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Decreased expression of hsa-miR-142-3p and hsa-miR-155-5p in common variable immunodeficiency and involvement of their target genes and biological pathways

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Abstract
Common variable immunodeficiency (CVID) is the most common symptomatic and heterogeneous type of inborn errors of immunity (IEI). However, the pathogenesis process of this disease is often unknown. Epigenetic modifications may be involved in unresolved patients. MiR-142 and miR-155 were identified as immune system modulators and dysregulated in autoimmune and inflammatory diseases. We assessed hsa-miR-142-3p and hsa-miR-155-5p expression in a selected cohort of unresolved CVID cases and identified experimentally validated targets of these miRNAs. We constructed a protein-protein interaction (PPI) network from the common targets of two miRNAs and determined the hub genes. The hub genes' expression was investigated in GEO datasets. Gene ontology (GO) and pathway enrichment analysis were done for target genes. Hsa-miR-142-3p and hsa-miR-155-5p expression were significantly reduced in CVID patients. Evaluation of the PPI network demonstrated some hub genes in which pathogenic mutations have been reported in IEI, and other hub genes directly contribute to immune responses and the pathophysiology of IEI. Expression analysis of hub genes showed that they were significantly dysregulated in validating the CVID cohort. The pathway enrichment analysis indicated the involvement of the FOXO-mediated signaling pathway, TGFß receptor complex, and VEGFR2-mediated vascular permeability. Considering the dysregulation of hsa-miR-142-3p and hsa-miR-155-5p in CVID and the known role of their target genes in the immune system, their involvement in the pathogenesis of CVID can be suggested.

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Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic and heterogeneous type of inborn errors of immunity (IEI). It has a high level of genetic and clinical variability which is associated with challenges in diagnosis and determining optimal treatments for patients.[1] CVID is characterized by impaired B-cell activity that results in decreased immunoglobulin synthesis as hypogammaglobulinemia. CVID is also associated with recurrent infections, and autoimmune and lymphoproliferative manifestations.[2,3]

Monogenic mutations with Mendelian inheritance have been found in a minority of CVID patients, and it has been hypothesized that in the remaining cases CVID manifests as a complex disease.[4,5] It has been recently proposed that epigenetic modifications might have a role in CVID pathogenesis in genetically unresolved patients.[6] MicroRNAs (miRNAs) are a new class of epigenetic modulators that have essential functions in complex diseases.[7] MiRNAs participate in many biological processes and have an integral function in immunity, including regulation of the survival and function of B and T lymphocytes, and epigenetic control of gene expression in the B-cell's differentiation and maturation.[8] Since in most cases of CVID, the pathogenic mechanism and the molecular defect causing the disease are still unknown, it seems necessary to evaluate epigenetic factors such as miRNAs and their gene targets as an important factor involved in the disease. Several studies have reported changes in the DNA methylation pattern in CVID patients while only a few research looked into the profile of miRNA in CVID.[9-11]

In knock-out (KO) mouse models, deletion of miR-155 and miR-142 has been demonstrated to cause CVID-like phenotype, including immunodeficiency of adaptive immunity, hypogammaglobulinemia, polyclonal proliferation, lung disease, and intestinal inflammation. In humans, the dysregulation of hsa-miR-142 and hsa-miR-155 has not yet been identified in CVID patients, but numerous studies have shown that miR-155 and miR-142 are involved in autoimmune and neoplastic complications that can be comorbidites of CVID, including natural killer cell lymphoma (NKTCL), MALT lymphoma (gastric), gastric cancer, and immune thrombocytopenia.[12] According to recent studies, human miRNA sequences and mice model miRNA sequences are similar.[13] The purpose of this study was to evaluate the expression of hsa-miR-142-3p and hsa-miR-155-5p in a new cohort of CVID patients. Also, we investigated the gene targets of these two miRNAs and evaluated their function in the immune system and PIDs pathogenesis. Then, by examining the biological pathways related to these gene targets, we searched for potential mechanisms in the pathogenesis of CVID.

Method

Study population

Figure 1 illustrates the different steps in the study design. Twenty-two individuals with a diagnosis of CVID were alive and available in the registry of IEI patients in Isfahan University of Medical Science. All CVID patients were diagnosed based on the European Society for Immunodeficiencies (ESID). ESID diagnostic criteria for CVID comprise at least one of the following conditions: increased susceptibility to infection, OR autoimmune manifestations, OR granulomatous disease, OR unexplained polyclonal lymphoproliferation, OR affected family member with antibody deficiency. AND, marked decrease of IgG and IgA with or without low IgM levels, AND at least one of the following: poor antibody response to vaccines (and/or absent isohemagglutinins) OR low switched memory B-cells (<70% of age-related normal value). Diagnosis was established after the fourth year of life and secondary causes of hypogammaglobulinemia and profound T-cell deficiency were excluded.[14] Ten age- and sex-matched healthy participants with no prior history of immunological disorders were also included as control groups in our study. All methods were conducted following the Declaration of Helsinki. The Research Council and Ethical Committee of Isfahan University of Medical Sciences approved the study (IR.MUI.REC.1401.026). We acquired informed consent from every study participant or their legal guardians.

DNA extraction and whole exome sequencing (WES)

Genomic DNA was extracted via the salting out method, and DNA purity was evaluated by calculating the ratio of spectrophotometric absorbance at 260 and 280 nm. The Paired-End DNA Sample Prep Kit v1 is utilized to construct DNA libraries for paired-end sequencing. Exome capturing was conducted using the SureSelect Human All Exon kit (Agilent Technologies). The HiSeq 2500 (Illumina) was used to perform massively parallel sequencing, generating 100 base reads.[15] The quality of output FASTQ files was evaluated using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimmomatic was used to eliminate the base and sequence adapters with low base quality.[16] Sequence alignment was done to the GRCh37 Genome Reference Consortium Human Build 37 (GRCh37) utilizing the Burrow-Wheeler Aligner (BWA).[17] Aligned BAM files were sorted by Samtools based on chromosomal coordinates.[18] Duplicate reads were identified by Picard MarkDuplicates (http://broadinstitute.github.io/picard/). Variant calling was performed using the GATK HaplotypeCaller (v.4.5.0.0). We assessed the pathogenicity of all candidate variations based on the updated guideline of the American College of Medical Genetics and Genomics (ACMG).[19] We evaluated the allele frequency of variants in population databases such as the Genome Aggregation Database (gnomAD; Broad Institute) and Iranome (https://www.iranome.com) and excluded the variants with an allele frequency greater than 0.01%. We excluded patients exhibiting pathogenic mutation identified through WES in established IEI genes panel based on the International Union of Immunological Societies (IUIS) classification[20] or candidate IEI genes.[21]

Total RNA extraction and cDNA synthesis

We collected whole peripheral blood samples from our study population, and RNA extraction was conducted via

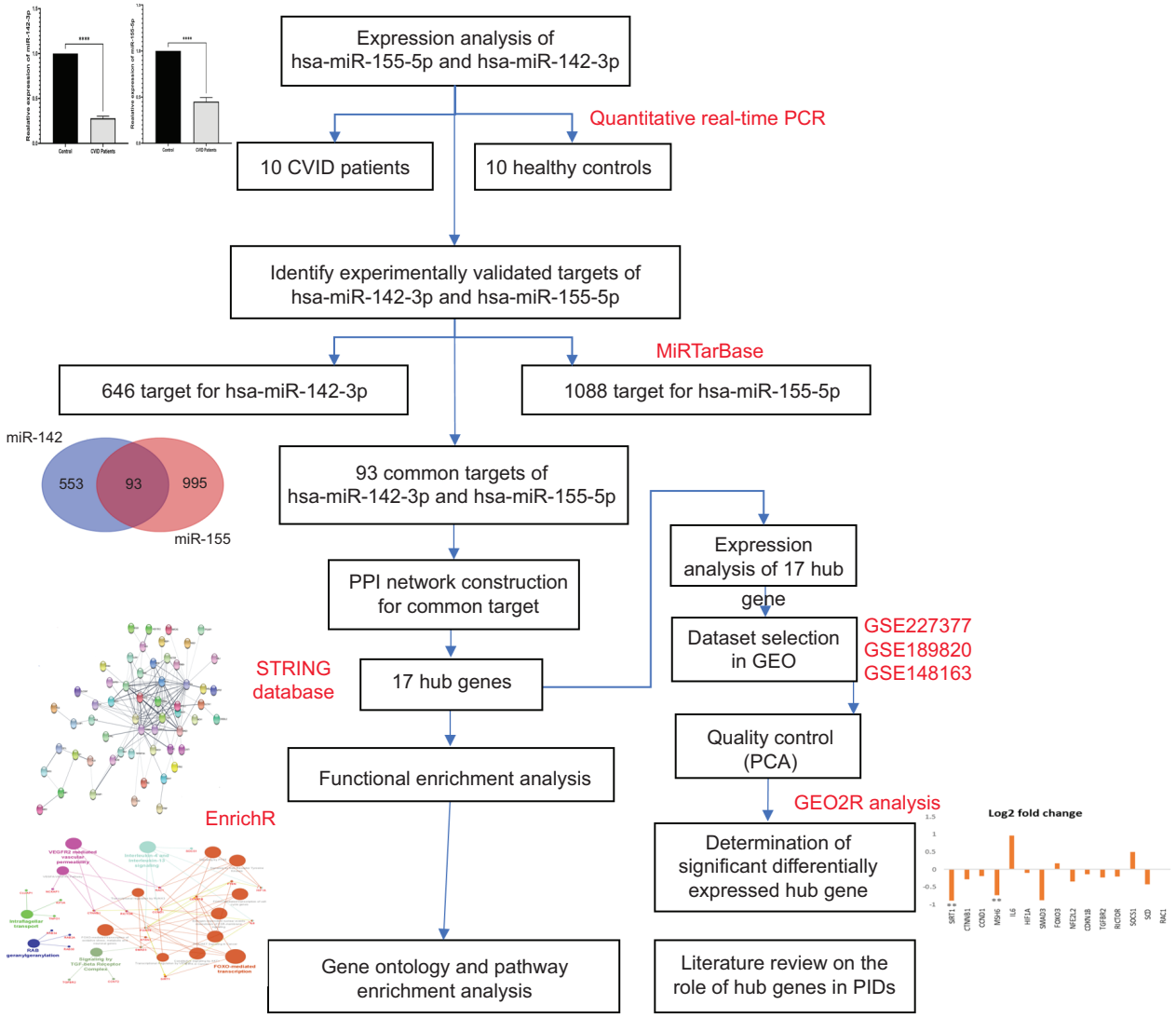


Figure 1 Flow chart showing each of the stages of the research design.

a blood RNA extraction kit (Parstous, Mashhad, Iran) following the instructions provided by the manufacturer. The concentration and purity of the total RNA were measured using a nanodrop spectrophotometer, and the RNA quality was assessed using 1% agarose gel electrophoresis. cDNA was made of total RNA with a 50 nt miRNA-specific stem-loop primer by easy cDNA synthesis kit (Parstous, Mashhad, Iran), and acquired cDNA was kept at −20°C.

