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*Original article*

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# Regulatory Role of circRNA-0067835 in Behcet Disease through Targeting Micro RNA-155: Implication of *ATG1*, *AKT* and *MTOR*

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## Abstract

***Background:*** Autophagy has been proven to contribute to maintaining eukaryotic cells' normal intracellular homeostasis, whereas autophagy malfunction may predispose to Behcet Disease (BD). The accumulation of the products of autophagic degradation as well as impairment in autophagic flux in cases with BD, may be attributed to dysregulated miRNA-155 expression. This study attempts to determine the contribution of circRNA-0067835 in miRNA-155-mediated modulation of the autophagy axis as well as to investigate its impact on the production of pro-inflammatory cytokines in BD.

***Methods:*** This study was carried out on 40 cases with BD and 40 healthy control subjects. The collection of serum samples was done before performing a real-time PCR to estimate the relative gene expression of ATG1, AKT, miRNA-155, mTOR, TAB2, and circRNA-0067835. Additionally, IL-1β, IL-17, and TNF-α serum levels were determined by ELISA.

***Results:*** Behcet Disease (BD) patients had significantly upregulated circRNA-0067835, with subsequent downregulation of its target gene, miRNA-155 than controls ($P<0.05$). In addition, decreased miRNA-155 gene expression was correlated with significantly increased TAB2 gene expression levels in BD patients compared to the controls ($P<0.05$). Furthermore, enhanced production of pro-inflammatory cytokines was detected in cases with BD than in controls.

***Conclusions:*** The correlation between circRNA-0067835 and miRNA-155 fairly contributes to the regulation of cytokine production in BD via the modulation of autophagy. The investigation of the circRNA-0067835 and the microRNA-155 and their downstream adaptor molecules could be a potential therapeutic agent for BD.

**Keywords:** Autophagy, Auto-inflammation, Behcet disease (BD), Circular RNA-0067803, Microrna-155.

## Introduction

Behcet disease (BD) is an immune-mediated systemic vasculitis, which main symptoms are eye and skin lesions, genital ulcerations, and oral aphthous ulcers. Eye involvement is accompanied by pan uveitis or posterior with occlusive retinal vasculitis (1). Currently, BD is regarded as an auto-inflammatory disease. Numerous investigations have shown that dendritic cells play a crucial role in controlling the aberrant immune response during the progression of BD (2). The gastrointestinal, vascular, eyes, and nervous systems are the major organs involved in BD (3).

miRNAs are a family of non-coding RNAs that can bond with their target mRNAs to contribute to regulating their downstream signaling molecules. miRNAs repress gene expression by the RNA-induced silencing complex (4). They may target protein-coding mRNAs at the post-transcriptional level,

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resulting in target mRNA translational inhibition or degradation (5,6). Previous research demonstrated that miRNA-155 expression in BD patients' dendritic cells was reduced. The exact etiological basis of BD and the mechanism of how miRNA-155 is implicated in the pathogenesis of BD are still not well clarified, but it might be due to the possible role of autophagy (7,8).

Autophagy is one of the evolutionarily catabolic pathways degrading damaged organelles, abnormal proteins, as well as recycled cellular components (9). Autophagy has a vital role in maintaining eukaryotic cells' normal intracellular homeostasis, as well as autophagy malfunction could be involved in the pathogenesis of several diseases, such as autoimmune diseases, inflammation, neurodegenerative, and cancer diseases (10). In addition, miRNA-155 may modulate the autophagy process and contribute to the progression of multiple diseases, including BD (7,11).

circRNA is a form of long non-coding RNA with intrinsically closed loops with a natural resistance against exoribonucleases (12). circRNAs are produced by noncanonical splicing processes and influence gene transcription and expression by competing with endogenous RNA (13).

circRNAs have miRNA-binding sites bound by circRNA that do not have the capacity to target mRNA and lose their capability to inhibit gene expression, increasing mRNA. circRNA might have numerous binding sites for the same miRNA to alleviate the targeted mRNA repression (14,15). A recent study showed that miRNA-155 has a complementary sequence to circRNA-0067835. Therefore, it was determined as the competing endogenous RNA for circRNA-0067835 (16).

Our study was designed to detect the role of circRNA-0067835 in miRNA-155-mediated modulation of the autophagy axis, as well as to investigate its impact on the production of inflammatory cytokines in BD.

## Materials and Methods

### *Ethical approval*

All measures were carried out following the ethical considerations of the Research Committee of Kasr Al-Ainy, Cairo University Hospitals, as well as the ethical standards of the 1964 Declaration of Helsinki (approval number; MD 362-2020). All subjects provided informed consent prior to data collection and following the explanation of research objectives.

