10005

Abstract. Chronic rhinosinusitis with nasal polyps (CRSwNP) is one of the most prevalent chronic diseases. In patients with CRSwNP, the present study performed comprehensive bioinformatics analyses to characterize the transcriptome profiles of mRNAs and long non-coding RNAs (lncRNAs). A total of 265 differentially expressed lncRNAs and 994 mRNAs were identified. The majority of up- and downregulated differentially expressed genes were significantly enriched in the biological process of 'signal transduction'. The most significantly enriched molecular function was 'protein binding' and the most significantly enriched cellular component was 'membrane'. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis led to identification of several significantly enriched pathways [false discovery rate (FDR)<0.05], including 'cytokine-cytokine receptor interaction' (FDR=$3.94x10^{16}$) and 'cell adhesion molecules' (CAMs) (FDR=$1.28x10^{-5}$). Key differentially expressed lncRNAs were identified, including lncRNA XLOC_010280, which regulates chemokine (C-C motif) ligand 18 (CCL18) and inflammation, and RP11-798M19.6, which regulates polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7) and cell proliferation. Based on the results of reverse transcription-quantitative polymerase chain reaction, except for CCL8, neural precursor cell expressed developmentally downregulated gene 4-like and GALNT7, the expression of 3 other selected genes was consistent with the results of integrated analysis. The results of the present study provide a foundation for future investigations into mRNAs and lncRNAs as diagnostic and therapeutic targets in CRSwNP.

Correspondence to: Ms. Yanhua Lei, Department of Otolaryngology, Head and Neck Surgery, The First People's Hospital of Jining, 6 Jiankang Road, Central District, Jining, Shandong 272000, P.R. China
E-mail: 13953769663@163.com

*Contributed equally

Key words: chronic rhinosinusitis with nasal polyps, long non-coding RNA, mRNA, integrated analysis, differentially expressed genes, biomarker

Introduction

Chronic rhinosinusitis (CRS) is characterized by inflammation of the nose and the sinus mucosa, and has a morbidity rate of approximately 14% (1). CRS is one of the most common conditions associated with upper airway illness and severely affects patients' quality of life. According to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS), CRS can be classified into the following two categories: CRS without nasal polyposis (CRSsNP) and CRS with nasal polyposis (CRSwNP) (2,3).

Although CRSsNP is more prevalent, CRSwNP accounts for ~20% of all CRS cases. CRSwNP, which is often accompanied by asthma, fungal rhinosinusitis and aspirin-exacerbated respiratory disease, is considered more difficult to treat compared with CRSsNP (4). The disease often requires a combination of surgical and medical treatments. However, CRSwNP often recurs even after therapy. Despite numerous studies in this field, the pathophysiological mechanisms of human CRS with nasal polyps (CRSwNP) remain to be fully elucidated (5,6).

Development of human diseases can proceed through the accumulation of different genetic alterations affecting the structure and function of the genome. Combined analyses of molecular data at multiple levels, including DNA copy-number alteration, and mRNA, long non-coding RNA (lncRNA) and miRNA expression, can clarify biological functions and pathways dysregulated in various diseases (7). The rapid development of high-throughput sequencing technology has provided a more comprehensive and efficient analytical method for the study of the pathogenesis of CRSwNP. In our study, 994 differentially expressed mRNAs [false discovery rate (FDR) <0.05] and 265 differentially expressed lncRNAs (P<0.05) were identified. By constructing an lncRNA-mRNA interaction network, and performing GO functional and KEGG pathway enrichment analyses of target genes regulated by lncRNAs, this study aimed to understand the mechanism of CRSwNP and screen potential lncRNAs and mRNAs for determining the prognosis of CRSwNP patients.

Materials and methods

Eligible CRSwNP gene expression profiles. Gene Expression Omnibus database (GEO, www.ncbi.nlm.nih.gov/geo) is the

largest available database of high-throughput gene expression data and we selected GEO datasets containing gene expression profiles of patients with CRSwNP (7). The following search key words were used: 'rhinosinusitis' [All Fields] AND 'Homo sapiens'[porgn] AND 'gse' [Filter]. The study types were limited to 'profiling by array' and the following inclusion criteria were used: (1) The selected dataset must contain genome-wide mRNA transcriptome data; (2) Data must originate from CRSwNP nasal polyp tissue (NP) samples and normal control nasal mucosa tissue samples; (3) Both normalized and raw datasets were considered. According to these requirements, two CRSwNP datasets [GSE36830 (case: Normal ratio=6:6) and GSE72713 (case: Normal ratio=6:3)] were obtained.

Identification of differentially expressed mRNAs and lncRNAs in CRSwNP. We performed background correction for the raw data. Using metaMA package analysis (version 3.1.2; Guille mette Marot), P-values of differentially expressed genes were calculated by moderated t-tests (Limma version 3.30.13) for each dataset and combined by the inverse normal method. The adopted standard for determination of DEGs was P<0.05, and all datasets had the same direction of differential expression. Finally, the differentially expressed mRNAs and lncRNAs between case and normal control samples were identified (8,9).

Functional annotation. We performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) classification according to the GO categories of molecular function (MF), biological process (BP) and cellular component, to identify the enriched functions and pathways of DEGs, using the online software GeneCoDis 3 (genecodis.cnb.csic.es/analysis) (10). We set FDR<0.05 as the threshold of statistical significance. A total of 58 mRNAs were subjected to GO and KEGG enrichment analyses using the R language (GSEABase package), P-value <0.01 (P<0.01 is considered to indicate a statistically significant difference).

