İSTANBUL ÜNİVERSİTESİ YAYINEVİ

ARAŞTIRMA / RESEARCH
DOI: 10.26650/IUITFD.2019.0023
İst Tıp Fak Derg 2020 / J Ist Faculty Med 2020

THE *miR-196a2*T/C VARIANT AS A POSSIBLE PREDISPOSING FACTOR FOR ANKYLOSING SPONDYLITIS IN A TURKISH POPULATION

TÜRK POPÜLASYONUNDA ANKİLOZAN SPONDİLİTE YATKINLIK SAĞLAYICI FAKTÖR OLARAK *miR-196a2*T/C VARYANTI

Sacide PEHLİVAN[1], Savaş GÜRSOY[2], Ayşe Feyda NURSAL[3], Mazlum Serdar AKALTUN[2], Kürşat ÖZDİLLİ[4], Mustafa PEHLİVAN[5]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey
Gaziantep University, Faculty of Medicine, [2]Department of Physical Therapy and Rehabilitation, [5]Department of Internal Medicine Division of Hematology, Gaziantep, Turkey
[3]Hitit University, Faculty of Medicine, Department of Medical Genetics, Corum, Turkey
[4]Medipol University, Medipol University Hospital, Pediatric Bone Marrow Unit, Istanbul, Turkey

ORCID IDs of the authors: S.P. 0000-0003-1272-5845; S.G. 0000-0002-1673-9905; A.F.N. 0000-0001-7639-1122; M.S.A. 0000-0002-9666-9483; K.Ö. 0000-0002-7129-5024; M.P. 0000-0002-6692-085X

Cite this article as: Pehlivan S, Gursoy S, Nursal AF, Akaltun MS, Ozdilli K, Pehlivan M. The *miR-196a2*T/C variant as a possible predisposing factor for ankylosing spondylitis in a turkish population. J Ist Faculty Med 2020;83(2):81-5. doi: 10.26650/IUITFD.2019.0023

ABSTRACT

Objective: Ankylosing spondylitis (AS) is a chronic inflammatory disorder. MicroRNAs (miRNAs) can function as either oncogenes or tumor suppressor genes. Altered miRNA expression has been implicated in the pathogenesis of several diseases. Therefore, we aimed to explore the effects of *miR-196a2*T/C (rs11614913) variant profile on susceptibility to AS in a Turkish population.

Materials and Methods: Blood samples were collected from 78 AS patients and 79 healthy controls. *miR-196a2*T/C variant was genotyped by PCR-RFLP. Odds ratio (OR) with 95% confidence interval (95%CI) were calculated using the χ^2 test.

Results: The frequency of T/C and T/T genotypes of the *miR-196a2*T/C were higher in AS patients compared to healthy controls (*p=0.034* and *p=0.028*, respectively). The subjects carrying the *miR-196a2*T/C variant T/T genotype showed a 2.542-fold increased AS risk than the control group. However, no difference was observed in the allele frequencies of *miR-196a2*T/C between AS patients and the controls. It was found that C/C genotype of *miR-196a2*T/C variant was more frequent in AS patients with enthesitis than AS patients without enthesitis (*p=0.042*).

Conclusion: The *miR-196a2*T/C variant represents a genetic risk

ÖZET

Amaç: Ankilozan spondilit (AS) kronik, enflamatuar bir hastalıktır. MikroRNA'lar (miRNA',lar) onkogen veya tümör baskılayıcı genler olarak fonksiyon yapabilirler. Çeşitli hastalıkların patogenezinde miRNA ekspresyonu'nun değişmesi bulunmaktadır. Bu nedenle, Türk toplumunda AS yatkınlığında *miR-196a2*T/C (rs11614913) varyant profilinin etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: 78 AS hastası ve 79 kontrolden kan örnekleri toplandı. *miR-196a2*T/C varyantı PCR-RFLP ile genotiplendi. χ^2 testi kullanılarak %95 güven aralığı ile Odds oranı (OR) hesaplandı.