### *Subjects*

The present study included 80 subjects who were assigned to two groups. The first group included 40 Egyptian BD patients diagnosed based on Behçet's Disease Current Activity Form (ICBD) (17,18), with 35 males as well as five female subjects aged 16-74 years (mean 34.36 ± 10.95 years). These patients were recruited from the Faculty of Medicine's Rheumatology and Rehabilitation Department, Cairo University, in the interval between January 2020 and January 2022. The second cohort (the control group) included 40 age- and sex-matched healthy participants, with seven males and females ranging between 20 to 45 years (mean 30.88 ± 6.21 years). All measures were carried out following the ethical considerations of the Research Committee of Kasr Al-Ainy, Cairo University Hospitals, as well as the ethical standards of the 1964 Declaration of Helsinki (approval number; MD 362-2020). All subjects provided informed consent prior to data collection and following the explanation of research objectives.

Behcet disease (BD) cases with malignant tumors, severe infections, evidence of diseases like cardiovascular disease, or any nervous system, renal, pulmonary, hepatic, gastrointestinal, or endocrine disorders diagnosed prior to the onset of BD or other autoimmune diseases were excluded. All BD cases underwent full history taking as well as clinical examination, such as assessment of disease activity utilizing the Behçet Disease Current Activity Form (BDCAF) (19,20).



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Behcet disease (BD) severity levels were graded as severe, moderate, and mild (21). All controls as well as BD cases were subjected to routine laboratory investigations such as C-reactive protein (CRP), kidney function tests (urea and creatinine), liver function tests (AST and ALT), erythrocyte sedimentation rate (ESR), as well as complete blood count. Estimation of gene expression of circRNA0067835, miRNA155, transforming growth factor β-activated kinase 1-binding protein 2 (TAB2), mTOR, AKT and ATG1 were done by real-time-PCR and determination of IL-17, IL-1β, and TNF-α serum levels were performed utilizing ELISA.

Peripheral blood samples (5 mL) were collected in serum vacutainer tubes and allowed to clot for 15 minutes, then centrifuged at 4000 xg for 10 minutes. Serum samples were kept at -80 °C for further analysis.

***Estimation of relative gene expression of miRNA-155 by QRT-PCR***

Isolation and purification of small RNA-containing total RNA, including miRNAs, was performed from serum samples using the mirvana®kit (Thermo Fisher, MA, USA) according to the guidelines of the manufacturer. For Quantitation, Nanodrop® spectrophotometer was utilized for measuring the isolated miRNA absorbance at 260 nm. High-specificity stem-loop reverse transcription (RT) was done using the stem-loop TaqMan® MicroRNA RT Kit (Applied Biosystems, USA) based on the guidelines of the manufacturer. Additionally, real-time qPCR amplification was performed using high sensitivity TaqMan® MicroRNA assay kit (Applied Biosystems, USA), using the U6 snRNA as an endogenous control for normalization. The comparative CT method (ΔΔCT) was utilized to determine the relative expression of the miRNA-155 gene within the patient and control groups (22).

***Estimation of expression levels of circRNA-006856, TAB2, mTOR, ATG1, and AKT genes***

Isolation of total RNA was done utilizing a Qiagen kit (Qiagen, USA) in accordance with the manufacturer's guidelines. Quantification was conducted as described earlier. Total RNA was converted to cDNA utilizing a high-capacity cDNA reverse transcription kit (Fermentas, USA). Real-time qPCR analysis and amplification were conducted utilizing an Applied Biosystem with software version 3.1 (StepOne™, USA). The optimization of the qPCR assay with the primer sets was done at the annealing temperature in Applied Biosystems in accordance with the following protocol: one cycle of initial denaturation for two 2 min at 95 °C as well as 40 cycles for 15 s at 95 °C, 60°C for 60 s as well as 72 °C for 60 s. The primer sequences are depicted in Table 1. The expression of the relative gene was determined in accordance with Applied Biosystems software relative to the β-actin gene as an internal reference.

***Estimation of IL-1β, TNF-α, and IL-17 serum levels using ELISA***

The kits used were supplied by cloud clone corp (CCC, USA) for human IL-17 quantitative detection (Catalog Number: SEB955Hu), IL-1β (Catalog Number: SEA56HU), and TNF-α (Catalog Number: SEA133Ra).