Protein-protein interaction (PPI) network construction in CRSwNP. To further research the biological functions of DEGs, we constructed a PPI network based on the top 100 DEGs in CRSwNP using Biological General Repository for Interaction Datasets (BioGRID; thebiogrid.org) and Cytoscape software version 3.5.0 (11,12). Based on the existing protein interaction data included in the BioGRID database, Cytoscape was used to search for common and regulatory mRNAs with opposite effects in case vs. normal samples (13). After removing non-differentially expressed genes, a protein interaction network was mapped. Such a network consists of nodes and edges. The nodes in the network represent the proteins and edges represent the interactions between them (14).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) confirmation. Based on the results of the integrated high-throughput transcriptome data analysis, five DEGs and one DElncRNA with higher degrees between case and normal control groups were identified and subsequently screened as candidate genes. The sequences of the primers used to detect the differentially expressed genes/lncRNA and the endogenous control were listed in Table I. Blood samples from patients with CRSwNP and healthy individuals from the First People's Hospital of Jining (Jining, China) were collected on Nov 24th, 2017. All individuals provided signed informed consent for use of their samples in this present study. The present study was approved by the Ethics Committee of the First People's Hospital of Jining [committee's reference no. 2016(012)]. RNA samples were used to verify the differential expression of candidate genes in disease and control groups using RT-qPCR (15). Blood samples were collected from 3 patients with CRSwNP (cases 1-3) and 3NCs and were frozen at -80˚C within 2 h. We performed RNA isolation using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) after thawing the frozen samples at room temperature. We generated cDNA from 1 μg extracted RNA using SuperScript® III Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.). qPCR reactions were performed in an ABI 7500 real-time PCR system with Power SYBR® Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). We analyzed relative gene expression using the $2^{-\Delta\Delta Cq}$ method (16). We selected the human 18srRNA as an endogenous control for mRNA expression analysis (17). The thermocycling conditions were as follows: 95˚C for 15 min, followed by 40 cycles of 95˚C for 10 sec, 55˚C for 30 sec and 72˚C for 32 sec, and then 95˚C for 15 sec, 60˚C for 60 sec and 95˚C for 15 sec.

Statistical analysis. The metaMA package in R version 3.1.2 (cran.r-project.org/web/packages/metaMA/index.html; Guille mette Marot) was used to combine data from multiple microarray datasets and obtain the individual P-values in the present study. DEGs in CRSwNP compared with normal control were identified with P<0.05. Values were presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

Differential expression analysis of genes in CRSwNP. The probes corresponding to multiple genes were removed and average values of expression were calculated for genes corresponding to multiple probes. Subsequently, lncRNAs included in the GSE72713 dataset were screened out to obtain 1,747 lncRNAs (1483 were newly identified and 264 were previously known). A total of 15,996 mRNAs were overlapping between the two datasets. Compared with the normal control group, 994 DEmRNAs in CRSwNP were identified with P<0.05, among which, 354 genes were upregulated and 640 genes were downregulated. The top 20 most significantly up- or downregulated genes were listed in Table II. The top 100 DEmRNAs were included in the cluster analysis. The heatmap was shown in Fig. 1.

Limma package was used for differential expression analysis. Using the standard P<0.05, a total of 265 differentially expressed lncRNAs were obtained, including 56 upregulated and 209 downregulated genes. The top 20 significant differences in expression of lncRNA are shown in Table III. The

Table I. Sequence of primers.

Name	Sequence (5'-3')	Size of product (bp)
GAPDH-F (endogenous control)	GGAGCGAGATCCCTCCAAAAT	197
GAPDH-R (endogenous control)	GGCTGTTGTCATACTTCTCATGG	
CCL18-F	AGCATCATGAAGGGCCTTGC	209
CCL18-R	TGCCGGCCTCTCTTGGTTAG	
CCL8-F	TGGAGAGCTACACAAGAATCACC	133
CCL8-R	TGGTCCAGATGCTTCATGGAA	
CUL4B-F	TTTACAACCCAGGGATTCGGC	154
CUL4B-R	GGATTCCTCAGCCATCTTCGC	
NEDD4L-F	CTGGGAAATGAGGATAGCGCC	191
NEDD4L-R	AAAACGTTCGGCCATCCAAGT	
GALNT7-F	GGTTCATCTTACGCAGTTTGCT	140
GALNT7-R	GGGCATGGGGTCATTGACA	
RP11-798M19.6-F	AAAGTTTTGGGAAGCTGGCAAG	192
RP11-798M19.6-R	GGAGAAAGCAATCAGGGCACA	

CCL, chemokine (C-C motif) ligand; CUL4B, cullin-4B; NEDD4L, neural precursor cell expressed developmentally downregulated gene 4-like; GALNT7, polypeptide N-acetylgalactosaminyltransferase 7; F, forward; R, reverse.

Table II. Top 20 differentially expressed mRNAs.