Bulgular: *miR-196a2*T/C T/C ve T/T genotipleri AS hastalarında sağlıklı kontrollere kıyasla daha yüksekti (sırasıyla, *p=0.034* and *p=0.028*). *miR-196a2*T/C varyant T/T genotipini taşıyan kişiler kontrol grubundan 2,542 kat artmış AS riski gösterdiler. Ancak, *miR-196a2*T/C allele frekansları açısından AS hastaları ve kontroller arasında fark saptanmadı. *miR-196a2*T/C varyant C/C genotipinin entezitli AS hastalarında entezit bulunmayan AS hastalarından daha çok olduğu bulundu (*p=0.042*).

Sonuç: *miR-196a2*T/C varyantı Türk toplumunda AS ve hastalığın entezit ile ilişkisine yatkınlık sağlayan genetik risk faktörü rolü göstermektedir.

Corresponding author/İletişim kurulacak yazar: sacide.pehlivan@istanbul.edu.tr

Submitted/Başvuru: 12.03.2019 • **Revision Requested/Revizyon Talebi:** 01.07.2019 •
Last Revision Received/Son Revizyon: 22.07.2019 • **Accepted/Kabul:** 22.08.2019 • **Published Online/Online Yayın:** 30.09.2019

factor for increased susceptibility to AS and is associated with enthesitis in the Turkish population.

Keywords: Ankylosing spondylitis, *miR-196a2*, variant, enthesis

Anahtar Kelimeler: Ankilozan spondilit, *miR-196a2*, varyant, entezit

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis which involves mainly the vertebrae and sacroiliac joints, resulting in severe pain and loss of mobility (1). The general prevalence of AS is 1% and this disease primarily influences young men older than 15 years (2). It can cause structural and functional deterioration. Even though the etiopathogenesis of AS remains unclear, AS seems to be a multifactorial illness, affected by several genetic and environmental factors. It is reasonable that the major histocompatibility complex (MHC) class I allele human leukocyte antigen B27 (HLA-B27) gene constitutes the most crucial risk factor for development of this disease (3).

MicroRNAs (miRNAs) are a group of endogenous, tiny, noncoding single-stranded RNAs of about 22 nucleotides that play a role as post-transcriptional gene regulatory factors for up to 30% of all human genes. MiRNAs exert their effect by binding to the 3'-untranslated region of specific messenger RNAs (mRNAs) and target them for breaking down or translational inhibition (4). Several miRNAs play a role in modulation of numerous physiological functions, including proliferation, apoptosis, differentiation, immune response, and inflammation. MiRNAs are thought to be tissue-specifically expressed and it has been suggested that alteration in their expression is related with illness (5). It has been reported that miRNAs are involved in the pathogenesis of several human diseases like autoimmune or autoinflammatory disorders (6). *miR-196a* has been defined as an upstream regulator of Hoxb8 and Sonic hedgehog (Shh) in limb development (7). *miR-196a2*T/C (rs11614913) variant causes a change from cytosine to thymine. This variant is found at the 3p mature *miR-196a* region and has an impact on the transformation of pre-miR-196a2 to its mature form. *miR-196a2*T/C have been widely scrutinized in several types of cancer, ischemic stroke, Moyamoya disease, premature ovarian failure, and spontaneous abortion (8). In previous studies, it has been reported that different miRNA variants are associated with AS development. Hence, we aimed to clarify the effect of the *miR-196a2*T/C variant on AS risk. Furthermore, we investigated whether the *miR-196a2*T/C variant is linked with clinical-pathological findings of AS.

MATERIALS AND METHODS

Study Population

A total of 78 unrelated AS patients and 79 healthy controls were enrolled for analysis of the possible effects of the *miR-1962a*T/C variant. All samples were obtained from patients and controls who had been admitted to Gaziantep University, Faculty of Medicine, Department of Physical Therapy and Rehabilitation. They were diagnosed with AS after routine tests, X-ray, CT and nuclear magnetic resonance examinations according to the Modified New York Criteria for AS (9). Patients with type 2 diabetes mellitus, liver disease, renal failure and chronic infectious disease were excluded. A clinical data collection form was designed, and age, body mass index (BMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Radiology Index (BASRI), AS-specific Quality of life (AS-QoL), HLA-B27 and enthesis were recorded. For comparison, a control group included individuals with similar ethnic backgrounds and living in the same geographic area with the patients. The controls had no history of arthritis. Informed written consent was obtained from all patients and subjects before enrollment to the study, according to the ethical guidelines of the 2008 Declaration of Helsinki. Ethical approval was attained from the Local Human Research Ethics Committee.