***Statistical Analysis***

Data were encoded and entered utilizing the 22$^{nd}$ version of the statistical package SPSS. For quantitative variables, data were expressed utilizing standard deviation and mean, while for categorical variables, data were expressed utilizing relative frequencies (percentages) as well as frequencies (cases' numbers). When comparing two groups, the unpaired test was used, while the Chi test was utilized to compare categorical data. Utilizing the Pearson correlation coefficient, associations between quantitative variables were analyzed (23). $P<0.05$ was considered significant.



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**Table 1.** The studied genes' primer sequences.

| Gene symbol | NCBI code | Primer sequence from 5′- 3′<br>F: Forward primer, R: Reverse primer |
|---|---|---|
| **mTOR** | **NM_004958.4** | (F) 5′CTCATCAGCATTAATAATAAGC 3′<br>(R) 5′GTGTCCATTTTCTTGTCATAG 3′ |
| **CirRNA0067835 (IFT80)** | **NC_000003.12** | (F) 5′CCGCCCACTGTACAATTCAC 3′<br>(R) 5′TCTTCAGCAGTAGTCCAGCC 3′ |
| **B actin (housekeeping gene)** | **NM_007393** | (F) 5′CACCATTGGCAATGAGCGGTTC3′<br>(R) 5′AGGTCTTTGCGGATGTCCACGT3′ |
| **MiRNA155** | **NR_030784** | (F) 5′TGCTAATCGTGATAGGGG 3′<br>(R) 5′GAACATGTCTGCGTATCTC 3′ |
| **U6 (housekeeping gene)** | **NR_004394** | (F) 5′-GCTTCGGCAGCACATATACTAAAAT-3′<br>(R) 5′-CGCTTCACGAATTTGCGTGTCAT-3′ |
| **TAB2** | **NM_001292034** | (F) 5′GGUGCAUGUUACAGAAUAAdTdT<br>3′(R) 5′UUAUUCUGUAACAUGCACCdTdT 3′ |
| **AKT** | **NM_005163** | (F)5′CTTCTTTGCCGGTATCGTGT3′<br>(R)5′CTGGCCGAGTAGGAGAACTG 3′ |
| **ATG1(ULK1)** | **NM_003565.4** | (F) 5′CGCCACATAACAGACAAAAATACAC 3′<br>(R) 5′ CCCCACAAGGTGAGAATAAAGC 3′ |

## Results

### *Comparisons of the demographic features among the studied groups*

In our study, the age of BD cases ranged between 16 and 74 years, with a mean of 34.37±10.96 years, whereas the age of control subjects ranged from 20-46 years, with a mean of 30.89±6.22 years. They are age matched with no substantial variations were found between both groups (P=0.2). With respect to sex, the incidence of BD was most commonly occurring among males (87.5%) compared to females (12.5%), with a highly statistically significant difference as compared to females (p-value< 0.001). The control subjects were sex-matched with the BD patients (p= 0.7), with a larger percentage of males (82.5%) than females (17.5%).

### *Age of onset and disease severity and duration*

The mean age of onset of BD in the patients' group was 25.47±7.22 years, and the mean disease duration was 8.89±0.75 years. Of the 40 BD cases, there were 6 (15%) mild, 7 (17.5%) moderate, and 27 (67.5%) severe patients.

### *Clinical data*

The frequency and the percentage of BD manifestations among cases it was as follows: 38(95%) of cases had oral ulcers, 34(85%) had genital ulcers, 11(27.5%) showed skin manifestations, while 11(27.5%) and 6(15%) suffered from arthralgia and arthritis respectively. Furthermore, 4(10%) of cases complained of GIT manifestations, 3(7.5%) with diarrhea, and 1(2.5%) with bleeding per rectum. The percentage of the eye, vascular, and CNS manifestations was found to be 62.5%, 22.5% and 10%, consecutively.

### *Laboratory findings*

There was a substantial variation in the ESR level among cases with BD 21.54±18.57 and controls 7.41±2.85 (P<0.001) and a significant difference in platelet count between BD patients268.33±83.61 and controls 236.52±55.32(P=0.043) but no significant difference as regards routine laboratory markers (kidney function tests (urea and creatinine), complete blood count (CBC) and liver function tests (ALT and AST).



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## Biochemical and molecular results

### *Crosstalk between circRNA-0067835 and miRNA155 genes expression*

Our results demonstrate a substantial elevation in circRNA- 0067835 and TAB2 gene expression and a substantial decline in miRNA-155 gene expression in BD cases than controls (P<0.001) (Fig. 1).