Quantitative real-time PCR

In several studies, hsa-miR-423-5p was proposed as an optimal endogenous reference gene for quantification of circulating miRNAs;[22-25] so, hsa-miR-423-5p was applied as an endogenous reference gene in this study. miRBase database was used to retrieve the miRNA sequence (http://www.mirbase.org/). MiRNA Primer Design Tool was applied to design primer sequences (http://genomics.dote.hu/), and the NCBI BLAST tool was used to check the specificity of the primers. The forward primer sequences used in this experiment are as follows: 5′- GTTGGTGTAGTGTTTCCTACTT -3′ for hsa-miR-142-3p, 5′- GGGGTTAATGCTAATCGTGATA-3′ for hsa-miR-155-5p, and 5′-TTGTGAGGGGCAGAGAGC -3′ for hsa-miR-423-5p, and the universal reverse primer sequences was 5′- GTGCAGGGTCCGAGGT -3′. The primers were generated by Metabion Company (Germany). We utilized SYBR Green Master Mixes (Parstous, Mashhad, Iran) to conduct real-time PCR. The PCR mixture included 1 µL of cDNA, 1 µM of both forward and reverse primers, 5 µL of SYBR Green Master Mix, and 3 µL of sterilized water. PCR conditions were 94°C for 10 min for initial denaturation, followed by 40 cycles (15 s at 95°C, 1 min at 58°C, and 1 min at 72°C). The quantitative detection of fluorescence emission of SYBR Green bound to target DNA sequences was conducted using StepOnePlus™ Real-Time PCR system (Applied Biosystems, USA). After running, melting-curve analysis has been employed to confirm the specificity of the reactions and detect primer-dimer artifacts in real-time PCR reactions.

Normalization of data and statistical analysis

Relative quantification of the expressions of hsa-miR-142-3p and hsa-miR-155-5p was presented as the fold change = $2^{-\Delta\Delta Ct}$, and data were normalized to the expression of the hsa-miR-423-5p as an endogenous control. GraphPad Prism version 9.0.0 was applied for statistical analysis. Shapiro-Wilk test was applied to evaluate the normality of the variables. Subsequently, parametric and nonparametric tests were performed based on this assumption. Two-tailed Student's *t*-test was used for analysis and a P-value of less than 0.05 was deemed significant. The data were presented as mean ± standard error of the mean (SEM). The values were reported as the frequency (percentage and numbers) with mean ± standard deviation in normal distributed data and median (interquartile range, IQR) in nonnormal distributions.

Construction of protein-protein interactions (PPIs) and hub gene analysis

MiRTarBase (Release 7) database was applied to identify experimentally validated targets of hsa-miR-142-3p and hsa-miR-155-5p. Afterward, the common target genes of hsa-miR-142-3p and hsa-miR-155-5p were found using Venn diagram. The STRING database (version 10) was utilized for PPI network construction for common target genes of both miRNAs.[26] Cytoscape software was applied for the analysis and visualization of PPI networks. After analysis of the constructed PPI network, using the cytoHubba plugin, hub genes for the common target genes were determined based on their centrality metrics.[27,28]

Validation cohort and identification of deferentially expressed hub genes

To assess the expression changes of target genes of hsa-miR-155-5p and hsa-miR-142-3p in CVID disease, the expression of the hub genes that are determined in the PPI network of target genes was evaluated in gene expression datasets of CVID patients. For this purpose, gene expression profiling of CVID patients from RNA sequencing datasets accessible through Gene Expression Omnibus (GEO) has been applied. The GEO accessions of the datasets were GSE148163, GSE189820, and GSE227377. The quality of data was evaluated using principal component analysis (PCA) and hierarchical clustering. The GEO2R tool was applied for the determination of differentially expressed genes (DEGs). The identified hub genes were screened among the DEGs, and Log2 fold change expression measures for the deferentially expressed hub genes were identified.

GO and pathway enrichment analysis

GO enrichment analysis was conducted for target genes of hsa-miR-155-5p and hsa-miR-142-3p. Pathway enrichment analysis was done separately for target genes of hsa-miR-142-3p and hsa-miR-155-5p, and also for common targets of hsa-miR-142-3p and hsa-miR-155-5p. Target genes of hsa-miR-142-3p and hsa-miR-155-5p were uploaded to the EnrichR server and enriched Reactome pathways and the GO terms were extracted. The enrichment results were visualized by the Cytoscape software (version 3.8.2), together with the ClueGO and EnrichmentMap plugins (version 3.3). The EnrichmentMap is a plugin of Cytoscape that could help understanding the enrichment results.

Result

Patients

Among the 22 CVID cases, we excluded 12 patients exhibiting pathogenic mutation identified through WES in established IEI genes panel or candidate IEI genes. Ten unsolved CVID patients (five females and five males with a mean age of 5.5 years at onset were included for further evaluations. Ten healthy participants who had no prior history of immunological disorders were also included as control groups in our study. The mean age of the CVID patients at the time of the study was about 40.1 years, and all these patients had the same treatment with Intravenous Immunoglobulin (IVIg). The sampling time was before receiving IVIg, and the patients did not receive any immunomodulatory or immunosuppressive drugs at the time of sampling.

Among the ten unsolved CVID patients, four patients (40%) were the offspring of consanguineous parents. Table 1 illustrates the demographic and immunological characteristics of the examined patients. The most common clinical manifestations identified in our patients were recurrent infections (100%), and the respiratory tract infection was the most prevalent (80%). Two patients had non-Hodgkin Lymphoma and autoimmune manifestations were not observed in any of the patients. Some of the patients experienced otitis, sinusitis, recurrent diarrhea, pneumonia, bronchiectasis, allergy, asthma (30%), and lymphoproliferation (40%) (Table 2).

Table 1 Demographics and immunological characteristics of CVID patients.

Parameters	Total (N = 10)
Sex (M/F), N (%)	5/5 (50/50)
Consanguinity; N (%)	4 (40%)
Age at the study time, years Mean (SD)	40.1 (14.94)
Age of onset, years Mean (SD)	5.5 (2.00)
IgG*, mg/dL Mean (SD)	341.20 (144.04)
IgM*, mg/dL Mean (SD)	242.90 (277.25)
IgA*, mg/dL Mean (SD)	92.40 (134.38)
IgE*, IU/mL Mean (SD)	3.31 (4.25)
CD19 percentage Mean (SD)	9.33 (5.50)
CD20 percentage Median (Q1, Q3)	6 (6,17)
CD16 percentage Mean (SD)	11.7 (2.48)
CD56 percentageMean (SD)	6.71 (2.75)

Quantitative variables are reported as mean (SD) in normal and median (Q1, Q3) in nonnormal distributions. Qualitative variables are reported as numbers (N (percentages)).

Table 2 Clinical manifestations of CVID patients.

Parameters	Total (N = 10)
Recurrent infections	10 (100%)
Recurrent respiratory infections, N (%)	8 (80%)
Recurrent nonrespiratory infection, N (%)	3 (30%)
Otitis, N (%)	3 (30%)
Sinusitis, N (%)	3 (30%)
Pneumonia, N (%)	3 (30%)
Bronchiectasis, N (%)	3 (30%)
Allergy, N (%)	3 (30%)
Asthma, N (%)	1 (10%)
Splenomegaly/Hepatomegaly, N (%)	4 (40%)
Lymphadenopathy, N (%)	1 (10%)
Autoimmunity, N (%)	0 (0 %)
Arthritis, N (%)	1 (10%)
Recurrent diarrhea, N (%)	1 (10%)
Malignancy (Non-Hodgkin Lymphoma), N (%)	2 (20%)

Qualitative variables are reported as numbers (N (percentages)).

Measurement of hsa-miR-142-3p and hsa-miR-155-5p expression

We assessed the expression of hsa-miR-142-3p and hsa-miR-155-5p between 10 CVID patients and 10 controls. Our findings demonstrated that the expression of hsa-miR-142-3p and hsa-miR-155-5p was significantly decreased in CVID patients in comparison to healthy controls ($P < 0.05$, Figure 2). Also, the relative expression of each patient compared to the control group was measured separately by log2 fold change. For the hsa-miR-142-3p, the expression was decreased in all patients (P1-P10). For the hsa-miR-155-5p, the expression was decreased in all patients compared to the mean of expression in control groups, except for one patient (P3) (Figure 3).

PPI construction and hub gene identification

According to the MiRTarBase database, there are 646 experimentally validated targets for hsa-miR-142-3p and 1088 targets for hsa-miR-155-5p. A Venn diagram identified 93 common

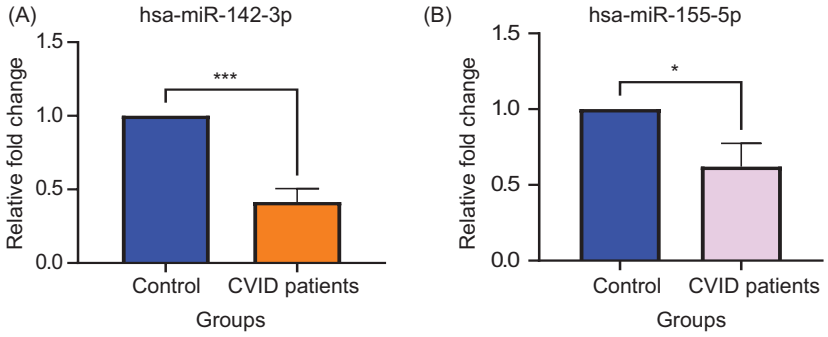


Figure 2 Relative fold change was calculated using by quantitative real-time PCR (qRT-PCR) analysis. Expression of hsa-miR-142-3p (A) and hsa-miR-155-5p (B) downregulated in blood samples of CVID patients in comparison to control subjects. The error bars indicate SEM. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, and ****: $P \leq 0.0001$).

