BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2021, VOL. 78, NO. 2, 63–66
https://doi.org/10.1080/09674845.2020.1786284

Association of miR-146a rs57095329 with Behçet's disease and its complications

OO Abdelaleem[a], NA Fouad[b], OG Shaker[c], HA Hussein[d], FA Ahmed[e], DY Ali[f] and HS Elsayed[a]

[a]Medical Biochemistry and Molecular Biology, Faculty of Medicine, Fayoum University, Al Fayyum, Egypt; [b]Rheumatology and Rehabilitation Department, Fayoum University, Al Fayyum, Egypt; [c]Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt; [d]Internal Medicine Department, Fayoum University, Al Fayyum, Egypt; [e]Microbiology Department, Fayoum University, Al Fayyum, Egypt; [f]Clinical Pathology, Faculty of Medicine, Fayoum University, Al Fayyum, Egypt

ABSTRACT

Background: Behçet's disease is a chronic relapsing and remitting autoimmune multisystem inflammatory disease characterised by oral aphthae, genital ulcers, skin lesions, gastrointestinal involvement, arthritis, vascular lesions and neurological manifestations. We hypothesised a link between rs57095329 of miR-146a and Behçet's disease, with further links with common clinical features.

Methods: We tested our hypothesis in 130 Behçet's disease patients and 131 age and sex-matched healthy controls. Behcet's disease current activity index (BDCAI) was used to assess patients' disease activity status. MiR-146a (rs57095329) was genotyped in all participants using RT-PCR and results in patients analysed according to clinical features.

Results: The frequency of the GG and AG genotypes in rs57095329 were strongly associated with Behçet's disease (adjusted OR 8.05, 95% CI 3.63–17.82; $P < 0.001$ and OR 2.26, 95% CI 1.27–4.04; $P = 0.006$, respectively), and in dominant (GG+AG > AA) and recessive (GG > AA+AG) models (both $P < 0.001$). Additionally, G allele distribution was significantly greater in Behçet's disease compared with controls (OR 2.85, 95% CI 1.98–4.11, $P < 0.001$). The AA genotype and A allele were linked to oral ulcers, the GG genotype and G allele to neurological disease, and the GG genotype and G allele to ocular disease (all $P < 0.01$). There were no links with genital ulceration, skin lesions, vascular disease or the result of the pathergy test.

Conclusion: The miR-146a (rs57095329) is associated with Behçet's disease and certain genotypes and alleles with oral ulcers, and with ocular and neurological manifestations.

ARTICLE HISTORY
Received 18 May 2020
Accepted 10 June 2020

KEYWORDS
Behçet's disease; rs57095329; miR-146a

Introduction

Behçet's disease is a chronic relapsing autoimmune, multisystem inflammatory disease characterized by oral aphthae in nearly all patients, genital ulcers and skin lesions, and commonly associated with systemic symptoms, including gastrointestinal involvement, arthritis, vascular lesions, pulmonary artery aneurysm and neurological manifestations [1–3]. Many studies have proposed that genetic factors, aberrant innate immune responses and endothelium dysfunction are associated with Behçet's disease. However, the pathogenesis and the underlying aetiology remain unclear [4].

MicroRNAs (miRNAs) are a group of non-coding potential transcripts that are 19–25 nucleotides in length. Recently, much attention has been focused on the action of miRNAs and the development of autoimmune and inflammatory diseases due to the disruption in the miRNA intermediated regulation of the immune cell development and function. Expression of several protein-encoding genes is found to be negatively regulated by microRNAs at the post-transcriptional level through inducing messenger RNA (mRNA) translational repression or degradation [5].

MiR-146a has been implicated in both the development and functions of innate immune cells, and mature miRNA-146a can bind to numerous target mRNAs, such as those for tumour necrosis factor receptor-associated factor 6 (TRAF-6), interleukin-1 receptor-associated kinase 1 (IRAK-1), IRAK-2 and many transcripts related to inflammatory signalling [6]. Furthermore, several studies suggest it is linked to autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis and rheumatoid arthritis (RA) [7–9]. In a large study of Chinese patients with ocular Behçet's disease, Zhou et al. [10] found a slightly decreased frequency in the CC genotype and C allele of miR-146a SNP rs2910164, but no difference in SNP rs57095329. We tested the hypothesis of a link between miR-164a SNP rs57095329 and Behçet's disease in a Caucasian population, and that any such differences were linked to the major clinical features of the disease.