Genotyping

Genomic DNA was obtained from peripheral blood by the standard salting method developed by Miller et al. (10). The isolated DNA was stored at -20°C until further analysis. The *miR-1962a*T/C variant was genotyped in all subjects by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) analysis, as described previously (11). The following primers were used to amplify the rs11614913T/C polymorphism site in the miR-196a-2 precursor: F 5'-CCC CTT CCC TTC TCC TCC AGA TA-3' and R 5'-CGA AAA CCG ACT GAT GTA ACT CCG-3'. PCR was performed in a 25-mL reaction mixture with 10X PCR buffer, 3 mL 25 mM MgCl2, 1.5 mL 10 pM of both primers, 2 mL 10 mM each dNTP, 2 mL (20-40 ng/mL) DNA, and 1 U Taq DNA polymerase. Reaction parameters were as follows: initial denaturation at 94°C for 5 min; followed by 35 cycles at 91°C for 60 s, 62°C for 60 s, and 72°C for 60 s, and a final extension at 72°C for 5 min. The

PCR amplification products were digested using *MspI* restriction enzymes. For *miR-196a2*T/C variant, allele C was cuttable, producing two fragments of 24 bp and 125 bp, allele T was uncuttable and the fragment was still 146 bp.

Statistical Analysis

All statistical analyses were done using SPSS Statistical Program Version 16.0 and OpenEpi info 2.2 software package programs. Continuous data was given as mean±SD (standard deviation) and (min/max). Chi^2 test was used to determine the significance of differences in the allele frequency and genotype distribution between the two study groups. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. P value $p<0.05$ was considered statistically significant.

RESULTS

In this study, a total of 78 unrelated Turkish patients with AS and 79 healthy subjects were evaluated for the *miR-196a2*T/C variant. The distribution of the genotype and allele of the patients and healthy controls for the *miR-196a2*T/C variant are presented in Table 1. A significant difference between the patients and the controls was observed for genotype distribution of the *miR-196a2*T/C variant. The frequency of T/C and T/T genotypes of the *miR-196a2*T/C variant was much higher in AS patients than in healthy controls (*p=0.034*, OR=0.379, 95%CI, 1.155-0.929; *p=0.028,* OR=2.542, 95%CI, 1.108-5.834, respectively). The allele frequencies of the *miR-196a2*T/C variant showed no statistically significant difference between AS patients and the controls (*p>0.05*).

We then evaluated the associations between the clinical features of AS and *miR-196a2*T/C genotype distribution (Table 1). *miR-196a2*T/C variant genotype distributions showed no significant association with various clinical parameters including BMI, BASDAI, BASMI, BASFI, BASRI, AS- AS-QoL, and HLA-B27 (*p>0.05* and data not shown). There was a significant association between the *miR-196a2*T/C variant and enthesitis. The CC genotype of the *miR-196a2*T/C variant was more frequent in patients with enthesitis than patients without enthesitis (*p=0.042*, OR=2.798, 95%CI=1.036-7.554) (Table 2).

DISCUSSION

AS is the most frequent form of spondyloarthropathy. Of all rheumatic disorders, AS is closely related with genetic factors. High monozygotic twin concordance (63%) and familial aggregation studies suggest a heritability of over 90% in cases with AS (12). HLA-B27 creates a major genetic risk, present in about 6% of the US population, and more than 90% of patients with AS. Overall, the prevalence of AS in various parts of the world correlates with the prevalence of HLA-B27.

MiRNAs are a group of regulatory RNA that acts as a principal control factor in gene expression. During past decades, it has become increasingly evident that miRNAs play a crucial role not only in normal development and physiology, but also in the pathologies of autoimmune disorders, cancer, heart disease, and inflammatory conditions (13). The significance of miRNAs in regulating bone homeostasis and metabolism has been well understood.