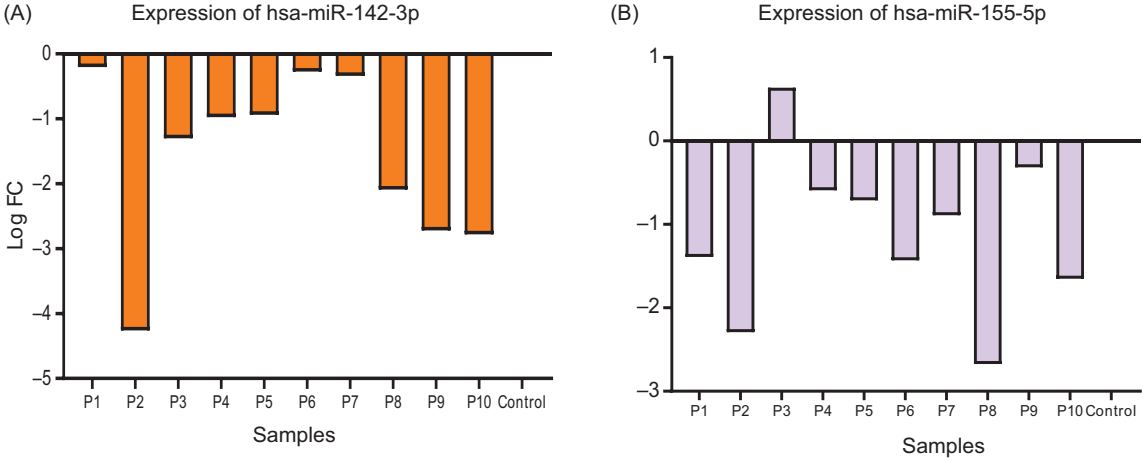
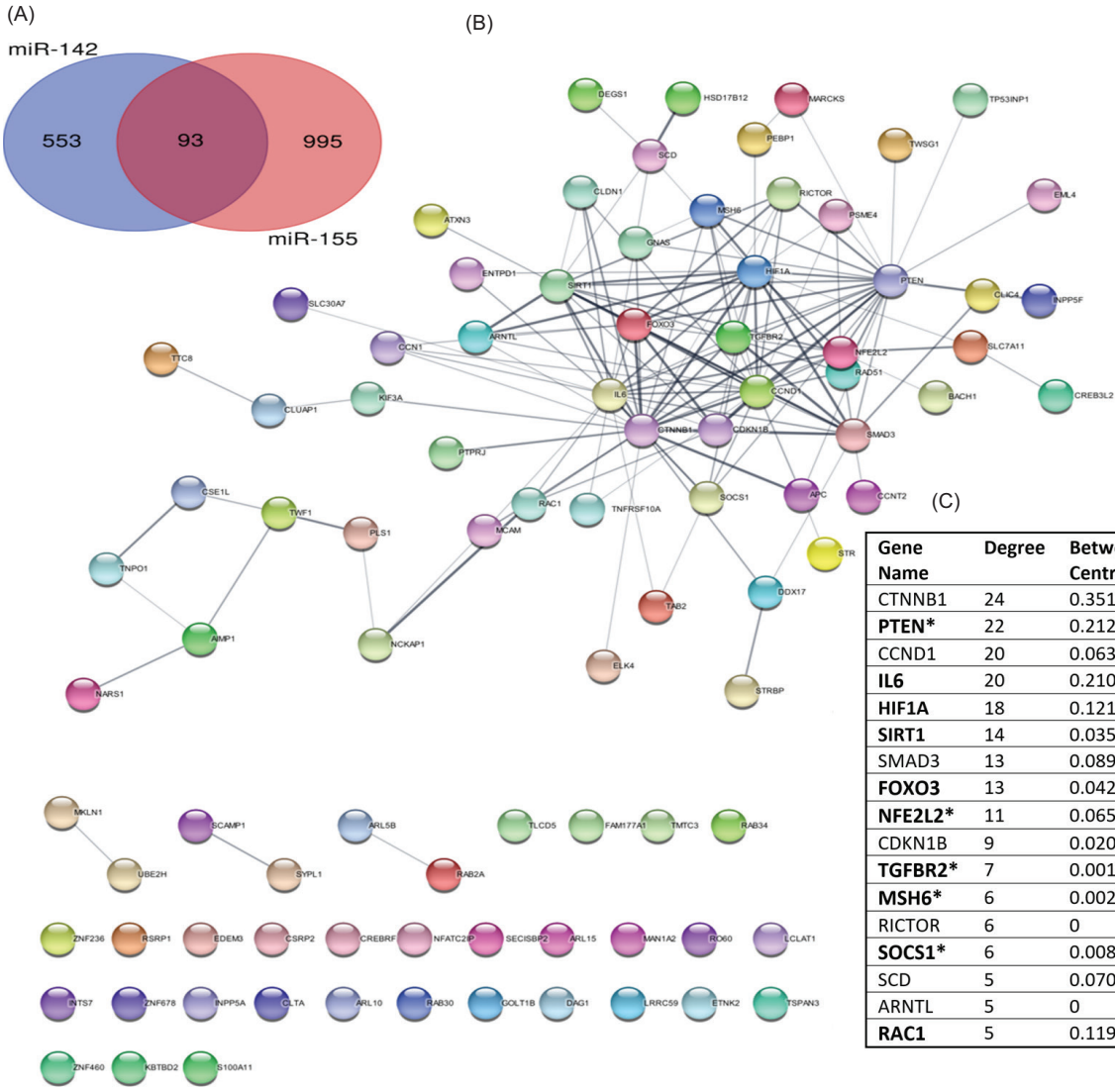


Figure 3 Log2 fold change (logFC) of hsa-miR-142-3p expression (A) and hsa-miR-155-5p (B) in each CVID patient 1-10 (P1-P10) compared with control. The error bars indicate SEM.



Gene Name	Degree	Betweenness Centrality	Closeness Centrality
CTNNB1	24	0.351752	0.513761
PTEN*	22	0.212147	0.46281
CCND1	20	0.0631	0.455285
IL6	20	0.210487	0.486957
HIF1A	18	0.121096	0.455285
SIRT1	14	0.035947	0.4375
SMAD3	13	0.089464	0.430769
FOXO3	13	0.042008	0.427481
NFE2L2*	11	0.065754	0.424242
CDKN1B	9	0.020045	0.434109
TGFBR2*	7	0.001869	0.402878
MSH6*	6	0.002956	0.370861
RICTOR	6	0	0.391608
SOCS1*	6	0.00803	0.394366
SCD	5	0.070779	0.356688
ARNTL	5	0	0.375839
RAC1	5	0.119663	0.4

Figure 4 Venn diagram of the common gene targets of hsa-miR-142-3p and hsa-miR-155-5p (A). The protein-protein interaction (B), and hub genes for the common targets of hsa-miR-142-3p and hsa-miR-155-5p (C). Network analysis results: Hub genes in which pathogenic mutations are known to cause inborn errors of immunity (IEI) are in bold and marked with an asterisk (*). Hub genes that are implicated in IEI pathogenesis or contribute to the immune and inflammatory responses are in bold (C).

target genes between hsa-miR-142-3p and hsa-miR-155-5p (Figure 4A). The PPI network has been drawn for the 93 common targets (Figure 4B) and the 17 hub genes in the PPI network have been identified, in such a way that the genes whose degrees are greater than 5 were determined as hub genes and their betweenness, and closeness scores were determined (Figure 4C). Then the role of hub genes in the immune system and pathogenesis of IEI, especially CVID, is investigated in the literature. It is interesting to note that among these 17 hub genes, there are 5 genes in which pathogenic mutations have been reported in IEI, including PTEN, NFE2L2, TGFBR2, MSH6, and SOCS1, and especially pathogenic mutations of PTEN, MSH6, and SOCS1 were reported in CVID patients.[20,29-33] Other hub genes, that is, RAC1 and IL6 genes are directly involved in the pathogenesis of IEI diseases.[34,35] FOXO3, HIF1A, and SIRT1 genes contribute to the immune and inflammatory responses.[36-38]

GO enrichment analysis

The top 10 significantly enriched GO terms separately for targets of hsa-miR-142-3p and hsa-miR-155-5p have been illustrated in Table 3. Some of the enriched GO terms were shared between the targets of hsa-miR-142-3p and hsa-miR-155-5p. Kinase binding and Kinase activity were the two main GO terms in the molecular function group for the target genes of hsa-miR-142-3p, and ubiquitin protein ligase binding and DNA binding were the two main molecular function-enriched terms for the target genes of hsa-miR-155-5p. Moreover, the results of the cellular component GO enrichment indicate that intracellular membrane-bounded organelle, nucleus, cell-substrate junction, and focal adhesion are commonly enriched GO terms in targets of hsa-miR-142-3p and hsa-miR-155-5p. Biological process GO terms for hsa-miR-142-3p targets and hsa-miR-155-5p targets were

Table 3 Top 10 significantly (adjusted p-value<0.05) enriched GO terms in hsa-miR-155-5p and hsa-miR-142-3p targets.

		Term	Overlap	Adjusted P-value	Odds Ratio
hsa-miR-142-3p	Biological process	Positive Regulation of Nucleic Acid-Templated Transcription (GO:1903508)	40/557	0.004006	2.429787
		Positive Regulation of DNA-templated Transcription (GO:0045893)	70/1243	0.004006	1.904085
		Regulation of DNA-templated Transcription (GO:0006355)	97/1922	0.004006	1.716385
		Positive Regulation of Transcription by RNA Polymerase II (GO:0045944)	55/938	0.008719	1.967332
		Negative Regulation of Cell Population Proliferation (GO:0008285)	29/379	0.00903	2.577928
		Regulation Of Transcription by RNA Polymerase II (GO:0006357)	97/2028	0.017741	1.61236
		Regulation of Protein Serine/Threonine Kinase Activity (GO:0071900)	13/112	0.024287	4.033847
		Negative Regulation of DNA-templated Transcription (GO:0045892)	56/1025	0.024287	1.819939
		Negative Regulation of Cell Migration (GO:0030336)	16/163	0.024287	3.351299
		Negative Regulation of Cell Motility (GO:2000146)	14/133	0.028031	3.61605
	Cellular component	Intracellular Membrane-Bounded Organelle (GO:0043231)	227/5175	8.18E-06	1.600923
		Nucleus (GO:0005634)	198/4487	3.57E-05	1.574088
		Focal Adhesion (GO:0005925)	32/387	9.17E-05	2.817643
		Cell-Substrate Junction (GO:0030055)	32/395	1.07E-04	2.754386
		Trans-Golgi Network (GO:0005802)	18/241	0.042024	2.483425
		Autophagosome (GO:0005776)	9/81	0.058624	3.717266
		Chromosome (GO:0005694)	13/164	0.091693	2.637568
		Recycling Endosome (GO:0055037)	12/159	0.147395	2.497465
		Golgi Cisterna (GO:0031985)	6/51	0.147395	4.06204
		COPI Vesicle Coat (GO:0030126)	3/12	0.147395	10.12611
	Molecular function	Kinase Activity (GO:0016301)	13/106	0.01827	4.295433
		Purine Ribonucleoside Triphosphate Binding (GO:0035639)	31/476	0.022456	2.163669
		Kinase Binding (GO:0019900)	30/460	0.022456	2.165078
		Protein Kinase Binding (GO:0019901)	32/511	0.022456	2.074607
		C3HC4-type RING Finger Domain Binding (GO:0055131)	3/5	0.022456	45.58399
		DNA-binding Transcription Factor Binding (GO:0140297)	21/282	0.022456	2.482554
		Nuclear Receptor Binding (GO:0016922)	12/115	0.022456	3.572506
		Transcription Regulatory Region Nucleic Acid Binding (GO:0001067)	18/224	0.022456	2.690756
		Protein Serine/Threonine Kinase Activator Activity (GO:0043539)	8/56	0.023079	5.092827
		Transforming Growth Factor Beta Binding (GO:0050431)	5/21	0.023658	9.519685
hsa-miR-155-5p	Biological process	Positive Regulation of DNA-templated Transcription (GO:0045893)	131/1243	2.47E-11	2.294511
		Regulation of Transcription by RNA Polymerase II (GO:0006357)	183/2028	1.15E-10	1.963994
		Positive Regulation of Transcription by RNA Polymerase II (GO:0045944)	104/938	2.92E-10	2.396017
		Negative Regulation of Programmed Cell Death (GO:0043069)	57/381	6.85E-10	3.310428
		Positive Regulation of miRNA Metabolic Process (GO:2000630)	19/49	1.26E-09	11.65814
		Negative Regulation of Apoptotic Process (GO:0043066)	65/482	1.39E-09	2.942265
		Positive Regulation of miRNA Transcription (GO:1902895)	17/43	9.29E-09	12.0149
		Regulation of DNA-templated Transcription (GO:0006355)	165/1922	3.59E-08	1.830927
		Regulation of Cell Population Proliferation (GO:0042127)	84/766	4.01E-08	2.336851
		Positive Regulation of Nucleic Acid-Templated Transcription (GO:1903508)	67/557	6.55E-08	2.576054

(*continues*)

Table 3 Continued.