ID	Symbol	Combined.ES	P-value	FDR	Up/down
8564	KMO	3.53	5.66×10^{-8}	4.14×10^{-4}	Up
28959	TMEM176B	3.26	1.54×10^{-7}	4.64×10^{-4}	Up
3426	CFI	3.59	1.60×10^{-7}	4.64×10^{-4}	Up
2206	MS4A2	3.27	2.51×10^{-7}	5.75×10^{-4}	Up
23406	COTL1	3.25	4.93×10^{-7}	7.82×10^{-4}	Up
2207	FCER1G	3.06	5.38×10^{-7}	7.82×10^{-4}	Up
6355	CCL8	3.20	6.33×10^{-7}	8.44×10^{-4}	Up
85329	LGALS12	2.89	1.13×10^{-6}	1.32×10^{-3}	Up
6275	S100A4	2.89	1.15×10^{-6}	1.32×10^{-3}	Up
151258	SLC38A11	2.81	1.40×10^{-6}	1.49×10^{-3}	Up
342574	KRT27	-3.99	1.13×10^{-8}	1.81×10^{-4}	Down
140597	TCEAL2	-3.80	7.76×10^{-8}	4.14×10^{-4}	Down
372	ARCN1	-3.34	1.74×10^{-7}	4.64×10^{-4}	Down
54682	MANSC1	-3.22	3.36×10^{-7}	6.72×10^{-4}	Down
5523	PPP2R3A	-3.06	4.88×10^{-7}	7.82×10^{-4}	Down
64816	CYP3A43	-2.97	1.72×10^{-6}	1.63×10^{-3}	Down
138046	RALYL	-2.76	1.78×10^{-6}	1.63×10^{-3}	Down
6663	SOX10	-2.75	1.83×10^{-6}	1.63×10^{-3}	Down
56994	CHPT1	-2.73	2.19×10^{-6}	1.65×10^{-3}	Down
81621	KAZALD1	-2.96	2.23×10^{-6}	1.65×10^{-3}	Down

FDR, false discovery rate; CCL, chemokine (C-C motif) ligand.

differential expression of lncRNAs was presented using heat map in Fig. 2.

Functional annotation. As demonstrated in Fig. 3, several GO categories were enriched among the upregulated and downregulated DEmRNAs. 'Signal transduction' (including 81 genes, FDR=1.18×10^{-9}) and 'multicellular organismal development' (including 62 genes, FDR=1.55×10^{-6}) were the most significantly enriched BP; the top ranked cellular component (CC) GO categories were 'membrane' (including 304 genes, FDR=4.87×10^{56}) and 'cytoplasm' (including 305 genes, FDR=4.87×10^{56}); and 'protein

Table III. Top 20 differentially expressed long non-coding RNAs.

ID	Symbol	Log2FC	P-value	FDR	Up/down
XLOC_003006	-	6.63	$4.19x10^{-7}$	$1.83x10^{-4}$	Up
XLOC_016248	-	5.91	$4.63x10^{-6}$	$1.62x10^{-3}$	Up
ENSG00000253339.1	RP11-434I12.3	4.48	$8.09x10^{-4}$	$8.83x10^{-2}$	Up
XLOC_017561	-	2.46	$9.52x10^{-4}$	$9.23x10^{-2}$	Up
XLOC_011814	-	2.44	$2.05x10^{-3}$	$1.43x10^{-1}$	Up
XLOC_018649	-	3.99	$2.32x10^{-3}$	$1.43x10^{-1}$	Up
XLOC_015500	-	5.85	$2.39x10^{-3}$	$1.43x10^{-1}$	Up
ENSG00000248810.1	RP11-362F19.1	1.50	$2.82x10^{-3}$	$1.43x10^{-1}$	Up
XLOC_018891	-	1.87	$4.59x10^{-3}$	$1.78x10^{-1}$	Up
XLOC_000122	-	5.61	$5.16x10^{-3}$	$1.88x10^{-1}$	Up
XLOC_010540	-	-9.00	$1.79x10^{-9}$	$3.13x10^{-6}$	Down
XLOC_010305	-	-6.89	$7.56x10^{-9}$	$6.60x10^{-6}$	Down
ENSG00000250360.1	CTD-2089N3.1	-5.53	$8.81x10^{-8}$	$5.13x10^{-5}$	Down
XLOC_018529	-	-2.83	$6.32x10^{-5}$	$1.84x10^{-2}$	Down
XLOC_025155	-	-2.98	$7.93x10^{-5}$	$1.98x10^{-2}$	Down
XLOC_005882	-	-4.35	$1.39x10^{-4}$	$3.04x10^{-2}$	Down
XLOC_018024	-	-2.16	$2.09x10^{-4}$	$4.06x10^{-2}$	Down
XLOC_015712	-	-2.44	$3.15x10^{-4}$	$5.50x10^{-2}$	Down
XLOC_019396	-	-1.70	$3.72x10^{-4}$	$5.91x10^{-2}$	Down
ENSG00000181123.4	RP4-539M6.14	-1.83	$5.77x10^{-4}$	$7.80x10^{-2}$	Down

FDR, false discovery rate.

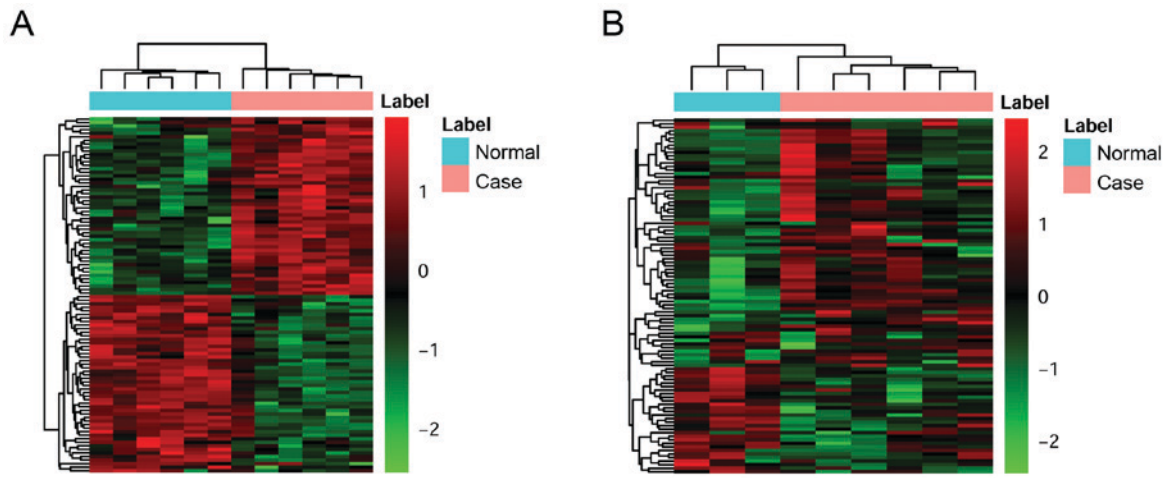

Figure 1. Heatmap images displaying genes that were significantly up- or downregulated (P<0.05) in chronic rhinosinusitis with nasal polyps when compared with normal controls. (A) GSE36830 and (B) GSE72713 datasets.