Table 1. The distribution of genotypes and alleles of *miR-196a2*T/C variant in AS patients and controls

miR-196a2		**Patients**	**Controls**	**OR**	**95%CI**	**p**
Genotypes		**n=78 (%)**	**n=79 (%)**			
	CC	25 (32.1)	27 (34.2)	0.467*	0.181-1.205*	0.115*
	TC	32 (41.0)	42 (53.2)	0.379*	0.155-0.929*	**0.034***
	TT	21 (26.9)	10 (12.6)	2.542&	1.108-5.834&	**0.028&**
Alleles						
	C	82 (52.6)	96 (60.8)	1.397&	0.893-2.187&	0.172&
	T	74 (47.4)	62 (39.2)			

*OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

Table 2. The distribution of genotypes *miR-196a2*T/C variant in AS patients with and without enthesis.

	Enthesis (+)	**Enthesis (-)**	**OR***	**95%CI**	**p**
Genotypes	**n=25 (%)**	**n=53 (%)**			
CC	13 (52)	15 (28.3)			
TC/TT	12 (48)	38 (71.7)	2.798&	1.036-7.554&	**0.042&**

*OR (95%CI) was adjusted by age and sex, &Fisher's Exact Test.

A number of studies have shown the regulation of miRNA expression during osteoblast and osteoclast production and neatly defined the variation of miRNA expression in primary osteocytes (14). Increasing evidence has recently demonstrated an essential role of miRNAs in the occurrence of several bone diseases (15). It has been shown that miRNA expression could be modified in synovia, peripheral blood mononuclear cells (PBMCs) or T cells from patients who suffer from various arthritic diseases, such as RA, osteoarthritis (OA) and AS (16). Yang et al. (17) reported that the frequency of low copy numbers of *miR-143, miR-146a, miR-9-3,* and *miR-205* and high copy numbers of *miR-301a* and *miR-23a* was increased in cases with acute anterior uveitis in patients with AS. Qian et al. (18) identified that *miR-146a* and *miR-155* expressions were significantly upregulated in AS patients.

Single nucleotide polymorphisms (SNPs) constitute the most frequently encountered sequence variation in the human genome. SNPs in miRNA-coding genes may influence processing and binding capacity of miRNAs by changing the secondary structure of miRNA precursors, leading to distorted expression of a series of target genes (19). This altered gene expression might cause change in phenotypes (20). In a meta analysis, Park et al. (21) reported that the *miR-146a* SNPs rs2910164, rs57095329, rs2431697 are associated with susceptibility to some autoimmune diseases. Fattah et al. (22) showed that C allele of *Pre-miRNA* rs3746444 polymorphism contributes to heritability of susceptibility to RA compared to T allele in the Egyptian population. Recently, Xu et al. (16) reported that the frequency of the GG genotype and G allele of *miR-146a* rs2910164 G/C variant was markedly higher in the AS patients than in the healthy controls ($p=0.014$ and $p=0.005$, respectively). However, Niu et al. (3) found no association between three *miR146A* variants (rs2910164, rs2431697 and rs57095329) in Chinese AS cases and controls. The *miR-196a2*T/C variant might influence the binding capacity of *miR-196a2* to its target mRNA or it might have an impact on transforming the pre-miRNA into its mature form (23). *miR-196a2*T/C has been widely scrutinized in several types of diseases including cancer, ischemic stroke, and spontaneous abortion (24-27). Qi et al. (28) reported that there was no relation between pre-miR-196a2 rs11614913 and acute anterior uveitis (AAU) associated with AS.

In this preliminary study, we scrutinized for the first time the *miR-196a2*T/C variant with AS susceptibility in a Turkish population. We found that the *miR-196a2*T/C variant was associated with increased risk of AS. The TC and TT genotypes of the *miR-196a2*T/C variant was significantly higher in AS patients than in healthy controls ($p=0.034$, $p=0.02$, respectively) (Table 1) The subjects carrying the *miR-196a2*T/C variant TT genotype showed a 2.542-fold increased AS risk (OR: 2.542, 95% CI: 1.108-5.834) than the control group. Additionally, our results showed a significant association of *miR-196a2*T/C variant with enthesis, one of the main clinical findings of AS. The CC genotype of *miR-196a2*T/C variant was more frequent in AS patients with enthesitis than in AS patients without enthesitis. To our knowledge, our study establishes the first evidence that *miR-196a2*T/C can be used as a possible diagnostic biomarker for AS (Table 2).