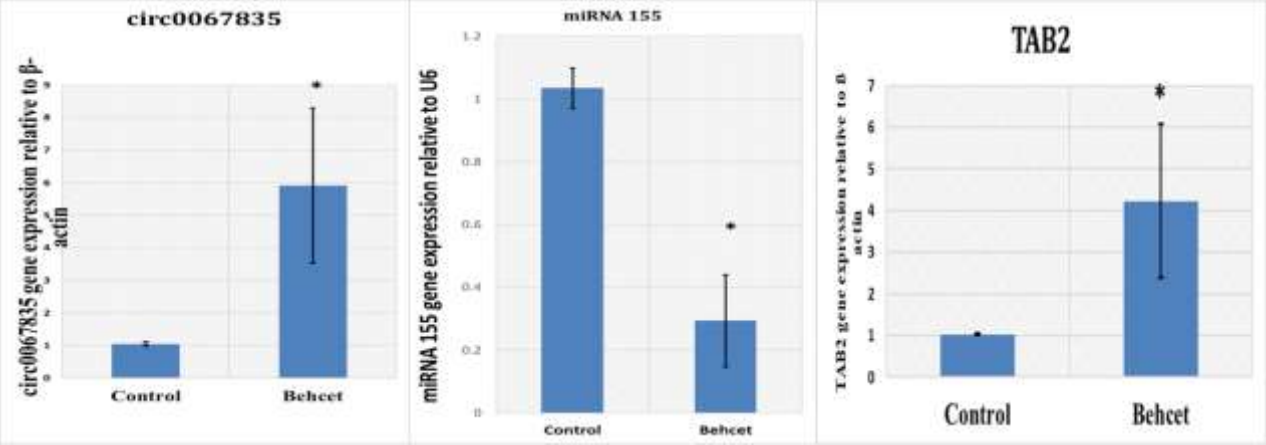


**Fig. 1.** CircRNA0067835, miRNA155, and TAB2 gene expression among studied groups. This figure demonstrates a highly significant increase in circRNA-0067835 and TAB2 gene expression and a highly significant decrease in miRNA-155 gene expression in BD cases than controls (P<0.001). *Denotes significant difference versus the control group. P<0.05 is considered significant.

### *ATG1, AKT, and mTOR gene expression*

Results showed a significant elevation in Akt, mTOR, and ATG1 gene expression in BD patients than controls (P<0.001) (Fig. 2).