binding' (including 229 genes, FDR=$3.94x10^{16}$) was the most significantly enriched MF. The most significantly enriched BP of upregulated and downregulated DEGs was 'signal transduction' and the most significantly enriched MF was 'protein binding'. The most significantly enriched cellular component (CC) was 'membrane'. Several pathways were significantly enriched after the KEGG pathway enrichment analysis (FDR<0.05, Fig. 4), including 'cytokine-cytokine receptor interaction' (including 26 genes, FDR=$3.94x10^{16}$) and 'cell adhesion molecules' (CAMs) (including 17 genes, FDR=$1.28x10^{-5}$).

Ppi network of differentially expressed mRNAs. Based on the existing protein interaction data included in the BioGRID database, Cytoscape was used to search the top 100 upregulated and top 100 downregulated differentially expressed mRNAs. After removing the non-differentially expressed genes from analysis, the protein interaction network map was drawn (Fig. 5). A total of 144 nodes and 123 edges were identified. Among them, the genes with the highest degree were CUL4B (degree=20), COPS5 (degree=8), NEDD4L (degree=8), ITGA4 (degree=5), CSF1R (degree=5), EPRS (degree=5), HSP90AA1 (degree=5).

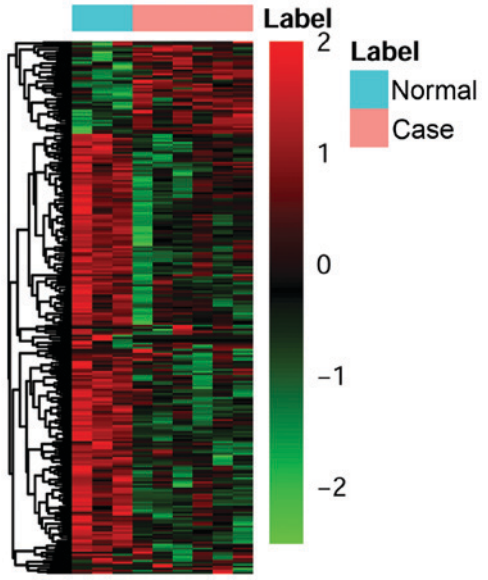

Figure 2. Heatmap image displaying long non-coding RNAs that were significantly up- or downregulated (P<0.05) in chronic rhinosinusitis with nasal polyps when compared with normal controls.

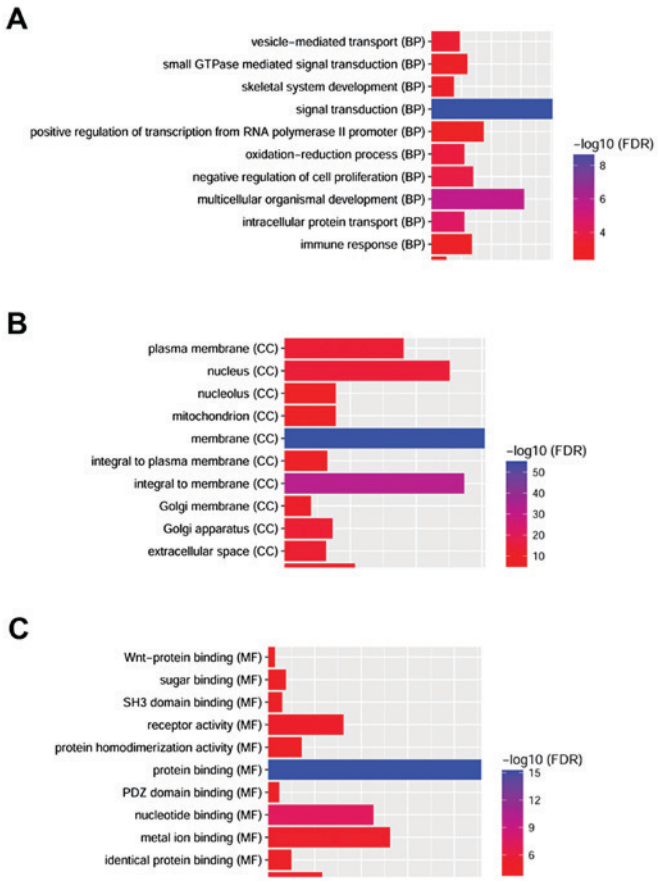

Figure 3. Gene Ontology pathway analyses of dysregulated protein-coding genes. (A) BP, (B) CC and (C) MF. BP, biological process; CC, cellular component; MF, molecular functions; FDR, false discovery rate.