CONTACT OO Abdelaleem dr.omayma@yahoo.com Faculty of Medicine, Fayoum University, Al Fayyum, Egypt
This article has been republished with minor changes. These changes do not impact the academic content of the article.

Patients and methods

We tested our hypothesis in a group of 130 patients comprising 17 females and 113 males, mean [standard deviation: SD] age 33.6 [7.8] years, attending the Department of Rheumatology and Rehabilitation, Fayoum University Hospital, Egypt. They were diagnosed in accordance with the International Study Group criteria for Behçet's disease [11], certain clinical features were determined, and the pathergy test applied (appearance of an ulcer or papules indicating a positive result). A group of 131 healthy subjects of 20 females and 111 males (chi-squared P = 0.530 to cases) aged 34.2 [8.7] (t-test P= 0.612 to cases) comprised a control group. Written signed consents were obtained from all participants. The study followed the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee in Fayoum, Faculty of Medicine. Any participant who had malignancy or other inflammatory-autoimmune disease was excluded. Behçet's disease current activity index (BDCAI) was used to assess Patients' disease activity status [12].

DNA extraction and genotyping of miR-146a rs57095329 were as follows. A 2 ml sample of venous blood was collected by sterile venepuncture into EDTA vacutainer tubes and were kept at −20°C till the time of DNA isolation and genotyping of rs57095329 using real-time PCR. Genomic DNA was extracted from whole blood from patients and controls using the QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) under manufacturer's guidelines. Samples were digested by proteinase K, ethanol was mixed with the lysate which was loaded onto a purification column, where the DNA attaches to the silica membrane. Elution Buffer was used to elute genomic DNA under low ionic strength conditions. The extracted DNA quantity was measured utilizing NanoDrop1000 (Thermo Scientific, Waltham, MA, USA). Genotyping was done using real-time polymerase chain reaction with TaqMan allelic discrimination assay (Applied Biosystems, Thermo Scientific, Waltham, MA, USA). A predesigned primer/probe set for miR-146a rs57095329 (A/G) [C_90078480_10] was used (Applied Biosystems). The PCR primers used for the rs57095329 SNP were as follows: Forward and reverse primers Context Sequence [VIC/FAM] CCCCGCGGGGCTGCG GAGAGTACAG [A/G] CAGGAAGCCTGGGGACCCAGC GCCT with probes VIC for the A allele and FAM for the G allele. Amplification of DNA was achieved in a 25 µl volume containing 12.5 µl Taq Man master mix, 1.25 µl primer/probe, 1 µl DNA and 10.25 µlH2O. Rotor gene Q System (Qiagen) was used to perform Real-time PCR with the following conditions: 95°C for 10 min then 45 cycles at 92°C for 15 s and finally, 60°C for 90 s. Fluorescence was calculated at the end of every cycle and at the endpoint.

SPSS version 25 was used to carry out statistical analyses. Chi-square (χ2) testing was used to compare frequencies of categorical data, t-test for continuously variable data. Logistic regression was performed to detect the odds ratio (OR) with 95% confidence intervals with adjustment for age and sex as possible confounders. $P < 0.05$ was considered statistically significant.

Results

The details of the clinical characteristics of the patients with Behçet's disease are shown in Table 1. MiR-146a (rs57095329) genotype was in accordance with the Hardy–Weinberg equilibrium (all $P > 0.05$) in Behçet's disease patients and controls. The genotype distributions of rs57095329 in all participants are shown in Table 2. Compared with healthy controls, both GG and AG (taking AA as reference) were strongly associated with Behçet's disease. This significance extended to dominant (GG+AG versus AA) and recessive (GG versus AG+GG) models. Presence of the G allele distribution was significantly higher in Behçet's disease compared with control individuals.

Table 1. Demographic and clinical data of Behçet's patients.