	Term	Overlap	Adjusted P-value	Odds Ratio
Cellular component	Intracellular Membrane-Bounded Organelle (GO:0043231)	396/5175	5.54E-16	1.804137
	Nucleus (GO:0005634)	348/4487	1.88E-14	1.781882
	Cell-Substrate Junction (GO:0030055)	45/395	8.95E-05	2.38704
	Focal Adhesion (GO:0005925)	44/387	9.37E-05	2.380148
	Nuclear Lumen (GO:0031981)	70/780	4.02E-04	1.840947
	Cell-Cell Junction (GO:0005911)	35/299	4.02E-04	2.448328
	Secretory Granule Membrane (GO:0030667)	32/279	0.001147	2.387635
	Intracellular Nonmembrane-Bounded Organelle (GO:0043232)	95/1195	0.001147	1.61959
	RSC-type Complex (GO:0016586)	6/14	0.001535	13.64914
	Clathrin-Coated Vesicle (GO:0030136)	14/78	0.001539	3.99997
Molecular function	Cadherin Binding (GO:0045296)	52/319	1.63E-10	3.657509
	Ubiquitin-Like Protein Ligase Binding (GO:0044389)	43/289	1.91E-07	3.256895
	Ubiquitin Protein Ligase Binding (GO:0031625)	41/271	1.93E-07	3.317672
	DNA Binding (GO:0003677)	86/846	4.00E-07	2.142228
	Kinase Binding (GO:0019900)	55/460	1.24E-06	2.539178
	RNA Polymerase II-specific DNA Binding Transcription Factor Binding (GO:0061629)	32/228	3.91E-05	3.017111
	DNA-binding Transcription Factor Binding (GO:0140297)	35/282	1.85E-04	2.619217
	Protein Kinase Binding (GO:0019901)	52/511	2.82E-04	2.105709
	RNA Polymerase II Cis-Regulatory Region Sequence-Specific DNA Binding (GO:0000978)	94/1122	2.82E-04	1.719891
	Purine Ribonucleoside Triphosphate Binding (GO:0035639)	47/476	0.001491	2.029436

mainly related to the regulation of transcription. Also, regulation of proliferation, motility, and migration of the cells were other enriched GO terms for hsa-miR-142-3p targets and regulation of apoptotic process and miRNA metabolic process for hsa-miR-155-5p targets (Table 3).

Functional enrichment analysis

Functional enrichment analysis was done for hsa-miR-142-3p target genes and hsa-miR-155-5p target genes separately (Table 4) and also for common target genes of hsa-miR-142-3p and hsa-miR-155-5p (Figure 5). According to the Reactome pathway analysis, target genes of hsa-miR-142-3p were mostly enriched in some pathways including signal transduction, membrane trafficking, vesicle-mediated transport, and signaling by Rho GTPases (Table 4). Target genes of hsa-miR-155-5p were mostly enriched in signal transduction, immune system, and cytokine signaling in the immune system, by interleukins (ILs), IL-4, and IL-13 (Table 4). On the other hand, the significant enriched Reactome pathways for the 93 common targets of hsa-miR-142-3p and hsa-miR-155-5p suggest the involvement of biological pathways such as FoxO-mediated signaling pathway, TGF-ß receptor complex, VEGFR2-mediated vascular permeability, IL-4 and IL-13 signaling (Figure 5).

Expression of the hub genes in GEO datasets

Expression of 17 hub genes was evaluated in 3 datasets. Detailed information about the chosen GEO datasets is given in Table 5. In GSE227377, 14 hub genes are differentially expressed among them, MSH6 was significantly downregulated and FOXO3 was significantly upregulated (Figure 6A). In GSE189820, 14 hub genes are differentially expressed. Among them, FOXO3 was significantly downregulated and SOCS1 was significantly upregulated (Figure 6B). In GSE148163, 15 hub genes are differentially expressed and SIRT1 and MSH6 are significantly downregulated (Figure 6C).

Discussion

Epigenetic modification is a potential factor contributing to CVID disease. This study aims to investigate miRNA expression as an epigenetic component in a selected cohort of unresolved CVID cases. Recent research indicates that epigenetic abnormalities significantly contribute to dysregulating subsets of B-cells in CVID patients,[6] and the regulation of gene expression by miRNA plays a significant impact in modulating the activation and function of B-cells.[8] However, it is still unclear as to how particular miRNAs function in this process. The goal of this study was to investigate the expression of hsa-miR-142-3p and hsa-miR-155-5p, as an epigenetic modulator, in CVID cases, and we observed that hsa-miR-142-3p and hsa-miR-155-5p expression levels in blood samples of CVID cases were decreased compared with healthy controls.

MiR-142 is extensively expressed in hematopoietic tissues and is involved in lineage differentiation in these tissues.[39,40] Multiple studies have indicated the essential function of miR-142 in the immunological and

Table 4 Top 10 significantly (adjusted p-value<0.05) enriched Reactome pathways in hsa-miR-155-5p and hsa-miR-142-3p targets.

miRNA	Term	Overlap	Adjusted P-value	Odds Ratio
miR-142-3p	Membrane Trafficking R-HSA-199991	43/599	8.47E-04	2.435957
	Vesicle-Mediated Transport R-HSA-5653656	44/637	8.82E-04	2.336397
	Signaling By Rho GTPases, Miro GTPases, and RHOBTB3 R-HSA-9716542	43/660	0.002096	2.188004
	RNA Polymerase II Transcription R-HSA-73857	71/1312	0.002096	1.821833
	Signal Transduction R-HSA-162582	116/2465	0.002096	1.603148
	Signaling By Rho GTPases R-HSA-194315	42/644	0.002096	2.188458
	Intra-Golgi and Retrograde Golgi-to-ER Traffic R-HSA-6811442	18/181	0.003515	3.408222
	Generic Transcription Pathway R-HSA-212436	64/1190	0.004493	1.79929
	Gene Expression (Transcription) R-HSA-74160	74/1449	0.004883	1.710106
	Deubiquitination R-HSA-5688426	23/279	0.004493	2.781807
miR-155-5p	RNA Polymerase II Transcription R-HSA-73857	141/1312	7.58E-14	2.363277
	Gene Expression (Transcription) R-HSA-74160	151/1449	7.58E-14	2.29225
	Generic Transcription Pathway R-HSA-212436	126/1190	7.68E-12	2.300146
	Immune System R-HSA-168256	175/1943	1.03E-10	1.950693
	Signal Transduction R-HSA-162582	207/2465	3.53E-10	1.822022
	Signaling By Interleukins R-HSA-449147	61/453	2.69E-09	2.929329
	Cytokine Signaling in Immune System R-HSA-1280215	81/702	3.02E-09	2.47529
	Disease R-HSA-1643685	155/1736	3.02E-09	1.909347
	Interleukin-4 And Interleukin-13 Signaling R-HSA-6785807	26/107	5.15E-09	5.933084

inflammatory responses. Furthermore, the expression of miR-142-3p is increased in immune cells after the induction of inflammation.[41,42] MiR-142 is known as a crucial modulator of lymphopoiesis, and it is necessary for the normal functioning and development of dendritic cells (DCs), mast cells, and megakaryocytes in mice.[43-45] MiR-142 is also essential for the functioning of mature B- and T-cells in addition to normal lymphocyte development.[46] Studies on mice models with miR-142 deficiency show that miR-142 is essential for the homeostasis of lymphocytes, and miR-142 deletion in mice models leads to defects in marginal zone B (MZB) cells, B1 B-cells, and peripheral T-cell development.[47] Ablation of miR-142 in mice models causes defects in humoral immune response regardless of a large expansion of the B lymphocyte compartment, suggesting that miR-142 is essential for efficient B-cell function.[47] It has been observed that mice models with miR-142 deficiency fail to mount specific antibody responses after exposure to antigen and exhibit defective formation of germinal center (GC) B-cells and differentiation of plasma B-cells.[48] Evidence has also shown that miR-142-3p acts as a critical gene controlling the expression profile of mature B-cells, and miR-142-3p may control cell homeostasis through the B-cell activating factor receptor (BAFF-R).[47]

Another miRNA examined in this study is miR-155, whose key role has been identified in various studies as a critical modulator of immune response.[49,50] MiR-155 is one of the particular miRNAs for the hematopoietic cell system and is mainly expressed in the spleen and thymus, especially within both B and T lymphocytes. It controls various biological functions in the immune system by regulating the transcriptome in lymphocytes.[51,52] Deregulation of miR-155 has been identified in chronic inflammation, autoimmunity, neoplasms, and fibrosis. Also, miR-155 overexpression enhances the proliferation of pre-B-cells and promotes B-cell lymphoma.[49,53] MiR-155 is required for proper DCs activation and functions as a positive modulator of the inflammatory cytokines production and contributes to inhibiting the migration of neutrophils and enhancing degranulation.[54] MiR-155 is required for the maintenance, maturation, and effector response of NK cells and regulates the production of IFN-γ in response to various stimulations.[55] MiR-155 is required for optimal activity of CD8+ T lymphocytes to eliminate tumoral cells and pathogens and to develop memory response.[56] Expression of miR-155 is required for the maintenance of normal functioning of B-cells and differentiation to antibody-producing cells.[57] It has been found that miR-155 is a positive modulator of proliferation and differentiation of Tfh cells and promotes TH17 and Th1 differentiation.[58] Also, Th2 cells express miR-155 which is involved in immune response in these cells.[59]

It has been observed that miR-142-/- and miR-155-/- mice KO models demonstrate CVID phenotype with hypogammaglobulinemia, immunodeficiency, lung disease, inflammation of the intestine, and polyclonal lymphoproliferation, highlighting the critical contribution of miRNAs in the control of B-cell activity.[47,50] Several findings have shown that deregulation of miR-142 and miR-155 is implicated in most comorbidities found in CVID cases such as the autoimmune and neoplastic clinical complications.[12] MiR-142 and miR-155 have evolutionary conservation between mouse species and humans, which allows for clinical use and transfer of studies in mouse models to humans.[13,60] CVID phenotype in miR-142-/- and miR-155-/- mice KO models is in accordance with our results, and the expression of these two miRNAs is decreased in CVID patients.