DElncRNAs, and GO and KEGG analyses of mRNAs in CRSwNP. Twenty-six pairs formed by lncRNAs and their neighboring genes (including 23 lncRNAs and 25 mRNAs) were searched to identify differentially expressed genes

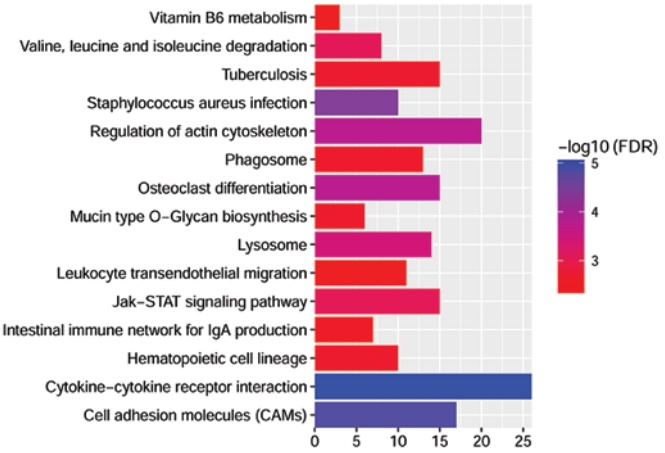

Figure 4. Kyoto Encyclopedia of Genes and Genomes pathway analyses of dysregulated protein-coding genes. FDR, false discovery rate; Jak, c-Jun N-terminal kinase; STAT, signal transducer and activator of transcription.

upstream and downstream of lncRNAs, as shown in Table IV. GO and KEGG enrichment analyses of the differentially expressed genes were performed using GeneCoDis3 (genecodis.cnb.csic.es/analysis). KEGG enrichment results were obtained using an FDR cut off of 0.05 and are shown in Table V.

RT-qPCR confirmation. To validate the results of the integrated analysis, the expression levels of five genes including (CCL18, GALNT7, CCL8, CUL4B, NEDD4L and the lncRNA RP11-798M19.6 were selected and verified by RT-qPCR. In Fig. 6, with the exception of CCL8, NEDD4L and GALNT7, the expression of the other 3 selected genes detected using RT-qPCR was consistent with the results of our integrated analysis.

Discussion

Although many studies have found that CRSwNP has various inflammatory phenotypes, drug sensitivities and prognoses, the detailed molecular mechanisms remain unclear (18). Since effective treatment is lacking, finding novel treatment strategies for CRS has long been a research objective. In our study, 994 DEmRNAs (354 upregulated and 640 downregulated) and 265 DElncRNAs (56 upregulated and 209 downregulated) were identified. Based on the results of integrated high-throughput transcriptome data analysis and a literature search, we selected five differentially expressed genes (CCL18, GALNT7, CCL8, CUL4B, NEDD4L) and one lncRNA (RP11-798M19.6) and the expression of these candidate genes was verified by RT-qPCR in disease and control groups. Expression profiles of CCL8, CUL4B and RP11-798M19.6 were consistent with the results of high-throughput sequencing. However, the RT-qPCR results for CCL18, NEDD4L and GALNT7 were inconsistent with our integrated analysis, which may be due to the small sample size.

GO and KEGG analyses were performed to identify the biological functions enriched in the DEmRNAs. Following the analysis of the dysregulated mRNAs, we found that two central

Table IV. Differentially expressed long non-coding RNAs and adjacent mRNAs.

	Long non-coding RNA			mRNA		
ID	Symbol	Start-100 kb	End+100 kb	Symbol	Start	End
ENSG00000204380.2	PKP4-AS1	159,435,168	159,691,330	PKP4	159,313,476	159,539,391
ENSG00000268001.1	CARD8-AS1	48,658,932	48,861,456	EMP3	48,824,766	48,833,810
ENSG00000272870.1	RP11-798M19.6	174,185,145	174,390,966	GALNT7	174,089,904	174,245,118
XLOC_001856	-	60,327,121	60,534,026	HOOK1	60,280,458	60,342,050
XLOC_002179	-	153,430,979	153,633,083	S100A2	153,533,584	153,540,366
XLOC_002179	-	153,430,979	153,633,083	S100A4	153,516,089	153,522,612
XLOC_003728	-	115,617,190	115,820,554	NHLRC2	115,614,420	115,676,953
XLOC_004001	-	34,532,537	34,734,403	EHF	34,642,640	34,682,604
XLOC_005757	-	108,928,967	109,130,444	SELPLG	109,016,053	109,027,735
XLOC_005757	-	108,928,967	109,130,444	CORO1C	109,038,885	109,125,372
XLOC_006494	-	118,455,546	118,673,787	PEBP1	118,573,663	118,583,389
XLOC_009628	-	20,654,577	20,875,447	ACSM3	20,621,565	20,808,903
XLOC_010280	-	34,304,118	34,520,635	CCL18	34,391,640	34,399,392
XLOC_010305	-	37,141,750	37,342,737	PLXDC1	37,219,556	37,310,647
XLOC_011271	-	72,625,994	72,827,011	CD300LF	72,690,450	72,709,117
XLOC_013363	-	58,726,344	58,938,866	RPS5	58,897,767	58,906,173
XLOC_013363	-	58,726,344	58,938,866	ZSCAN22	58,838,385	58,853,698
XLOC_014821	-	98,570,692	98,801,316	VWA3B	98,703,579	98,929,762
XLOC_016476	-	38,383,272	38,584,377	SOX10	38,366,693	38,383,429
XLOC_018137	-	128,110,058	128,311,374	GATA2	128,198,270	128,212,028
XLOC_018236	-	151,046,653	151,250,581	P2RY13	151,044,100	151,047,336
XLOC_019500	-	174,101,606	174,311,598	GALNT7	174,089,904	174,245,118
XLOC_019578	-	1,831,467	2,036,070	IRX4	1,877,527	1,887,350
XLOC_024517	-	134,039,147	134,241,605	WISP1	134,203,282	134,242,587
XLOC_025491	-	113,925,958	114,147,241	OR2K2	114,089,763	114,092,463
XLOC_026071	-	139,765,127	139,967,176	CDR1	139,864,570	139,867,036