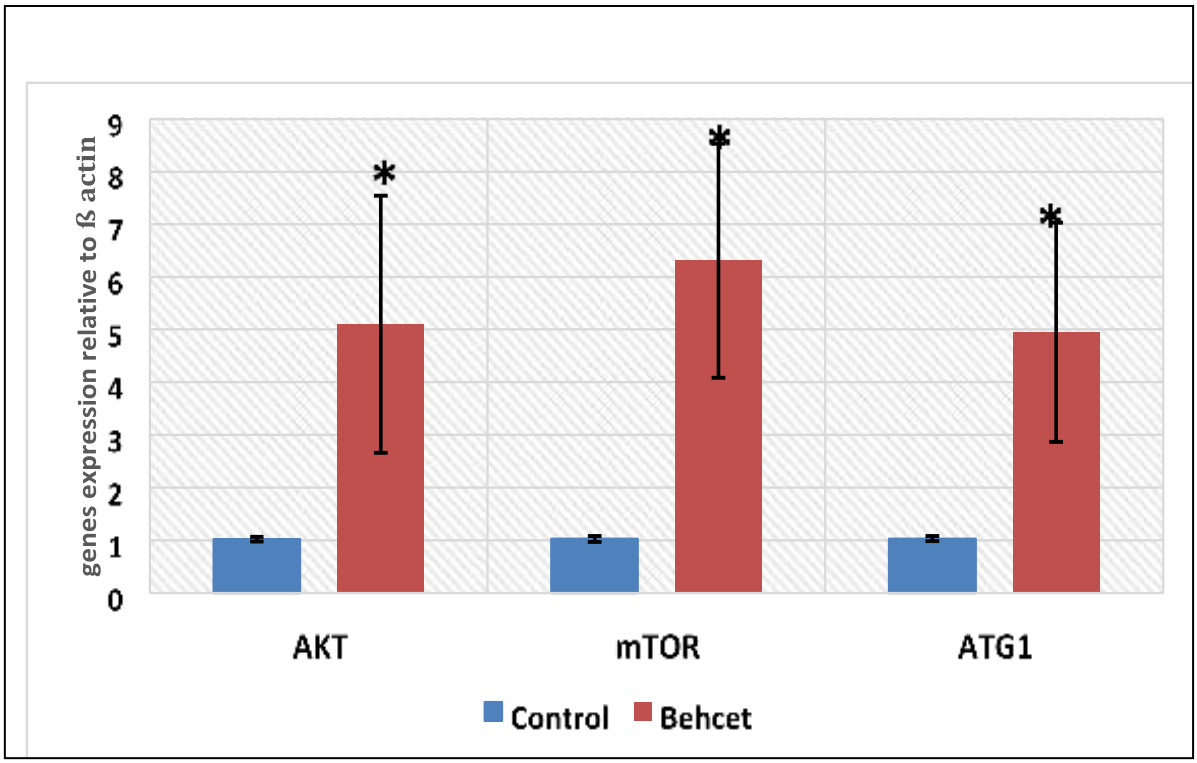


**Fig. 2.** AKT, mTOR and ATG1 gene expression among studied groups. A highly significant increase in AKT, mTOR, and ATG1 gene expression in BD cases than controls (P<0.001) is shown. *Denotes significant difference versus the control group. P <0.05 is considered significant.



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### *IL-17, IL-1β, and TNF-α serum levels*

The current results show a substantial increase in the protein level of TNF-α, IL-1β, and IL-17 in BD cases compared to controls (P<0.001) (Fig. 3).

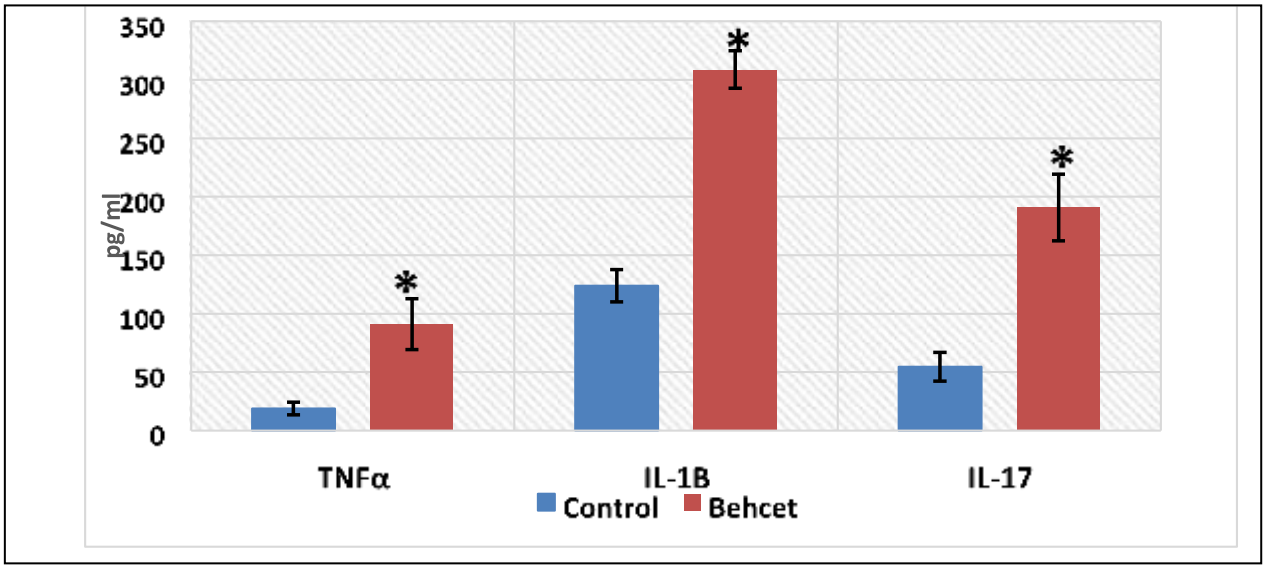


**Fig. 3.** TNFα, IL-1β and IL-17 in studied groups. *Denotes significant difference versus the control group. P <0.05 is considered significant. A highly significant increase in the protein level of TNF-α, IL-1β, and IL-17 in BD cases compared to controls (P<0.001) is shown.

### *Correlation between miRNA-155 and autophagy and inflammatory markers*

Our results show a significant negative correlation between miRNA-155 and IL-1β, (r=-0.437, p-value=0.005) (Fig. 4).

Furthermore, there were non-significant correlations between miRNA155 and AKT, mTOR, ATG1, TAB2, TNF-α,IL17, and circRNA-0067835(r=-0.197, p-value=0.223), (r=-0.136, p-value=0.404), (r=--0.126, p-value=0.438), (r=-0.295, p-value=0.064), (r=-0.214, p-value=0.184), (r=--0.001, p-value=0.995), (r=-0.011, p-value=0.946) respectively.