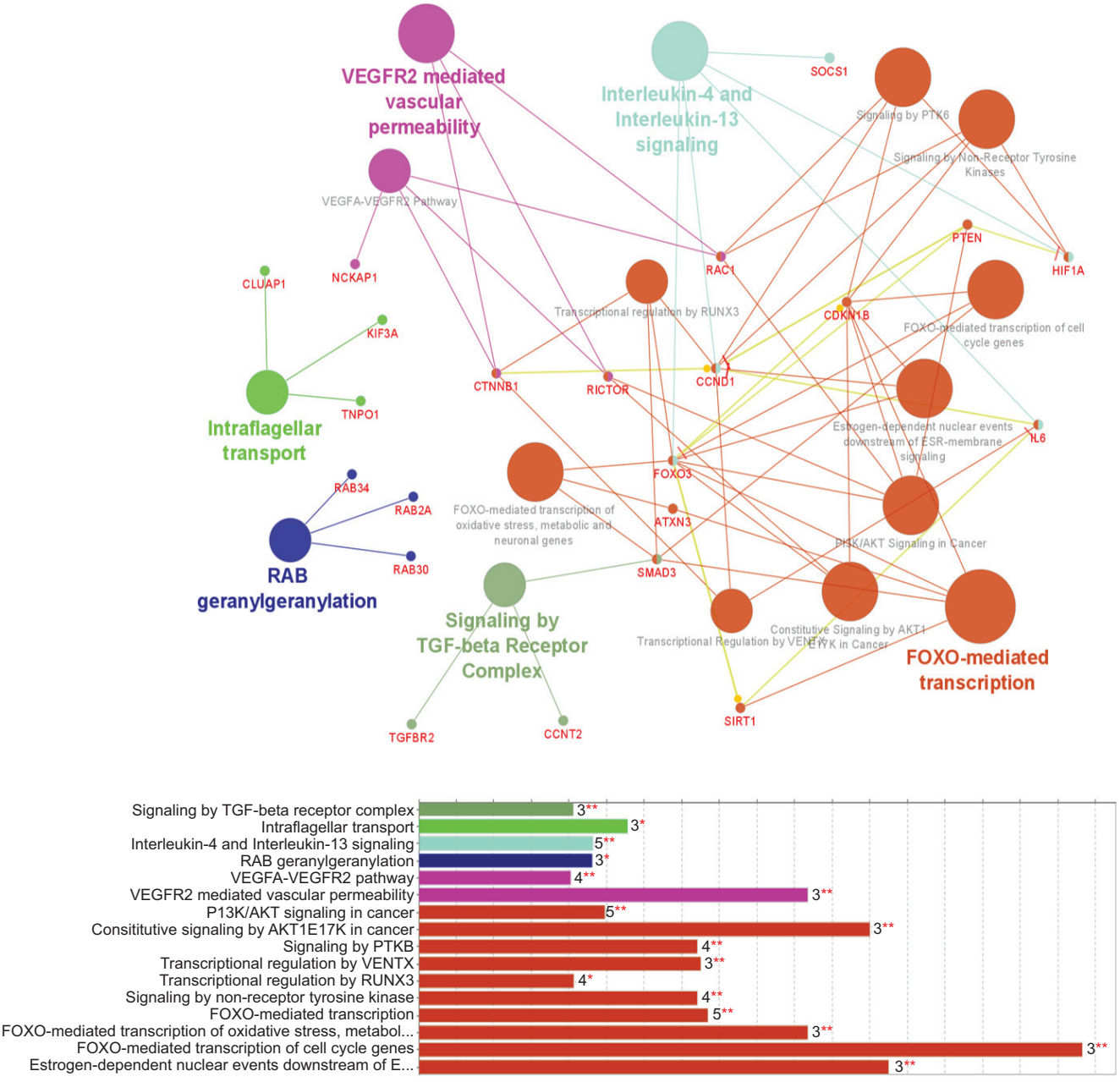


Figure 5 The significant enriched Reactome pathways and top genes for the common targets of hsa-miR-142-3p and hsa-miR-155-5p.

Table 5 Detailed information about the chosen GEO datasets.

GEO accession no.	Samples (CVID/Controls)	Platform	Ref.
GSE227377	n=15 (11/4)	Illumina NextSeq 500	(87)
GSE189820	n=21 (15/6)	Illumina NextSeq 500	(88)
GSE148163	n=7 (5/2)	NextSeq 550	(89)
Sum	31/12		

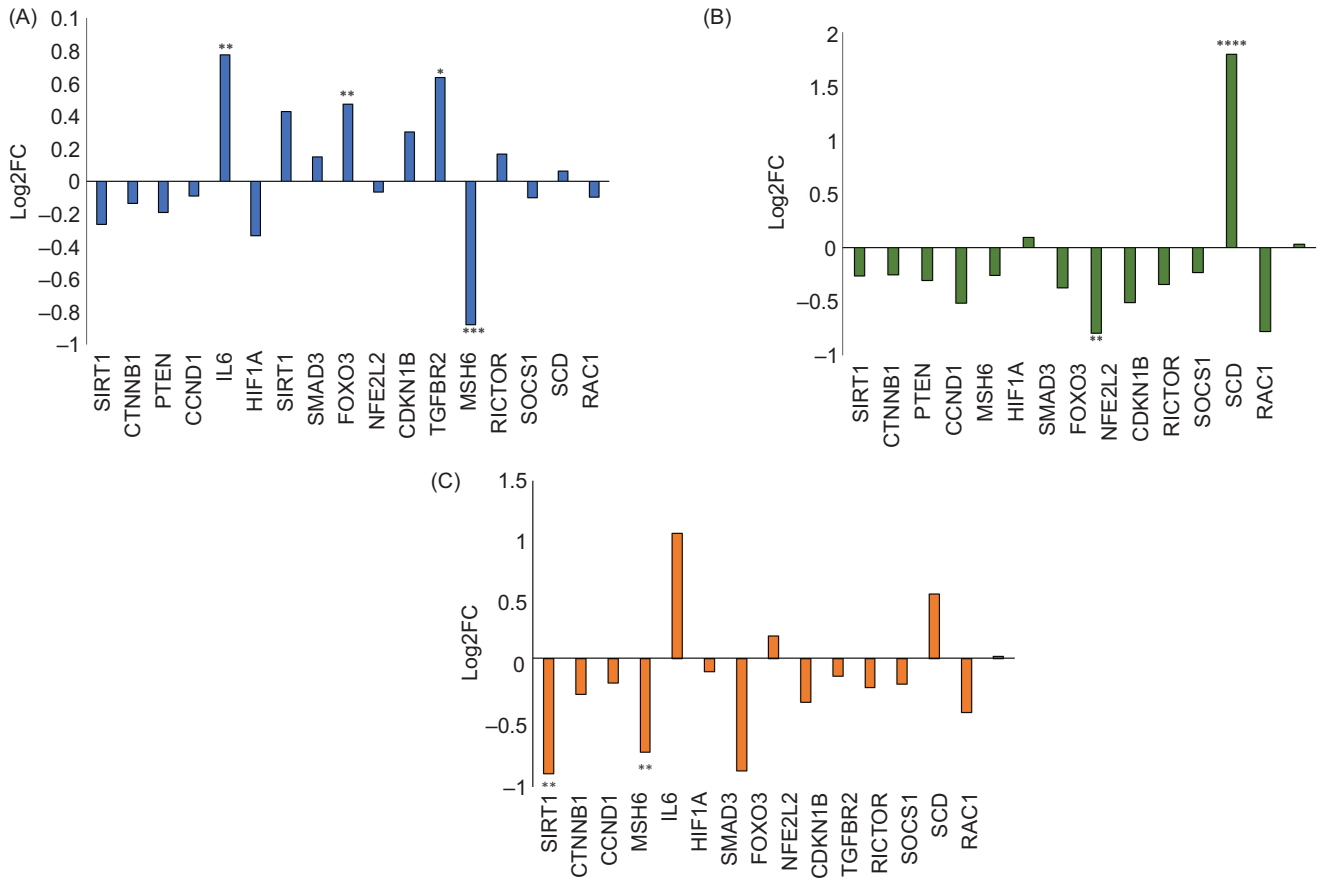


Figure 6 Analysis of differential gene expression of hub genes based on GEO database in CVID disease. (A) GSE227377, (B) GSE189820, and (C) GSE148163. Genes with significant differential gene expression have been marked with star signs (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, and ****: $P \leq 0.0001$).

Considering the activity of miR-155 and miR-142 in the immunity and function of B-cells, it can be concluded that the defect in miR-142 and miR-155 expression may cause disturbances in the function of B-cells. Also, according to the results of previous studies on the mice KO models and the decrease in the expression of these two miRNAs in CVID patients in this study, it can be suggested that the defect of miR-155 and miR-142 may be involved in the pathogenesis of CVID.

The evaluation of the clinical effect of miRNAs in CVID disease is at an initial step, and only limited studies have yet investigated the expression profile of miRNAs in CVID disease.[9-11] Although recent findings reveal the significance of varied expression of miRNAs and epigenetic regulation in defective differentiation of B-cells, further study is required to clarify the association of the expression of miRNAs with the development of B-cells and to evaluate whether these miRNAs might serve as novel potential biomarkers for CVID patient management and treatment.

Systems biology provides valuable strategies for identifying the underlying pathogenic mechanism of diseases and developing new therapeutic targets for the prevention and treatment of diseases.[21] In this study, the PPI network has been drawn for the common targets of hsa-miR-155 and hsa-miR-142, and the hub genes in PPI network have been identified. It is interesting to note that we identified five hub genes in which pathogenic mutations have been reported in IEI, including PTEN, NFE2L2, TGFBR2, MSH6, and SOCS1. Also, it has been reported that RAC1 and IL6 genes directly contribute to the pathogenesis of IEI diseases, and FOXO3, HIF1A, and SIRT1 genes contribute to the immune and inflammatory responses. Previous findings verify the significance of these hub genes in the immunity and IEI pathogenesis, which are mentioned below concerning each of these genes. PTEN is a tumor suppressor gene and is implicated in the homeostasis and development of B-cells.[61] PTEN deficiency impairs CSR and its pathogenic mutations identified in CVID cases.[29] NFE2L2 encodes a transcription factor that controls the expression of many genes involved in inflammatory and immunity responses.[62] NFE2L2 is crucial for controlling the antiviral and innate immune responses. NFE2L2 also inhibits the transcription of pro-inflammatory cytokines and the activation of IL6, which suppresses the inflammatory response of macrophages.[63] Autosomal dominant mutations of NFE2L2 were identified in patients presented with immunodeficiency, hypogammaglobulinemia, recurrent infections, and developmental delay.30 Another hub gene, TGFBR2, is a member of the TGF-ß receptor subfamily.[64] TGFß superfamily members act as crucial modulators of B-cell function at various stages of development in the bone marrow and in differentiation into plasma cells that secrete antibodies.[65]