characteristics of CRSwNP were significantly enriched. These included the inflammatory and immune response, such as 'cytokine-cytokine receptor interaction' (FDR=$3.94x10^{16}$), and regional microenvironment changes in nasal polyps, such as 'signal transduction' and 'protein binding'. We found that the most significantly enriched BP of upregulated and downregulated DEGs was 'signal transduction' and the most significantly enriched MF was 'protein binding'. The most significantly enriched cellular component (CC) was 'membrane'. Several pathways were demonstrated to be significantly enriched following the KEGG pathway enrichment analysis (FDR<0.05, Fig. 3), including 'cytokine-cytokine receptor interaction' (FDR=$3.94x10^{16}$), and 'cell adhesion molecules (CAMs)' (FDR=$1.28x10^{-5}$). KEGG analysis of DEmRNAs adjacent to DElncRNAs, revealed that DEmRNAs were enriched in 'mucin type O-Glycan biosynthesis (GALNT7)' and 'chemokine signaling pathway (CCL18)'. Chemokine receptors are potential targets for the treatment of many inflammatory diseases (19-21). Collectively these findings indicate that CCL18, closely related to chemokine signaling, is involved in CRSwNP.

CCL18, a 7.8 kDa protein composed of 69 amino acids, is known as pulmonary and activation-regulated chemokine (PARC) (22). Peterson *et al* (22) found that compared with normal control uncinate tissue (UT), CCL18 mRNA was significantly increased in NP (P<0.001) and UT (P<0.05) from patients with CRSwNP, but not in UT from patients with CRSsNP. And the author guessed that overproduction of CCL18 might contribute to the pathogenesis of CRSwNP (23). In our study, CCL18 was upregulated in patients with CRSwNP and the adjacent lncRNA XLOC_010280 was also upregulated. Wang *et al* (24) also found an important lncRNA, XLOC_010280, which was highly correlated with CCL18 in *cis* prediction of functions of lncRNAs. In our study, differentially expressed CCL18 was enriched in CCL18, and XLOC_010280 was determined as the adjacent lncRNA of CCL18. CCL18 and XLOC_010280 were both upregulated in patients with CRSwNP. We may conclude that lncRNA XLOC_010280 upregulates the mRNA expression of CCL18, and causes the inflammatory disease.

As part of the acetylgalactosaminyltransferase family, N-acetylgalactosaminyltransferase 7 (GALNT7) acts as a glycosyltransferase in protein O-GlcNAcylation. A previous study demonstrated that GGalNAc-transferase-7 (GALNT7) was involved in the regulation of cell proliferation and was related to tumorigenesis. miR-494 and GALNT7-small

Table V. Kyoto Encyclopedia of Genes and Genomes of differentially expressed mRNAs adjacent to differentially expressed long non-coding RNA.

ID	Term	FDR	Count	Symbols
00512	Mucin type O-Glycan biosynthesis	$3.59x10^{-10}$	1	GALNT7
00650	Butanoate metabolism	$3.59x10^{-10}$	1	ACSM3
05150	Staphylococcus aureus infection	$5.43x10^{-7}$	1	SELPLG
03010	Ribosome	$4.46x10^{-4}$	1	RPS5
04514	CAMs	$4.86x10^{-3}$	1	SELPLG
04062	Chemokine signaling pathway	$2.86x10^{-2}$	1	CCL18

ACSM3, acyl-coenzyme A synthetase medium chain family member 3; RPS5, ribosomal protein S5; SELPLG, selectin P ligand; GALNT7, polypeptide N-acetylgalactosaminyltransferase 7; CCL18, chemokine (C-C motif) ligand 18; FDR, false discovery rate; CAMs, cell adhesion molecules.