However, there are some limitations to this study. (i) The study population contained only Turkish subjects and our results may not reflect findings related to other ethnic populations. Therefore, our results need to be replicated and confirmed by further studies conducted on other ethnic groups since SNPs and haplotype configurations can vary among various ethnic populations. (ii) The number of subjects with AS was rather scarce in this case-control study. (iii) We were unable to exclude some other possible factors including exposure to different environmental factors and other genetic factors.

In conclusion, *miR-196a2*T/C is associated with AS susceptibility. Further research on *miR-196a2*T/C in AS will be helpful in understanding the impact of this variant.

Ethics Committee Approval: Ethics committee approval was received for this study from the local ethics committee.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study S.P., S.G., M.P.; Data Acquisition- S.G., S.M.A., M.P.; Data Analysis/Interpretation- M.P., K.Ö., A.F.N., S.P.; Drafting Manuscript- A.F.N., S.P.; Critical Revision of Manuscript- S.G., M.P., S.M.A., K.Ö.; Final Approval and Accountability- S.P., A.F.N., M.S.A., K.Ö., M.P.; Technical or Material Support- K.Ö., S.P.; Supervision- A.F.N., S.P., S.G.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Etik Komite Onayı: Etik komite onayı bu çalışma için, yerel etik komiteden alınmıştır.

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti/Tasarım- S.P., S.G., M.P.; Veri Toplama- S.G., S.M.A., M.P.; Veri Analizi/Yorumlama- M.P., K.Ö., A.F.N., S.P.; Yazı Taslağı- A.F.N., S.P.; İçeriğin Eleştirel İncelemesi- S.G., M.P., S.M.A., K.Ö.; Son Onay ve Sorumluluk- S.P., A.F.N., M.S.A., K.Ö., M.P.; Malzeme ve Teknik Destek- K.Ö., S.P.; Süpervizyon- A.F.N., S.P., S.G.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