Variables	Data
Family history	28 (21.5%)
Disease duration (months)	27.2 [9.2]
Genital ulceration	16 (12.3%)
Oral ulceration	49 (37.7%)
Positive pathergy test	59 (45.4%)
Neurological manifestations	5 (3.8%)
Ocular manifestations	44 (33.8%)
Vascular manifestations	17 (13.1%)
Arthritis	6 (4.6%)
Skin Lesions	39 (30%)
BDCAI	2 (1–3)

Data are presented as mean [SD], median (interquartile range) or n (%). BDCAI; Behçet's disease current activity index.

Table 2. MiR-146a (rs57095329) genotypes and alleles.

Genotype and allele		Behçet's disease	Controls	Adjusted OR (95% CI)
Genotypes	GG	42 (32.3)	12 (9.2)	8.05 (3.63–17.82)[a]
	AG	61 (46.9)	59 (45.0)	2.26 (1.27–4.04)[b]
	AA	27 (20.8)	60 (45.8)	1 (reference)
Dominant model	GG+AG	103 (79.2)	71 (54.2)	3.22 (1.86–5.55)[a]
	AA	27 (20.8)	60 (45.8)	1 (reference)
Recessive model	GG	42 (32.3)	12 (9.2)	4.98 (2.45–10.14)[a]
	AA+AG	88 (67.8)	119 (90.8)	1 (reference)
Alleles	G	145 (55.8)	83 (31.7)	2.85 (1.98–4.11)[a]
	A	115 (44.2)	179 (68.3)	1 (reference)

Data are n (%). OR; odds ratio, CI; confidence interval. [a]p<0.001, [b]p = 0.006.

Table 3. Association of miR-146a (rs57095329) in patients with clinical manifestations.

		Genotype			
		GG	AG	AA	*P* value
Oral ulcers	Yes	5(11.9)	23(37.7)	21(77.8)	<0.001
	No	37(88.1)	38(62.3)	6(22.2)	
Genital ulcers	Yes	5(11.9)	6(9.8)	5(18.5)	0.505
	No	37(88.1)	55(90.2)	22(81.5)	
Skin lesions	Yes	16(38.1)	17(27.9)	6(22.2)	0.329
	No	26(61.9)	44(72.1)	21(77.8)	
Pathergy test	+ve	15(35.7)	28(45.9)	16(59.3)	0.158
	-ve	27(64.3)	33(54.1)	11(40.7)	
Ocular disease	Yes	22(52.4)	22(36.1)	0(0)	<0.001
	No	20(47.6)	39(63.9)	27(100)	
Vascular disease	Yes	6(14.3)	11(18.0)	0(0)	0.066
	No	36(85.7)	50(82.0The)	27(100)	
Neurological disease	Yes	5(11.9)	0(0)	0(0)	0.004
	No	37(88.1)	61(100)	27(100)	

Data are presented as n (%). *P* values by chi-squared.

Table 3 shows miR164a variants according to clinical features. Patients with oral ulcers were more likely to carry the AA genotype and less likely to carry the GG genotype in a linear trend. Accordingly, oral ulceration was strongly linked to the A allele ($P < 0.001$). Similarly, patients with ocular disease were more likely to carry the GG genotype and less likely to carry the AA genotype in a linear trend, and overall the G allele was strongly linked to this complication ($P < 0.001$). Presence of neurological disease was associated with the GG genotype, and the G allele ($P < 0.001$). There were no links with genital ulcers, skin lesions, the pathergy test or vascular disease. Analysis for arthritis was not attempted as it lacked sufficient statistical power.

Discussion

We add to the literature in reporting that rs57095329 in miR-146a GG and AG genotypes and the G allele distribution are significantly higher in Behçet's disease compared with control individuals. Furthermore, we show links with clinical features. The GG and AG genotypes were associated with more frequent ocular involvement and neurological manifestations compared to the AA genotype. Conversely, the AA genotype and A allele were linked to oral ulceration.