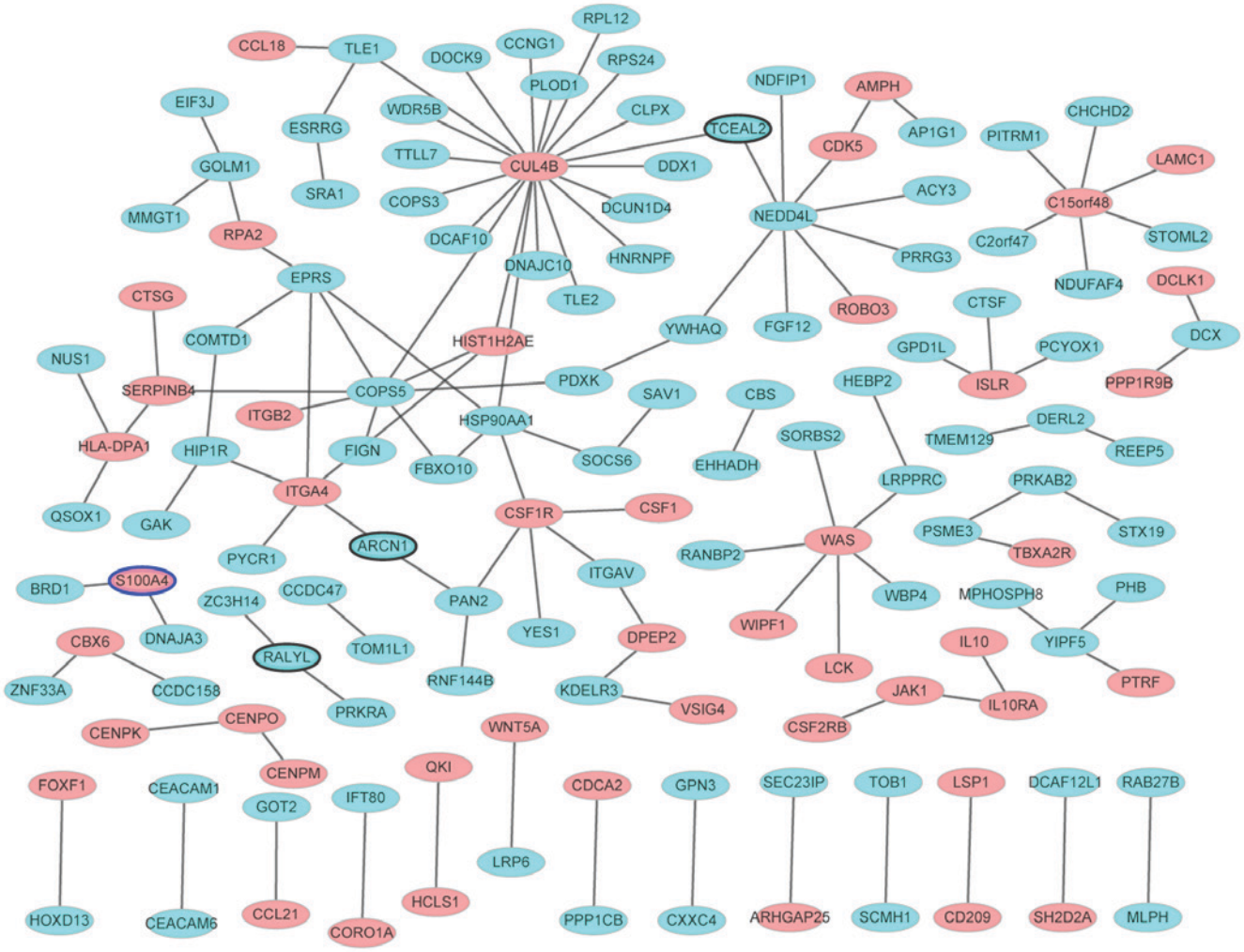

Figure 5. Chronic rhinosinusitis with nasal polyps-specific protein-protein interaction network. All points represent differentially expressed genes; green indicates downregulated, and red represents upregulated. The black border indicates the top 10 downregulated and the blue border indicates the top 10 upregulated.

interfering RNA (siRNA) have been shown to inhibit tumor growth in nude mice (25). In our study, GALNT7 was upregulated in patients with CRSwNP, and its adjacent lncRNA RP11-798M19.6 was downregulated in these patients. Besides, in the KEGG analysis, the mRNA of GALNT7 was enriched in 'mucin type O-Glycan biosynthesis', which may be associated with CRSwNP.

In conclusion, the present study provides further insight into the molecular aspects of CRSwNP, suggesting new molecular signatures and new targets for application as

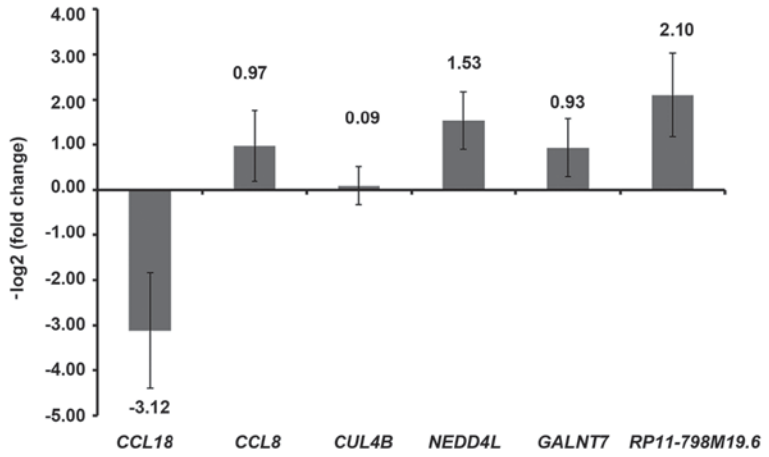

Figure 6. Reverse transcription-quantitative polymerase chain reaction results of differentially expressed mRNAs and differentially expressed long non-coding RNAs in chronic rhinosinusitis with nasal polyps. CCL, chemokine (C-C motif) ligand; CUL4B, cullin-4B; NEDD4L, neural precursor cell expressed developmentally downregulated gene 4-like; GALNT7, polypeptide N-acetylgalactosaminyltransferase 7.

specific biomarkers. In particular, our findings suggest that inflammation-related genes and cell proliferation-associated genes may be factors indicating poor prognosis of CRSwNP.

Acknowledgements

The authors would like to thank Beijing Medintell Bioinformatic Technology Co., Ltd. (Beijing, China) for assisting with the in high-throughput sequencing and data analysis.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

ML and YL supervised the research program, drafted the manuscript and revised it critically for important intellectual content. PG and JA participated in the acquisition, analysis and interpretation of data. CG contributed to the acquisition and analysis of data. FL and YL carried out the data analysis. ML and YL gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study has been approved by the Ethics Committee of The First People's Hospital of Jining.

Patient consent for publication

All individuals provided signed informed consent for the use of their samples in the present study.

Competing interests

The authors declare that they have no competing interests.