REFERENCES

1. Han R, Xia Q, Xu S, Fan D, Pan F. Interleukin-23 receptor polymorphism (rs10889677 A/C) in ankylosing spondylitis: Meta-analysis in Caucasian and Asian populations. Clin Chim Acta 2018;477:53-9. [CrossRef]
2. Wang Y, Yi XD, Lu HL. Influence of CYP2C9 and COX-2 Genetic Polymorphisms on Clinical Efficacy of Non-Steroidal Anti-Inflammatory Drugs in Treatment of Ankylosing Spondylitis. Med Sci Monit 2017;23:1775-82. [CrossRef]
3. Niu Z, Wang J, Zou H, Yang C, Huang W, Jin L. Common MIR146A Polymorphisms in Chinese Ankylosing Spondylitis Subjects and Controls. PLoS One 2015;10:e0137770. [CrossRef]
4. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102-14.
5. Lu J, Getz G, Miska EA, Alvarez-Salveda E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-38. [CrossRef]
6. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol 2010;10:111-22. [CrossRef]
7. Hornstein E, Mansfield JH, Yekta S, Hu JK, Harfe BD, McManus MT, et al. The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development. Nature 2005;438:671-74. [CrossRef]
8. Lim JJ, Shin DA, Jeon YJ, Kumar H, Sohn S, Min HS, et al. Association of miR-146a, miR-149, miR-196a2, and miR-499 Polymorphisms with Ossification of the Posterior Longitudinal Ligament of the Cervical Spine. PLoS One 2016;11:e0159756. [CrossRef]
9. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361-8. [CrossRef]
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215. [CrossRef]
11. Bodal VK, Sangwan S, Bal MS, Kaur M, Sharma S, Kaur B. Association between Microrna 146a and Microrna 196a2 Genes Polymorphism and Breast Cancer Risk in North Indian Women. Asian Pac J Cancer Prev 2017;18:2345-8.
12. Smith JA. Update on ankylosing spondylitis: current concepts in pathogenesis.Curr Allergy Asthma Rep 2015;15:489. [CrossRef]
13. Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. Cell 2009;136:26-36. [CrossRef]
14. Hackl M, Heilmeier U, Weilner S, Grillari J. Circulating microRNAs as novel biomarkers for bone diseases - Complex signatures for multifactorial diseases? Mol Cell Endocrinol 2016;432:83-95. [CrossRef]
15. Arfat Y, Xiao WZ, Ahmad M, Zhao F, Li DJ, Sun YL, et al. Role of microRNAs in osteoblasts differentiation and bone disorders. Curr Med Chem 2015;22:748-58. [CrossRef]
16. Xu HY, Wang ZY, Chen JF, Wang TY, Wang LL, Tang LL, et al. Association between Ankylosing Spondylitis and the miR-146a and miR-499 Polymorphisms. PLoS One 2015;10:e0122055. [CrossRef]
17. Yang L, Du L, Yue Y, Huang Y, Zhou Q, Cao S, et al. miRNA Copy Number Variants Confer Susceptibility to Acute Anterior Uveitis With or Without Ankylosing Spondylitis. Invest Ophthalmol Vis Sci 2017;58:1991-2001. [CrossRef]
18. Qian BP, Ji ML, Qiu Y, Wang B, Yu Y, Shi W, et al. Identification of Serum miR-146a and miR-155 as Novel Noninvasive Complementary Biomarkers for Ankylosing Spondylitis. Spine (Phila Pa 1976) 2016;41:735-42. [CrossRef]
19. Duan S, Mi S, Zhang W, Dolan ME. Comprehensive analysis of the impact of SNPs and CNVs on human microRNAs and their regulatory genes. RNA Biol 2009;6:412-25. [CrossRef]
20. Lu J, Clark AG. Impact of microRNA regulation on variation in human gene expression. Genome Res 2012;22:1243-54. [CrossRef]
21. Park R, Lee WJ, Ji JD. Association between the three functional miR-146a single-nucleotide polymorphisms, rs2910164, rs57095329, and rs2431697, and autoimmune disease susceptibility: A meta-analysis. Autoimmunity 2016;49:451-8. [CrossRef]
22. Fattah SA, Ghattas MH, Saleh SM, Abo-Elmatty DM. Pre-micro RNA-499 Gene Polymorphism rs3746444 T/C is Associated with Susceptibility to Rheumatoid Arthritis in Egyptian Population.Indian J Clin Biochem 2018;33:96-101. [CrossRef]
23. Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, et al. Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 2008;118:2600-8. [CrossRef]
24. Vinci S, Gelmini S, Pratesi N, Conti S, Malentacchi F, Simi L, et al. Genetic variants in miR-146a, miR-149, miR-196a2, miR-499 and their influence on relative expression in lung cancers. Clin Chem Lab Med 2011;49:2073-80. [CrossRef]
25. Ahn DH, Rah H, Choi YK, Jeon YJ, Min KT, Kwack K, et al. Association of the miR-146aC>G, miR-196a2C>T, and miR-499A>G polymorphisms with gastric cancer and survival in the Korean population. Mol Carcinog 2013;52(S1):E39-E51. [CrossRef]
26. Jeon YJ, Kim OJ, Kim SY, Oh SH, Oh D, Kim OJ, et al. Association of the miR-146a, miR-149, miR-196a2, and miR-499 polymorphisms with ischemic stroke and silent brain infarction risk. Arterioscler Thromb Vasc Biol 2013;33:420-30. [CrossRef]
27. Jeon YJ, Kim SY, Rah H, Choi DH, Cha SH, Yoon TK, et al. Association of the miR-146aC>G, miR-149T>C, miR-196a2T>C, and miR-499A>G polymorphisms with risk of spontaneously aborted fetuses. Am J Reprod Immunol 2012;68:408-17. [CrossRef]
28. Qi J, Hou S, Zhang Q, Liao D, Wei L, Fang J, et al. A functional variant of pre-miRNA-196a2 confers risk for Behcet's disease but not for Vogt-Koyanagi-Harada syndrome or AAU in ankylosing spondylitis. Hum Genet 2013;132:1395-404. [CrossRef]