Mutations of the TGFBR2 gene have been reported in IEI. Gain of function mutations in TGFBR1/2 results in hyper-IgE syndrome (HIES) and combined immunodeficiency (CID) with associated or syndromic features named Loeys-Dietz syndrome (LDS), and loss of function variant in TGFBR2 as a predisposing risk factor reported in generalized pustular psoriasis (GPP) and adult-onset immunodeficiency syndrome (AOID).[20,31] MSH6 as a DNA repair protein acts in somatic hypermutation (SHM) and class-switch recombination (CSR) by repairing double-strand breaks in DNA.[66] MSH6 deficiency is associated with defects in CSR and SHM processes and antibody production.[66] Studies have shown that genetic defects in the MSH6 gene contribute to IEI that are associated with defective production of switched isotypes immunoglobulin (IgG/IgA/IgE) such as CVID.[32] SOCS1 encodes a suppressor protein of cytokine signaling pathways and is a regulator of adaptive and innate immunity.[67] SOCS1 haploinsufficiency has been identified in the pleiotropic form of v with autosomal dominant inheritance and early onset autoimmunity.[33] Also, a heterozygous de novo variant in the SOCS1 gene has been known to be a pathogenic mutation in patients with CVID.[68] RAC1 and its homolog RAC2, the members of the family of Rho GTPases, are known to be involved in immune responses such as regulation of the homotypic adhesions of B lymphocytes, CSR, and the humoral-mediated response[69] Pathogenic mutations in some genes of the Rho GTPase family have been detected in IEI, such as the Rac2, Cdc42, and RhoH genes.[34] RAC2 mutations have been discovered in several patients with several forms of IEI, such as CVID, severe combined immunodeficiency (SCID), and defects of phagocytes.[70] Abnormal activation of Rac1 has been reported in IEI because of RhoGEF mutations that regulate the Rho GTPase family with guanine nucleotide exchange factor (GEF) activity.[34] According to the mutations found in members of the Rho GTPase family in IEI, especially the close homolog of RAC1, that is, RAC2, it seems necessary that pathogenic mutation in RAC1 should also be evaluated in IEI patients. IL6 encodes IL-6 with immunoregulatory effects produced by different immune cells.[71] Studies have shown the contribution of IL-6 in CVID, humoral immunodeficiency, and autoimmune disorders.[35] FOXO3, a member of the FOXO proteins family, is also identified as a significant hub gene in the PPI network of our study with a degree of 13. The FOXO proteins are a subgroup of the Forkhead transcription factors family that regulates various biological pathways and immune cell homeostasis, including B and T lymphocytes and other nonlymphoid lineages.[36] HIF1A is another hub gene that encodes hypoxia-inducible factor-1α (HIF-1α). The critical role of HIF-1α has been identified in the regulation of multiple aspects of the immunity system in DCs, neutrophils, macrophages, and B- and T-cells, including the differentiation, development, and function of different immune cells.[37] The implication of HIF is observed in multiple inflammatory diseases and according to its important role in the immune system, it may also be implicated in IEI pathogenesis. SIRT1 encodes a histone deacetylase that has anti-inflammatory activity and has been known to be an important modulator of innate and adaptive immunity.[38] SIRT1 deficiency can impair the differentiation of B-cells into plasma cells and enhance the secretion of pro-inflammatory cytokines of B-cells and autoantibody generation, which can be a possible cause of autoimmune disorders.[38,72,73] This information illustrates the important activity of hsa-miR-142 and hsa-miR-155 target genes in the immune system and IEI, which especially highlights the potential role of these two miRNAs in IEI and CVID pathogenesis.

In this study, the expression of 17 hub genes from the PPI network of target genes of hsa-miR-142-3p and hsa-miR-155-5p was investigated in three GEO datasets. In two datasets, the expression of MSH6 and FOXO3 was significantly differentially expressed and the expression of SOCS1 and SIRT1 was significantly differentially expressed in one of the datasets. According to the common perspective that miRNAs suppress the expression of their target genes, it is expected that with the downregulation of hsa-miR-142-3p and hsa-miR-155-5p, the expression of their target genes will increase. However, some studies have shown that with the dysregulation of miRNAs, the expression of their target genes can also be upregulated or downregulated.[74,75] Therefore, dysregulation (upregulation or downregulation) of target genes of hsa-miR-142-3p and hsa-miR-155-5p can be expected.

As mentioned in the results section, the significant enriched Reactome pathways for the common targets of hsa-miR-142-3p and hsa-miR-155-5p suggest the involvement of FoxO-mediated signaling pathway, TGF-ß receptor complex, VEGFR2-mediated vascular permeability, and IL-4 and IL-13 signaling. According to several studies mentioned below, it seems that the enriched pathway of common targets of the two miRNAs may have more important activity in the immune response and IEI pathogenesis. Considering that miR-142-3p and miR-155-5p have different biological activities in addition to regulating immune responses, the common gene targets of these two miRNAs may have a relatively more important role in immune system activities. FoxO signaling pathway has been significantly and repeatedly observed in the results of enrichment analysis. As mentioned earlier, FOXO3, a member of the FOXO proteins family, is also identified as a significant hub gene in the PPI network of our study with a degree of 13. Other critical members of the FOXO proteins family involved in the immune system include FoxO1, which is particularly critical for the differentiation, isotype switching, survival, and proliferation of B-cells, and it also has a critical function in GC development and the depletion of FoxO1 results in reduced SHM and isotype switching, leading to defective antibody production.[76] Foxp3 is another member of the FOXO family, and its mutation has been linked to immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX).[20] Recent findings have demonstrated that Foxp3 contributes to the development of CVID.[77] It has been shown that the expression of Foxp3 exerts a suppressive impact on Treg cells and hinders the B- and T-cells proliferation and activation. Also, a decline in the frequency of CD4+CD25highFoxp3+ regulatory T-cells and the level of FOXP3 expression has been reported in the peripheral blood of CVID patients with an autoimmune disorder, which may indicate the involvement of Treg lymphocytes in the pathogenesis of CVID.[77] Additional study is required to completely comprehend the activity of Treg lymphocytes and Foxp3 in the pathogenesis of CVID, but it appears that a reduction in Treg cell number and Foxp3 expression may indirectly promote CVID development.

In our finding, TGF-ß receptor complex has been observed as a significant pathway in the results of enrichment analysis, and TGFBR2 is recognized as a significant hub gene in the PPI network of our study. TGF-ß signaling is necessary for regulating different biological functions in the immune responses.[64] TGF-ß is an immunosuppressive mediator and its defect or in its receptors (TGFBR1/2) is associated with inflammatory and immunodeficiency disease, as before mentioned.[20,31] Differential gene expression analysis has shown that the TGFB1 gene is one of the genes that are dysregulated in CVID patients.[6] According to the reported evidence of the activity of the TGF-ß superfamily in immunity and its involvement in inflammatory and immunodeficiency diseases, the involvement of TGF-ß signaling in the pathogenic process of CVID is hypothesized, and it seems that the examination of pathogenic mutations in the genes implicated in the TGF-ß signaling pathway in CVID diseases is necessary. VEGF/VEGFR signaling is another biological pathway in our result of enrichment analysis that can be considered for further investigation as a potential mechanism in CVID pathogenesis. VEGF/VEGFR signaling modulates innate and adaptive immunity, and recent research in cancer has shown that VEGF has immunosuppressive activities in addition to proangiogenic properties.[78,79] Different types of VEGF receptors are expressed in the immune cells and regulate the activity of these cells.[80] VEGF inhibits CD3+ T-cell proliferation and reduces CD4+ and CD8+ T-cell numbers by inducing a decrease in hematopoietic progenitor cells.[79] Evidence has shown that the VEGF suppresses inflammation in bacterial infection by affecting macrophages and mediates the migration and infiltration of macrophages.[80] Other evidence has shown that VEGF inhibits the proangiogenic and immunologic function of macrophages in a rodent model of glioblastoma.[81] VEGF can also affect the function and maturation of DCs and can suppress DC differentiation.[82] Studies have reported that blockades of VEGF-C and VEGF-A can induce the activation of CD8+ and CD4+ cells, enhance the antigen-presenting properties of DCs, augment macrophage-mediated cytotoxicity, and activate the complement cascade.[83] The reported evidence indicates the effect of VEGF/VEGFR signaling on the immune system, but more studies are required to completely identify the mechanisms of its interaction and its possible involvement in the development of immunodeficiency diseases.

One of the limitations of this study was that we could not examine a larger number of patients. Although this problem is observed in other miRNA studies in CVID and less than 12 patients were evaluated in these studies[9-11], the problem of small sample size is one of the limitations of studying rare diseases. On the other hand, we examined the expression of hub genes only in the datasets, and for further studies, we suggest that the expression of hub genes be considered in CVID. Another limitation of our research is related to the variability in lymphocyte composition observed in whole blood samples from patients with CVID. CVID is marked by a dysregulation of lymphocytes, which may result in modified gene and miRNA expression profiles due to variations in the proportions of different lymphocyte subsets in the blood.[84] This variability can complicate the interpretation of miRNA expression data, as the changes detected may not exclusively indicate the biological processes linked to CVID but could also be influenced by the composition of lymphocyte populations. We suggest that future research focuses on validating our results by isolating specific lymphocyte subsets, such as naive and memory B-cells, and analyzing the expression of hsa-miR-142-3p and hsa-miR-155-5p at the single-cell level, so that researchers can elucidate the intricate regulatory mechanisms and provide a more targeted interpretation of miRNA expression changes. However, analyzing miRNAs at the single-cell level presents various challenges due to their small size, the relatively low abundance of miRNAs compared to other RNA types, technical variability, and cell-to-cell variation in miRNA expression levels in single-cell analysis techniques.[85-89]

Conclusion

In conclusion, the critical function of miR-142 and miR-155 as modulators in the immune system has been proven in many studies and their dysregulation has been observed in several diseases such as autoimmune and inflammatory diseases. Dysregulation of miR-142 and miR-155 in CVID mice models and their known function in immunity suggest the potential implication of these miRNAs in the pathogenesis of CVID. This study is the first evidence regarding the dysregulation of miR-142 and miR-155 in human CVID diseases. The literature review about the hub genes in PPI network of common targets of hsa-miR-142-3p and hsa-miR-155-5p showed that PTEN, NFE2L2, TGFBR2, MSH6, SOCS1, RAC, IL6, FOXO3, HIF1A and, SIRT1 are involved in the IEI pathogenesis or immune and inflammatory responses, which can support the activity of these miRNAs in the pathogenesis of CVID. We also suggest that these hub genes can be considered candidate genes in monogenic cases of CVID diseases and investigated for pathogenic mutations. On the other hand, evidence shows that some enriched biological pathways, including FoxO-mediated signaling pathway, TGF-ß receptor complex, VEGFR2-mediated vascular permeability, and IL-4 and IL-13 signaling, are implicated in the immune response, and the role of some components of these pathways in the pathogenesis of IEI has been revealed in previous studies. So, these pathways can be considered as potential pathways in the pathogenicity of CVID for more investigation in future studies.

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Authors Contribution

All authors contributed equally to this article.

Conflict of Interest

The authors declare no competing interests.

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Data Availability

The FASTQ files generated from WES are accessible and can be supplied upon request. The datasets analyzed in this study are available in the Gene Expression Omnibus repository (GEO, https://www.ncbi.nlm.nih.gov/geo/) by accession number GSE148163, GSE189820, and GSE227377.