References

1. Beule A: Epidemiology of chronic rhinosinusitis, selected risk factors, comorbidities, and economic burden. GMS Curr Top Otorhinolaryngol Head Neck Surg 14: Doc11, 2015.
2. Ma Z, Shen Y, Zeng Q, Liu J, Yang L, Fu R and Hu G: miR-150-5p regulates EGR2 to promote the development of chronic rhinosinusitis via the DC-Th axis. Int Immunopharmacol 54: 188-197, 2017.
3. Bohman A, Juodakis J, Oscarsson M, Bacelis J, Bende M and Torinsson Naluai Å: A family-based genome-wide association study of chronic rhinosinusitis with nasal polyps implicates several genes in the disease pathogenesis. PLoS One 12: e0185244, 2017.
4. Chaaban MR, Walsh EM and Woodworth BA: Epidemiology and differential diagnosis of nasal polyps. Am J Rhinol Allergy 27: 473-478, 2013.
5. Lin H, Li Z, Lin D, Zheng C and Zhang W: Role of NLRP3 inflammasome in eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps. Inflammation 39: 2045-2052, 2016.
6. Kim JY, Kim DK, Yu MS, Cha MJ, Yu SL and Kang J: Role of epigenetics in the pathogenesis of chronic rhinosinusitis with nasal polyps. Mol Med Rep 17: 1219-1227, 2018.
7. Cava C, Bertoli G and Castiglioni I: Integrating genetics and epigenetics in breast cancer: Biological insights, experimental, computational methods and therapeutic potential. BMC Syst Biol 9: 62, 2015.
8. Saris CG, Horvath S, van Vught PW, van Es MA, Blauw HM, Fuller TF, Langfelder P, DeYoung J, Wokke JH, Veldink JH, *et al*: Weighted gene co-expression network analysis of the peripheral blood from Amyotrophic Lateral Sclerosis patients. BMC Genomics 10: 405, 2009.
9. Li WB, Zhou J, Xu L, Su XL, Liu Q and Pang H: Identification of genes associated with papillary thyroid carcinoma (PTC) for diagnosis by integrated analysis. Horm Metab Res 48: 226-231, 2016.
10. Huang QX, Cui JY, Ma H, Jia XM, Huang FL and Jiang LX: Screening of potential biomarkers for cholangiocarcinoma by integrated analysis of microarray data sets. Cancer Gene Ther 23: 48-53, 2016.
11. Zhang L, Zhang J, Yang G, Wu D, Jiang L, Wen Z and Li M: Investigating the concordance of gene ontology terms reveals the intra- and inter-platform reproducibility of enrichment analysis. BMC Bioinformatics 14: 143, 2013.
12. Yang L, Feng S and Yang Y: Identification of transcription factors (TFs) and targets involved in the cholangiocarcinoma (CCA) by integrated analysis. Cancer Gene Ther 23: 439-445, 2016.

13. Zhang H, Zhang C, Feng R, Zhang H, Gao M and Ye L: Investigating the microRNA-mRNA regulatory network in acute myeloid leukemia. Oncol Lett 14: 3981-3988, 2017.
14. Wang F, Wang R, Li Q, Qu X, Hao Y, Yang J, Zhao H, Wang Q, Li G, Zhang F, *et al*: A transcriptome profile in hepatocellular carcinomas based on integrated analysis of microarray studies. Diagn Pathol 12: 4, 2017.
15. Wang J, Qu D, An J, Yuan G and Liu Y: Integrated microarray analysis provided novel insights to the pathogenesis of glaucoma. Mol Med Rep 16: 8735-8746, 2017.
16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
17. Wu T, Wu HD, Xu ZX, Han F, Zhang BQ, Sun J and Hu SJ: Abnormal expression of long non-coding RNAs in myocardial infarction. Heart Vessels 32: 1253-1261, 2017.
18. Zhao B, Wang M, Xu J, Li M and Yu Y: Identification of pathogenic genes and upstream regulators in age-related macular degeneration. BMC Ophthalmol 17: 102, 2017.
19. Okada N, Nakayama T, Asaka D, Inoue N, Tsurumoto T, Takaishi S, Otori N, Kojima H, Matsuda A, Oboki K, *et al*: Distinct gene expression profiles and regulation networks of nasal polyps in eosinophilic and non-eosinophilic chronic rhinosinusitis. Int Forum Allergy Rhinol 8: 592-604, 2018.
20. Charo IF and Ransohoff RM: The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 354: 610-621, 2006.
21. Viola A and Luster AD: Chemokines and their receptors: Drug targets in immunity and inflammation. Annu Rev Pharmacol Toxicol 48: 171-197, 2008.
22. Peterson S, Poposki JA, Nagarkar DR, Chustz RT, Peters AT, Suh LA, Carter R, Norton J, Harris KE, Grammer LC, *et al*: Increased expression of CC chemokine ligand 18 in patients with chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol 129: 119-127.e1-9, 2012.
23. Hieshima K, Imai T, Baba M, Shoudai K, Ishizuka K, Nakagawa T, Tsuruta J, Takeya M, Sakaki Y, Takatsuki K, *et al*: A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 alpha/LD78 alpha and chemotactic for T lymphocytes, but not for monocytes. J Immunol 159: 1140-1149, 1997.
24. Wang W, Gao Z, Wang H, Li T, He W, Lv W and Zhang J: Transcriptome analysis reveals distinct gene expression profiles in eosinophilic and noneosinophilic chronic rhinosinusitis with nasal polyps. Sci Rep 6: 26604, 2016.
25. Nie GH, Luo L, Duan HF, Li XQ, Yin MJ, Li Z and Zhang W: GALNT7, a target of miR-494, participates in the oncogenesis of nasopharyngeal carcinoma. Tumour Biol 37: 4559-4567, 2016.