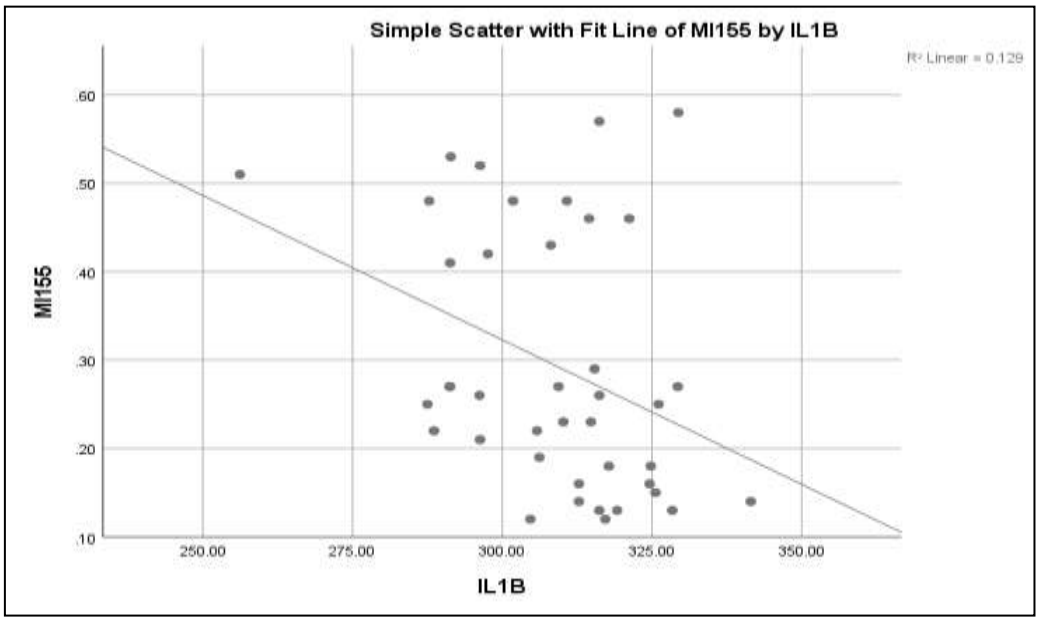


**Fig. 4.** Correlation between miRNA-155 and IL-1β. A significant negative correlation between miRNA-155 and IL-1β, (r=-0.437, p-value=0.005) is shown.



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***Association between miRNA-155/ circRNA-0067835 and BD severity***

The present results show a significant positive association between circRNA-0067835 gene expression and BD severity (p=0.03) (Table 2).

**Table 2.** Association between miRNA-155/ circRNA-0067835 and BD severity.

| severity | mild | moderate | severe | P value |
|---|---|---|---|---|
| **miRNA155** | 0.27±0.15 | 0.29±0.19 | 0.3±0.14 | 0.9 |
| **circRNA0067835** | 4.39±2.13 | 4.46±2.86 | 6.54±2.14 | 0.03 |

## Discussion

This study demonstrates a significant upregulation of circRNA-0067835, followed by downregulation of its target, miRNA-155, in BD patients than controls. This relationship highlights the sponge-like effect of circRNA-0067835 that leads to miRNA-155 sequestration. These findings align with (16) Zeng et al., 2021, who demonstrated that circRNA-0067835 could promote liver fibrosis by functioning as a miRNA-155 sponge, and increasing the transcription factor, Forkhead box protein O3 (FOXO3a) levels.

Another study by (24) Gong et al., 2018, revealed that circ-0067835 and miRNA-155 were involved in temporal lobe epilepsy (TLE). Moreover, circRNA-0067835 expression was substantially diminished, whereas miR-155 expression was elevated in the plasma and tissues of patients with TLE. Lower circRNA-0067835 is associated with elevated seizure frequency. Bioinformatics online programs suggested that circRNA-0067835 functions as a miRNA-155 sponge for the regulation of FOXO3a expression, which was proven utilizing luciferase reporter assay (25)**.**

Furthermore, in accordance with our results, *Liang and his colleagues* illustrated that miRNA-155 was diminished in DCs than in active BD cases (7)**.** They also illustrated that TAB2 expression is the direct target of miR-155, where TAB2 expression in DCs from active BD patients was elevated, and its expression could be inhibited by miR-155. These results are consistent with our findings, as our results show that decreased miRNA-155 gene expression correlates with significantly increase in TAB2 gene expression levels in BD cases than in controls.

We also elucidated that upregulated TAB2 in BD patients contributed to enhancing the production of the pro-inflammatory cytokines, IL-17, IL-1β, and TNF-α by inhibiting autophagy. This was clearly evident by the upregulation of the mTOR/AKT signaling pathway. Our findings demonstrated a substantial elevation in mTOR and AKT gene expression, with subsequent upregulation in ATG1 gene expression in BD cases compared to controls.