Ethics Approval

This study was approved by the Research Council and Ethical Committee of Isfahan University of Medical Sciences.

Consent to Participate

Informed consent was obtained from each participant included in the study.

References

1. Szczawinska-Poplonyk A, Schwartzmann E, Bukowska-Olech E, Biernat M, Gattner S, Korobacz T, et al. The pediatric common variable immunodeficiency-from genetics to therapy: A review. Eur J Pediatr. 2022:1-13. https://doi.org/10.1007/s00431-021-04287-6
2. Ramirez NJ, Posadas-Cantera S, Caballero-Oteyza A, Camacho-Ordonez N, Grimbacher B. There is no gene for CVID-novel monogenetic causes for primary antibody deficiency. Curr Opin Immunol. 2021;72:176-85. https://doi.org/10.1016/j.coi.2021.05.010
3. Tallmadge RL, Shen L, Tseng CT, Miller SC, Barry J, Felippe MJB. Bone marrow transcriptome and epigenome profiles of equine common variable immunodeficiency patients unveil block of B lymphocyte differentiation. Clin Immunol. 2015;160(2):261-76. https://doi.org/10.1016/j.clim.2015.05.005
4. de Valles-Ibáñez G, Esteve-Sole A, Piquer M, González-Navarro EA, Hernandez-Rodriguez J, Laayouni H, et al. Evaluating the genetics of common variable immunodeficiency: Monogenetic model and beyond. Front immunol. 2018;9:636. https://doi.org/10.3389/fimmu.2018.00636
5. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova J-L, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. Front immunol. 2016;7:220. https://doi.org/10.3389/fimmu.2016.00220
6. Rodríguez-Ubreva J, Arutyunyan A, Bonder MJ, Del Pino-Molina L, Clark SJ, de la Calle-Fabregat C, et al. Single-cell Atlas of common variable immunodeficiency shows germinal center-associated epigenetic dysregulation in B-cell responses. Nat Commun. 2022;13(1):1779. https://doi.org/10.1038/s41467-022-29450-x
7. Gareev I, Ramirez ME, Goncharov E, Ivliev D, Shumadalova A, Ilyasova T, et al. MiRNAs and lncRNAs in the regulation of innate immune signaling. Non-coding RNA Research. 2023. https://doi.org/10.1016/j.ncrna.2023.07.002
8. Rae W. Indications to epigenetic dysfunction in the pathogenesis of common variable immunodeficiency. Arch Immunol Ther Exp. 2017;65(2):101-10. https://doi.org/10.1007/s00005-016-0414-x
9. Babaha F, Yazdani R, Shahkarami S, Esfahani ZH, Abolhahassani H, Sadr M, et al. Evaluation of miR-210 expression in common variable immunodeficiency: patients with unsolved genetic defect. Allergol Immunopathol. 2021;49(2):84-93. https://doi.org/10.15586/aei.v49i2.39
10. De Felice B, Nigro E, Polito R, Rossi FW, Pecoraro A, Spadaro G, et al. Differently expressed microRNA in response to the first Ig replacement therapy in common variable immunodeficiency patients. Sci Rep. 2020;10(1):21482. https://doi.org/10.1038/s41598-020-77100-3
11. Esfahani ZH, Yazdani R, Shahkarami S, Babaha F, Abolhassani H, Sadr M, et al. Evaluation of microRNA-125b-5p and transcription factors BLIMP1 and IRF4 expression in unsolved common variable immunodeficiency patients. Iran J Allergy Asthma Immunol. 2021;20(6):700-10. https://doi.org/10.18502/ijaai.v20i6.8021
12. Amato G, Vita F, Quattrocchi P, Minciullo PL, Pioggia G, Gangemi S. Involvement of miR-142 and miR-155 in non-infectious complications of CVID. Mol. 2020;25(20):4760. https://doi.org/10.3390/molecules25204760
13. Gottmann P, Ouni M, Zellner L, Jähnert M, Rittig K, Walther D, et al. Polymorphisms in miRNA binding sites involved in metabolic diseases in mice and humans. Sci Rep. 2020;10(1):7202. https://doi.org/10.1038/s41598-020-64326-4
14. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract. 2019;7(6):1763-70. https://doi.org/10.1016/j.jaip.2019.02.004
15. Abolhassani H, Aghamohammadi A, Fang M, Rezaei N, Jiang C, Liu X, et al. Clinical implications of systematic phenotyping and exome sequencing in patients with primary antibody deficiency. Genet Med. 2019;21(1):243-51. https://doi.org/10.1038/s41436-018-0012-x
16. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for illumina sequence data. Bioinformatics. 2014;30(15):2114-20. https://doi.org/10.1093/bioinformatics/btu170
17. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. bioinformatics. 2009;25(14):1754-60. https://doi.org/10.1093/bioinformatics/btp324
18. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. bioinformatics. 2009;25(16):2078-9. https://doi.org/10.1093/bioinformatics/btp352
19. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-23. https://doi.org/10.1038/gim.2015.30
20. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. Human inborn errors of immunity: 2022 update on the classification from the international union of immunological societies expert committee. J Clin Immunol. 2022;42(7):1473-507. https://doi.org/10.1007/s10875-022-01289-3
21. Hojjati F, Roointan A, Gholaminejad A, Eshraghi Y, Gheisari Y. Identification of key genes and biological regulatory mechanisms in diabetic nephropathy: Meta-analysis of gene expression datasets. Nefrologia. 2023;43(5):575-86. https://doi.org/10.1016/j.nefro.2022.06.003

22. Wong L, Tsang YS, Kenny R, Lyburn M, McMahon LP. MiR-423-5p as optimal endogenous control for quantification of circulating microRNAs in patients With CKD. Kidney Int Rep. 2023;8(10):2150–2. https://doi.org/10.1016/j.ekir.2023.07.018
23. Wang S, Tao R, Ming T, Wang M, Liu J, He G, et al. Expression profile analysis and stability evaluation of 18 small RNAs in the Chinese Han population. Electrophoresis. 2020;41(23):2021–8. https://doi.org/10.1002/elps.202000058
24. Liu X, Zhang L, Cheng K, Wang X, Ren G, Xie P. Identification of suitable plasma-based reference genes for miRNAome analysis of major depressive disorder. J Affect Disord. 2014;163:133–9. https://doi.org/10.1016/j.jad.2013.12.035
25. Guo Y, Zhou X, Gao F, Wang M, Yang Q, Li X, et al. MiR-423-5p is a novel endogenous control for the quantification of circulating miRNAs in human esophageal squamous cell carcinoma. Heliyon. 2023;9(4). https://doi.org/10.1016/j.heliyon.2023.e14515
26. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res. 2010;39(suppl_1):D561–D8. https://doi.org/10.1093/nar/gkq973
27. Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y. CytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8 Suppl 4(Suppl 4): https://doi.org/10.1186/1752-0509-8-S4-S11
28. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303
29. Driessen GJ, IJspeert H, Wentink M, Yntema HG, van Hagen PM, van Strien A, et al. Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency. J Allergy Clin Immunol. 2016;138(6):1744–7. e5. https://doi.org/10.1016/j.jaci.2016.07.010
30. Redmond MT, Scherzer R, Prince BT. Novel genetic discoveries in primary immunodeficiency disorders. Clin Rev Allergy Immunol. 2022;63(1):55–74. https://doi.org/10.1007/s12016-021-08881-2
31. Kantaputra P, Daroontum T, Chuamanochan M, Chaowattanapanit S, Intachai W, Olsen B, et al. Loss of function TGFBR2 variant as a contributing factor in generalized pustular psoriasis and adult-onset immunodeficiency. Genes. 2022;14(1):103. https://doi.org/10.3390/genes14010103
32. Offer SM, Pan-Hammarström Q, Hammarström L, Harris RS. Unique DNA repair gene variations and potential associations with the primary antibody deficiency syndromes IgAD and CVID. PLoS One. 2010;5(8):e12260. https://doi.org/10.1371/journal.pone.0012260
33. Körholz J, Gabrielyan A, Sowerby JM, Boschann F, Chen L-S, Paul D, et al. One gene, many facets: Multiple immune pathway dysregulation in SOCS1 haploinsufficiency. Front immunol. 2021;12:680334. https://doi.org/10.3389/fimmu.2021.680334
34. Hashim IF, Mokhtar AMA. Small Rho GTPases and their associated RhoGEFs mutations promote immunological defects in primary immunodeficiencies. Int J Biochem Cell Biol. 2021;137:106034. https://doi.org/10.1016/j.biocel.2021.106034
35. Varzaneh FN, Keller B, Unger S, Aghamohammadi A, Warnatz K, Rezaei N. Cytokines in common variable immunodeficiency as signs of immune dysregulation and potential therapeutic targets-A review of the current knowledge. J Clin Immunol. 2014;34:524–43. https://doi.org/10.1007/s10875-014-0053-0
36. Peng S. Foxo in the immune system. Oncogene. 2008;27(16):2337–44. https://doi.org/10.1038/onc.2008.26
37. McGettrick AF, O'Neill LA. The role of HIF in immunity and inflammation. Cell Metab. 2020;32(4):524–36. https://doi.org/10.1016/j.cmet.2020.08.002
38. Kong S, McBurney MW, Fang D. Sirtuin 1 in immune regulation and autoimmunity. Immunol Cell Biol. 2012;90(1):6–13. https://doi.org/10.1038/icb.2011.102
39. Chen C-Z, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004;303(5654):83–6. https://doi.org/10.1126/science.1091903
40. Shrestha A, Mukhametshina RT, Taghizadeh S, Vásquez-Pacheco E, Cabrera-Fuentes H, Rizvanov A, et al. MicroRNA-142 is a multifaceted regulator in organogenesis, homeostasis, and disease. Dev Dyn. 2017;246(4):285–90. https://doi.org/10.1002/dvdy.24477
41. Fordham JB, Naqvi AR, Nares S. Regulation of miR-24, miR-30b, and miR-142-3p during macrophage and dendritic cell differentiation potentiates innate immunity. J Leukoc Biol. 2015;98(2):195–207. https://doi.org/10.1189/jlb.1A1014-519RR
42. Mandolesi G, De Vito F, Musella A, Gentile A, Bullitta S, Fresegna D, et al. miR-142-3p is a key regulator of IL-1ß-dependent synaptopathy in neuroinflammation. J Neurosci. 2017;37(3):546–61. https://doi.org/10.1523/JNEUROSCI.0851-16.2016
43. Chapnik E, Rivkin N, Mildner A, Beck G, Pasvolsky R, Metzl-Raz E, et al. miR-142 orchestrates a network of actin cytoskeleton regulators during megakaryopoiesis. Elife. 2014;3:e01964. https://doi.org/10.7554/eLife.01964
44. Mildner A, Chapnik E, Manor O, Yona S, Kim K-W, Aychek T, et al. Mononuclear phagocyte miRNome analysis identifies miR-142 as critical regulator of murine dendritic cell homeostasis. Blood. 2013;121(6):1016–27. https://doi.org/10.1182/blood-2012-07-445999
45. Yamada Y, Kosaka K, Miyazawa T, Kurata-Miura K, Yoshida T. miR-142-3p enhances FcεRI-mediated degranulation in mast cells. Biochem Biophys Res Commun. 2014;443(3):980–6. https://doi.org/10.1016/j.bbrc.2013.12.078
46. Talebi F, Ghorbani S, Chan WF, Boghozian R, Masoumi F, Ghasemi S, et al. MicroRNA-142 regulates inflammation and T cell differentiation in an animal model of multiple sclerosis. J Neuroinflammation. 2017;14:1–14. https://doi.org/10.1186/s12974-017-0832-7
47. Kramer NJ, Wang W-L, Reyes EY, Kumar B, Chen C-C, Ramakrishna C, et al. Altered lymphopoiesis and immunodeficiency in miR-142 null mice. Blood, Blood, Am J Hematol Jun 11;125(24):3720-30. doi: 10.1182/blood-2014-10-603951. Epub 2015 Apr 30
48. Graham NM, Wang W-L, Reyes E, Boldin MP. The role of microRNA-142 in B cell activation and effector functions. J Immunol. 2022;208(1_Supplement):168.02-.02. https://doi.org/10.4049/jimmunol.208.Supp.168.02
49. Alivernini S, Gremese E, McSharry C, Tolusso B, Ferraccioli G, McInnes IB, et al. MicroRNA-155-at the critical interface of innate and adaptive immunity in arthritis. Front immunol. 2018;8:1932. https://doi.org/10.3389/fimmu.2017.01932
50. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316(5824):608–11. https://doi.org/10.1126/science.1139253
51. Seddiki N, Brezar V, Ruffin N, Lévy Y, Swaminathan S. Role of mi R-155 in the regulation of lymphocyte immune function and disease. Immunol. 2014;142(1):32–8. https://doi.org/10.1111/imm.12227
52. Testa U, Pelosi E, Castelli G, Labbaye C. miR-146 and miR-155: Two key modulators of immune response and tumor development. Non-Coding RNA. 2017;3(3):22. https://doi.org/10.3390/ncrna3030022
53. Due H, Svendsen P, Bødker JS, Schmitz A, Bøgsted M, Johnsen HE, et al. miR-155 as a biomarker in B-cell