Behçet's disease is a chronic autoimmune disease, its main symptoms are recurring oral and genital aphthous ulcers, skin lesions and arthritis. Besides, involvement of the ophthalmic, vascular and central nervous systems are considered serious complications [1]. Both genetic and environmental factors have been proposed to cause immune dysfunction resulting in the appearance of the clinical manifestations of Behçet's disease [13]. Understanding the underlying genetic factors that contribute to disease pathogenesis may help to advance management strategies and novel prophylactic therapeutics [14]. Cloning and bioinformatics studies show that 30% of all human genes are regulated by miRNAs [6,15], and SNPs of miRNAs are rapidly gaining importance as modulators of miRNAs' regulatory role in inflammatory and autoimmune diseases [9,16]. During the past decade, miRNAs, including miR-146a, have become novel biomarkers in numerous autoimmune diseases [17].

The rs57095329 G allele has been linked to decreased protein-binding affinity of the transcription factor Ets-1, resulting in down-regulation of miR-146a and increased risk of SLE [18]. The aforementioned polymorphism is placed in the promoter of miR-146a. It was later established that rs57095329 interfered with V-Ets oncogene homologue 1 (Ets-1) binding to miR-146a, affecting the levels of expressed miR-146a contributing to the occurrence of the disease and which can result in genetic predisposition to diseases [10,18,19]. Stimulation of the innate immune system of the host leads to activation of toll-like receptor 4 (TLR4) in the NF-κB-signalling pathway. MiR-146a is induced upon stimulation of TLR4 resulting in reduced levels of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) [20]. Furthermore, polymorphisms in miR-146a genes lead to downregulation which negatively regulates its target genes TRAF-6 and IRAK-1 and this may explain the occurrence of inflammation in autoimmune disease including Behçet's disease [10,21]. In previous studies, miR-146a negatively correlated with inflammatory mediators (COX-2, TNF-α, IL-1β, IRAK1 and TRAF6) as well as oxidative stress status [22]. Furthermore, Wang et al. suggested that miR-146a inhibited NADPH oxidase 4 expression, resulting in endothelial inflammation [23]. Besides, rs57095329 was associated with the downregulation of miR-146a expression level. Kolahi et al. [24] failed to find an association between the expression of miR-146a and TNF-α and CTLA-4 genes.

Our data contrasts with that of Zhou et al. [10] who failed to find a link with miR-146a rs57095329 in Chinese patients with ocular Behçet's. In the same racial group, Hou et al. [25] failed to find a link between the copy number of miR-146a and Behçet's disease, whilst Kolahi et al., in a small study of Iranians, failed to link miR-146a to Behçet's disease, although they did find a link with phlebitis but not uveitis. These inconsistencies may be due to racial and ethnic differences as the prevalence of Behçet's varies widely, from up to 600/100,000 in parts of Turkey, 68/100,000 in Iran, 16/100,000 in Egypt and 14/100,000 in China [26]. Thus, further larger studies from various countries are needed to verify and explain the results of the present study.

We acknowledge the limitation of small numbers in parts of the clinical analyses, where the link with vascular disease may be a false negative, but the strong link with neurological disease suggests that this is not a false positive.

This work represents an advance in biomedical science because it shows that miR-146a (rs57095329) polymorphism is associated to Behçet's disease in a Caucasian population and is linked to mouth ulcers and both ocular and neurological manifestations.

Summary table

What is known about this subject:
- Behçet's disease is a chronic relapsing autoimmune, multisystem inflammatory disease.
- Data points to links between miRNAs in autoimmune disease, including Behçet's disease.
- Data from China fail to link miR-146a to Behçet's disease.

What at this study shows:
- Variants of rs57095329 of miR-146a are linked to Behçet'sdisease in a Caucasian population.
- Rs57095329 of miR-146a is associated with ocular involvement, vascular and neurological manifestations.
- Different genotypes and alleles are link to oral ulceration, ocular disease and neurological disease, but not to genital ulceration, skin lesions, the pathergy test or vascular disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work did not receive any funds from funding agencies.