According to a previous study, the kinase mTOR complex1 (mTORC1) negatively regulates autophagy by increasing the phosphorylation of ATG1, thereby inhibiting its activity by disrupting the interaction between ATG1 and AMP-activated protein kinase (AMPK). This interaction is essential for the induction of autophagy; therefore, the increased level of phosphorylated ATG1 in its free form, but not in its complex form, resulted in the inhibition of autophagy (25). Another study by (26) Giriş et al., 2017, illustrated that the expression levels of the anti-apoptotic components, Akt-1, and mTOR, increased in neuro BD.

Consistent with prior studies, we demonstrated that miRNA-155-3p is a potent inducer of autophagy via decreasing phosphorylation of mTOR and AKT in DCs (27,28). In contrast, a study by *Palizgir, et al., 2018* observed an insignificant decline in mTOR in macrophages in BD patients compared to healthy controls (29).



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Regarding the pro-inflammatory cytokines, our results indicated that defective autophagy regulation might stimulate cytokine production. *Liang and his colleagues* supported our results, as they found that IL-1β, IL-6, and TNF-α expression in DCs in active BD cases was substantially elevated than that of healthy controls. However, rapamycin, the mTOR inhibitor, decreased their expression levels by DCs. They also elucidated that in DCs transfected with miRNA-155, the expression level of TAB2 was substantially suppressed, and IL-1β, IL-6, and TNF-α expression significantly declined after the downregulation of TAB2. These findings show that autophagy acts as a negative regulator of inflammation by controlling the production of inflammatory cytokines (7). Another research demonstrated that rapamycin consistently alleviates inflammation of the retina. This mTOR inhibitor has also been demonstrated to be safe and effective in treating non-infectious uveitis (30).

The auto-inflammatory diseases, including BD, are considered IL-1-mediated diseases that respond to anti-IL-1 therapies. In fact, auto-inflammation is characterized by an over-activation of the innate immune system and by the overproduction of the pro-inflammatory cytokines, including IL-1β, IL-18, TNF, and type-1 interferons (31).

Yong et al., 2019 explained that the disturbed Treg/Th17 axis is involved in the pathogenesis of BD. The patients with clinically active BD were found to have decreased peripheral blood regulatory T cells. In contrast, Th17 cells were increased in BD patients in response to various inflammatory cytokines, and the pro-inflammatory cytokines (IL-1β, TNF-α, and IL-23) might be increased in response to Th17 cell proliferation (28).

In conclusion, the impaired autophagic flux in BD patients may be attributable to dysregulated expression of miRNA-155 via its effect on the mTOR/AKT signaling pathway. The interrelated relationship between circRNA-0067835 and miRNA-155 highlights the sponge-like effect of circRNA-0067835 that results in the sequestration of miRNA-155. The downregulation of miRNA-155 increases the expression of its target protein, TAB2, leading to an increase in the production of pro-inflammatory cytokines. There was a significant correlation between circRNA-0067835 and the severity of BD. circRNA-0067835 is potentially a novel biomarker for the diagnosis of BD. It also poses a new challenge for treatment via its effect on autophagy modulation through its suppressor effect on miRNA-155.

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## Conflict of Interest

All authors declare no conflicts of interest.

## References

1. Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci. 2004;24(20):4787-4795.
2. Wang C, Ye Z, Kijlstra A, Zhou Y, Yang P. Activation of the aryl hydrocarbon receptor affects activation and function of human monocyte-derived dendritic cells. Clin Exp Immunol. 2014;177(2):521-530.
3. Hatemi G, Yazici Y, Yazici H. Behçet's syndrome. Rheum Dis Clin North Am. 2013;39(2):245-261.
4. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol. 2013;14(11):699-712.
5. Lin PY, Yu SL, Yang PC. MicroRNA in lung cancer. Br J Cancer. 2010;103(8):1144–1148.