malignancies. Biomed Res Int. 2016;2016. https://doi.org/10.1155/2016/9513037
54. Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: An important role in inflammation response. J Immunol Res. 2022;2022. https://doi.org/10.1155/2022/7437281
55. Trotta R, Chen L, Ciarlariello D, Josyula S, Mao C, Costinean S, et al. miR-155 regulates IFN-γ production in natural killer cells. Blood. 2012;119(15):3478–85. https://doi.org/10.1182/blood-2011-12-398099
56. Dudda JC, Salaun B, Ji Y, Palmer DC, Monnot GC, Merck E, et al. MicroRNA-155 is required for effector CD8+ T cell responses to virus infection and cancer. Immunity. 2013;38(4):742–53. https://doi.org/10.1016/j.immuni.2012.12.006
57. Calame K. MicroRNA-155 function in B cells. Immunity. 2007;27(6):825–7. https://doi.org/10.1016/j.immuni.2007.11.010
58. Zhang J, Cheng Y, Cui W, Li M, Li B, Guo L. MicroRNA-155 modulates Th1 and Th17 cell differentiation and is associated with multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol. 2014;266(1-2):56–63. https://doi.org/10.1016/j.jneuroim.2013.09.019
59. Kim HJ, Park SO, Byeon HW, Eo JC, Choi JY, Tanveer M, et al. T cell-intrinsic miR-155 is required for Th2 and Th17-biased responses in acute and chronic airway inflammation by targeting several different transcription factors. Immunol. 2022;166(3):357–79. https://doi.org/10.1111/imm.13477
60. Sharma S. Immunomodulation: A definitive role of microRNA-142. Dev Comp Immunol. 2017;77:150–6. https://doi.org/10.1016/j.dci.2017.08.001
61. Suzuki A, Kaisho T, Ohishi M, Tsukio-Yamaguchi M, Tsubata T, Koni PA, et al. Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. J Exp Med. 2003;197(5):657–67. https://doi.org/10.1084/jem.20021101
62. He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond. Int J Mol Sci. 2020;21(13):4777. https://doi.org/10.3390/ijms21134777
63. Olagnier D, Brandtoft A, Gunderstofte C, Villadsen N, Krapp C, Thielke A, et al. Nrf2 negatively regulates STING indicating a link between antiviral sensing and metabolic reprogramming. Nat. Commun. 9, 3506. 2018. https://doi.org/10.1038/s41467-018-05861-7
64. Johnston CJ, Smyth DJ, Dresser DW, Maizels RM. TGF-ß in tolerance, development and regulation of immunity. Cell Immunol. 2016;299:14–22. https://doi.org/10.1016/j.cellimm.2015.10.006
65. Tamayo E, Alvarez P, Merino R. TGFß superfamily members as regulators of B cell development and function-implications for autoimmunity. Int J Mol Sci. 2018;19(12):3928. https://doi.org/10.3390/ijms19123928
66. Gardès P, Forveille M, Alyanakian M-A, Aucouturier P, Ilencikova D, Leroux D, et al. Human MSH6 deficiency is associated with impaired antibody maturation. J Immunol. 2012;188(4):2023–9. https://doi.org/10.4049/jimmunol.1102984
67. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol. 2007;7(6):454–65. https://doi.org/10.1038/nri2093
68. Thaventhiran JE, Lango Allen H, Burren OS, Rae W, Greene D, Staples E, et al. Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature. 2020;583(7814):90–5. https://doi.org/10.1038/s41586-020-2265-1
69. Gerasimčik N, He M, Dahlberg CI, Kuznetsov NV, Severinson E, Westerberg LS. The small Rho GTPases Rac1 and Rac2 are important for T-cell independent antigen responses and for suppressing switching to IgG2b in mice. Front immunol. 2017;8:1264. https://doi.org/10.3389/fimmu.2017.01264
70. Accetta D, Syverson G, Bonacci B, Reddy S, Bengtson C, Surfus J, et al. Human phagocyte defect caused by a Rac2 mutation detected by means of neonatal screening for T-cell lymphopenia. J Allergy Clin Immunol. 2011;127(2):535–8. e2. https://doi.org/10.1016/j.jaci.2010.10.013
71. Aliyu M, Zohora FT, Anka AU, Ali K, Maleknia S, Saffarioun M, et al. Interleukin-6 cytokine: An overview of the immune regulation, immune dysregulation, and therapeutic approach. Int Immunopharmacol. 2022;111:109130. https://doi.org/10.1016/j.intimp.2022.109130
72. Shen P, Deng X, Chen Z, Ba X, Qin K, Huang Y, et al. SIRT1: A potential therapeutic target in autoimmune diseases. Front immunol. 2021;12:779177. https://doi.org/10.3389/fimmu.2021.779177
73. Ghirotto B, Terra FF, Câmara NOS, Basso PJ. Sirtuins in B lymphocytes metabolism and function. World J Exp Med. 2019;9(1):1. https://doi.org/10.5493/wjem.v9.i1.1
74. Vasudevan S. Posttranscriptional upregulation by microRNAs. Wiley Interdiscip Rev RNA. 2012;3(3):311–30. https://doi.org/10.1002/wrna.121
75. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. Int J Genomics. 2014;2014. https://doi.org/10.1155/2014/970607
76. Kim ME, Kim DH, Lee JS. Foxo transcription factors: Applicability as a novel immune cell regulators and therapeutic targets in oxidative stress-related diseases. Int J Mol Sci. 2022;23(19):11877. https://doi.org/10.3390/ijms231911877
77. Mertowska P, Mertowski S, Podgajna M, Grywalska E. The Importance of the Transcription Factor Foxp3 in the Development of Primary Immunodeficiencies. J Clin Med. 2022;11(4):947. https://doi.org/10.3390/jcm11040947
78. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. Front immunol. 2018;9:978. https://doi.org/10.3389/fimmu.2018.00978
79. Geindreau M, Ghiringhelli F, Bruchard M. Vascular endothelial growth factor, a key modulator of the anti-tumor immune response. Int J Mol Sci. 2021;22(9):4871. https://doi.org/10.3390/ijms22094871
80. Li Y-L, Zhao H, Ren X-B. Relationship of VEGF/VEGFR with immune and cancer cells: Staggering or forward? Cancer Biol Med. 2016;13(2):206. https://doi.org/10.20892/j.issn.2095-3941.2015.0070
81. Turkowski K, Brandenburg S, Mueller A, Kremenetskaia I, Bungert AD, Blank A, et al. VEGF as a modulator of the innate immune response in glioblastoma. Glia. 2018;66(1):161–74. https://doi.org/10.1002/glia.23234
82. Mimura K, Kono K, Takahashi A, Kawaguchi Y, Fujii H. Vascular endothelial growth factor inhibits the function of human mature dendritic cells mediated by VEGF receptor-2. Cancer Immunol Immunother. 2007;56:761–70. https://doi.org/10.1007/s00262-006-0234-7
83. Li J, Li X-L, Li C-Q. Immunoregulation mechanism of VEGF signaling pathway inhibitors and its efficacy on the kidney. Am J Med Sci. 2023. https://doi.org/10.1016/j.amjms.2023.09.005
84. Tofighi Zavareh F, Mirshafiey A, Yazdani R, Keshtkar AA, Abolhassani H, Bagheri Y, et al. Lymphocytes subsets in correlation with clinical profile in CVID patients without monogenic defects. Expert Rev Clin Immunol. 2021;17(9):1041–51. https://doi.org/10.1080/1744666X.2021.1954908
85. Hücker SM, Fehlmann T, Werno C, Weidele K, Lüke F, Schlenska-Lange A, et al. Single-cell microRNA sequencing method comparison and application to cell lines and circulating lung tumor cells. Nat Commun. 2021;12(1):4316. https://doi.org/10.1038/s41467-021-24611-w
86. Ho V, Baker JR, Willison KR, Barnes PJ, Donnelly LE, Klug DR. Single cell quantification of microRNA from

small numbers of non-invasively sampled primary human cells. Communications Biology. 2023;6(1):458. https://doi.org/10.1038/s42003-023-04845-8

87. Friman V, Quinti I, Davydov AN, Shugay M, Farroni C, Engström E, et al. Defective peripheral B cell selection in common variable immune deficiency patients with autoimmune manifestations. Cell Rep. 2023;42(5). https://doi.org/10.1016/j.celrep.2023.112446
88. Strohmeier V, Andrieux G, Unger S, Pascual-Reguant A, Klocperk A, Seidl M, et al. Interferon-driven immune dysregulation in common variable immunodeficiency-associated villous atrophy and norovirus infection. J Clin Immunol. 2023;43(2):371–90. https://doi.org/10.1007/s10875-022-01379-2
89. Keller B, Strohmeier V, Harder I, Unger S, Payne KJ, Andrieux G, et al. The expansion of human T-bethighCD21low B cells is T cell dependent. Sci immunol. 2021;6(64):eabh0891.