References

[1] Direskeneli H. Autoimmunity vs autoinflammation in Behçet's disease: do we oversimplify a complex disorder? Rheumatology. 2006;45:1461–1465.
[2] Ideguchi H, Suda A, Takeno M, et al. Behçet disease: evolution of clinical manifestations. Medicine. 2011;90:125–132.
[3] Ceylan N, Bayraktaroglu S, Erturk SM, et al. Pulmonary and vascular manifestations of Behcet disease: imaging findings. Am J Roentgenol. 2010;194:W158–64.
[4] De Menthon M, Lavalley MP, Maldini C, et al. HLA-B51 /B5 and the risk of Behçet's disease: A systematic review and meta-analysis of case-control genetic association studies. Arthritis Rheum. 2009;61:1287–1296.
[5] Waller P, Blann AD. Non-coding RNAs – A primer for the laboratory scientist. Br J Biomed Sci. 2019;76:157–165.
[6] Janssens S, Beyaert R. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. Mol Cell. 2003;11:293–302.
[7] Singh S, Rai G, Aggarwal A. Association of microRNA-146a and its target gene IRAK1 polymorphism with enthesitis related arthritis category of juvenile idiopathic arthritis. Rheumatology Int. 2014;34:1395–1400.
[8] Ayeldeen G, Nassar Y, Ahmed H, et al. Possible use of miRNAs-146a and –499 expression and their polymorphisms as diagnostic markers for rheumatoid arthritis. Mol Cell Biochem. 2018;449:145–156.
[9] Apparailly F. Looking for microRNA polymorphisms as new rheumatoid arthritis risk loci? Joint Bone Spine. 2010;77:377–379.
[10] Zhou Q, Hou S, Liang L, et al. MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behçet's disease and Vogt–Koyanagi–Harada syndrome. Ann Rheum Dis. 2014;73:170–176.
[11] Davatchi F, Assaad-Khalil S, Calamia KT, et al. 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;28:338–347.
[12] Lawton G, Bhakta BB, Chamberlain MA, et al. The Behcet's disease activity index. Rheumatology. 2004;43:73–78.
[13] Pineton de Chambrun M, Wechsler B, Geri G, et al. New insights into the pathogenesis of Behcet's disease. Autoimmun Rev. 2012;11:687–698.
[14] Gheita TA, Gheita HA, Kenawy SA. The potential of genetically guided treatment in Behçet's disease. Pharmacogenomics. 2016;17:1165–1174.
[15] Lu J, Clark AG. Impact of microRNA regulation on variation in human gene expression. Genome Res. 2012;22:1243–1254.
[16] Zhao ZZ, Croft L, Nyholt DR, et al. Evaluation of polymorphisms in predicted target sites for micro RNAs differentially expressed in endometriosis. Mol Hum Reprod. 2011;17:92–103.
[17] Zeng L, Cui J, Wu H, et al. The emerging role of circulating microRNAs as biomarkers in autoimmune diseases. Autoimmunity. 2014;47:419–429.
[18] Luo X, Yang W, Ye DQ, et al. A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. PLoS Genet. 2011;7:e1002128.
[19] Jazdzewski K, Murray EL, Franssila K, et al. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A. 2008;105:7269–7274.
[20] Boldin MP, Baltimore D. MicroRNAs, new effectors and regulators of NF-κB. Immunol Rev. 2012;246:205–220.
[21] Ibrahim W, Sakr BR, Obaya E, et al. MicroRNA-146a expression and microRNA-146a rs2910164 polymorphism in Behçet's disease patients. Clin Rheumatol. 2019;38:397–402.
[22] Xie Y, Chu A, Feng Y, et al. MicroRNA-146a: a comprehensive indicator of inflammation and oxidative stress status induced in the brain of chronic T2DM rats. Front Pharmacol. 2018;9:478.
[23] Wang HJ, Huang YL, Shih YY, et al. MicroRNA-146a decreases high glucose/thrombin-induced endothelial inflammation by inhibiting NADPH oxidase 4 expression. Mediators Inflamm. 2014;2014:379537.
[24] Kolahi S, Farajzadeh MJ, Alipour S, et al. Determination of miR-155 and miR-146a expression rates and its association with expression level of TNF-α and CTLA4 genes in patients with Behçet'sdisease. Immunol Lets. 2018;204:55–59.
[25] Hou S, Ye Z, Liao D, et al. miR-23a, miR-146a and miR-301 confer predisposition to Vogt-Koyanagi-Harada syndrome but not to Behçet's disease. Sci Reps. 2016;6. DOI:10.1038/srep20057.
[26] Adeeb F, Stack AG, Fraser AD. Kitting the treads of silk through time: Behçet's disease – past, present and future. Int J Rheumatol. 2017;2017:1–13.