[ DOI: 10.61186/rbmb.12.1.195 ]

6. Shaker O, Mahfouz H, Salama A, Medhat E. Long Non-Coding HULC and miRNA-372 as Diagnostic Biomarkers in Hepatocellular Carcinoma. Rep Biochem Mol Biol. 2020;9(2):230-240.
7. Liang L, Zhou Q, Feng L. Decreased microRNA-155 in Behcet's disease leads to defective control of autophagy thereby stimulating excessive proinflammatory cytokine production. Arthritis Res Ther. 2021;23(1):135.
8. Adil A, Goyal A, Quint JM. Behcet Disease. Treasure Island (FL) StatPearls Publishing. 2023.
9. Hale AN, Ledbetter DJ, Gawriluk TR, Rucker EB. Autophagy: regulation and role in development. Autophagy. 2013;9(7):951-972.
10. Boutouja F, Stiehm CM, Platta HW. mTOR: A Cellular Regulator Interface in Health and Disease. Cells. 2019;8(1):18.
11. Park HJ, Son HJ, Sul OJ, Suh JH, Choi HS. 4-Phenylbutyric acid protects against lipopolysaccharide-induced bone loss by modulating autophagy in osteoclasts. Biochem Pharmacol. 2018;151:9-17.
12. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013;19(2):141-157.
13. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495(7441):333-338.
14. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495(7441):384-388.
15. Abdelgwad M, Zakaria R, Marzouk S, Sabry D, Ahmed R, Badary HA, Samir M. The Emerging Role of Circular RNA Homeodomain Interacting Protein Kinase 3 and Circular RNA 0046367 through Wnt/Beta-Catenin Pathway on the Pathogenesis of Nonalcoholic Steatohepatitis in Egyptian Patients. Rep Biochem Mol Biol. 2023;11(4):614-625.
16. Zeng X, Yuan X, Cai Q, Tang C, Gao J. Circular RNA as an epigenetic regulator in chronic liver diseases. Cells. 2021;10(8):1945.
17. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet. 1990;335(8697):1078-1080.
18. International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD). The International Criteria for Behçet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. J Eur Acad Dermatol Venereol. 2014;28(3):338-347.
19. Lawton G, Bhakta BB, Chamberlain MA, Tennant A. The Behcet's disease activity index. Rheumatology (Oxford). 2004;43(1):73-8
20. Gul FC, Nazik H, Cicek D, Demir B. Activity Criteria in Behçet's Disease [Internet]. Behcet's Disease. InTech; 2017.
21. Krause I, Uziel Y, Guedj D, Mukamel M, Harel L, Molad Y, Weinberger A. Childhood Behçet's disease: clinical features and comparison with adult-onset disease. Rheumatology (Oxford). 1999;38(5):457-62.
22. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.
23. Chan, Felix T, H J, Qi. An innovative performance measurement method for supply chain management. Supply Chain management. 2003;8(3-4):209-223.
24. Gong GH, An FM, Wang Y, Bian M, Wang D, Wei CX. Comprehensive Circular RNA Profiling Reveals the Regulatory Role of the CircRNA-0067835/miR-155 Pathway in Temporal Lobe Epilepsy. Cell Physiol Biochem. 2018;51(3):1399-1409.
25. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011;13(2):132-41.
26. Giriş M, Bireller S, Küçükali Cİ, Hanağasi H, Değirmencioğlu S, Tüzün E. Impact of Neuro-Behçet Disease Immunoglobulin G on Neuronal Apoptosis. Noro Psikiyatr Ars. 2017;54(1):67-71.
27. Xue H, Yuan G, Guo X, Liu Q, Zhang J, Gao X, et al. A novel tumor-promoting mechanism of IL6 and the therapeutic efficacy



[ DOI: 10.61186/rbmb.12.1.195 ]

of tocilizumab: Hypoxia-induced IL6 is a potent autophagy initiator in glioblastoma via the p-STAT3-MIR155-3p-CREBRF
pathway. Autophagy. 2016;12(7):1129-1152.
28. Yong C, Dan L, Chenhong L, Yan S, Jianfei C, Jianlong G, et al. Efficacy and safety of metformin for Behcet's disease and its effect on Treg/Th17 balance: a single-blinded, before-after study. Nan Fang Yi Ke Da Xue Xue Bao. 2019;39(2):127-133.
29. Palizgir MT, Akhtari M, Shahram F, Mostafaei S, Maassoomeh A, Sobhani S, Milad Mahmoudi Downregulation. of. autophagy-related genes in macrophages from patients with behcet's disease. Crescent J Med Biol Sci. 2017;5(1):14-20.
30. Okamoto T, Ozawa Y, Kamoshita M, Osada H, Toda E, Kurihara T, et al. The neuroprotective effect of rapamycin as a modulator of the mTOR-NF-KB axis during retinal inflammation. PloS one. 2016;11(1):e0146517.
31. Gratton R, Tricarico PM, D'adamo AP, Bianco AM, Moura R, Agrelli A, et al. Notch signaling regulation in autoinflammatory diseases. Int J of Mol Sci. 2020;21(22):8847.



[ DOI: 10.61186/rbmb.12.1.195 ]