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Research Article

关于白癜风病因的新见解，以及全身性和节段性白癜风中 lncRNA 和某些免疫学参数与黑色素浓度、激素和 瑟图因 1 基因表达的关联

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【摘要】：
背景：长链非编码 RNA（LncRNA）是一类非编码的遗传物质，长度超过 200bp，具有调节基因表达的能力。本研究旨在检测白癜风患者中 lncRNA SIRT-1、MCH、IL-17、IL-33、IFNγ 的表达水平，并探讨 LncRNA 与研究参数之间的可能相关性。方法：对 30 例全身性白癜风（GV）患者（患者接受治疗和未治疗）、30 例节段性白癜风（SV）患者（患者接受治疗和未治疗）和 25 名健康对照（HC）进行研究，采用 ELISE 法测定血清 IL-17、IL-33、IFNγ、SIRT-1 和 PMCH 浓度，并对 GV 和 SV 患者的 LncRNA SIRT-1 和 LncRNA PMCH 进行基因表达分析，以阐明它们在白癜风发病机制中的潜在作用。结果：LncRNA SIRT-1 在 GV 中的表达明显高于 SV（p=0.030，Mann-Whitney 检验），平均表达水平分别为 2.851（SE：1.052）和 0.507（SE：0.134）。而 LncRNA PMCH 在两种白癜风类型中的表达无明显差异。结论：本研究证实了 LncRNA SIRT-1 和 LncRNA PMCH 在白癜风患者中的表达失调，提示二者可能通过血清 SIRT-1、MCH 下调和 IL-17 上调参与了白癜风的发病过程。
【关键词】：白癜风、长链非编码 RNA、Sirtuin 1 基因、黑色素浓缩激素、白细胞介素。

New Insights on the Etiology of Vitiligo, Association of LncRNAs and Certain Immunological Parameters with the Expression of Melanin Concentration, Hormone, and Sirtuin 1 Genes in Generalized and Segmented Vitiligo

【Abstract】：
Background: Long non-coding RNAs (LncRNAs) are a type of non-coding, genetic material of LncRNAs that are more than 200 bp in length and have the ability to regulate gene expression. This study aimed to detect the expression levels of lncRNA SIRT-1, MCH, IL-17, IL-33, and IFNγ in subjects with vitiligo and to investigate the possible correlation between lncRNAs and the parameters of the study. **Methods:** The study was conducted on 30 patients with generalized vitiligo(GV) (patients were treated and untreated), 30 patients with segmented vitiligo (SV))(patients were treated and untreated), and 25 Healthy control (HC) serum concentration of IL-17, IL-33, IFNγ, SIRT 1, and PMCH were determined using ELISE and gene expression analysis of LncRNA SIRT-1 and LncRNA PMCH was performed in patients with GV and SV to elucidate their potential roles in the pathogenesis of vitiligo. **Results:** LncRNA SIRT-1 expression was significantly higher in GV than in SV (p=0.030, Mann-Whitney test), with mean expression levels of 2.851 (SE: 1.052) and 0.507 (SE: 0.134), respectively. In contrast, no significant difference in LncRNA PMCH expression was observed between the two vitiligo types. **Conclusion:** This study demonstrated the deregulated expression of expressions of LncRNA SIRT-1 and LncRNA PMCH in patients with vitiligo, suggesting that both contribute to the pathogenesis of vitiligo, perhaps through serum SIRT-1, MCH downregulation, and IL-17 upregulation.
【Key words】： Vitiligo, long noncoding RNAs, Sirtuin 1 gene, melanin-concentrating hormone, interleukins

1. Introduction

Vitiligo is a condition that causes the skin to lose its pigment, leading to patches of lighter skin. This is because the melanocytes responsible for producing melanin are exhausted in certain areas of the skin. A

noticeable sign of vitiligo is the presence of white spots with well-defined borders that are not scaly or dry [1].

Understanding of the etiology of vitiligo has advanced significantly in recent years. It is now known definitively that it is an autoimmune disorder related to oxidative stress and metabolism in addition to environmental and genetic factors [1]. Vitiligo, also known as GV, can appear on any part of the body but primarily on the hands, face, and fingers. This condition usually affects both sides of the body and presents as matching spots and patches [2]. Segmental vitiligo accounts for approximately 5-16% of all cases of vitiligo, and affects both men and women in almost equal numbers, although some studies suggest that there may be a slight tendency for more cases in females. One of the unique aspects of segmental vitiligo is that it typically only appears in a specific area of the body and creates a distinct division along the midline [3].

Long noncoding RNAs (LncRNAs) are noncoding transcripts with more than 200 nucleotides (200 nt), nonprotein coding transcripts that do not have any significant open reading frames[4] also description as 'mRNA-like' because they have polyadenylate and spliced, on the other hand not all LncRNAs have 7-methylguanosine capped or polyadenylated [5]. According to their location on chromosomes, LncRNAs are divided into many classifications: overlapping LncRNAs intergenic, antisense, bidirectional, and intronic; and antisense LncRNAs transcription from the opposite direction of the coding gene of their sense protein or a sense strand-derived RNA [6]. LncRNA molecules have an incredible ability to regulate gene expression by forming specific interactions with target genes to produce enzymes that can modify chromatin structure or enhancer RNA and sponge miRNA [7]. The MCH peptide consists of 19 amino acids expressed in the lateral hypothalamic brain area, and it has several physiological functions, such as food intake, memory formation, sleep-wake arousal, reproduction, and nutrient sensing [8]. Thus, we found that a balance between MCH and melanocortin system activity is likely required for skin pigmentation, and dysregulation of these pathways could underlie adverse human skin conditions. Exogenous application of MCH peptides induces melanosome aggregation, which can regulate skin pigmentation [9]. Human Sirtuin 1 gene (SIRT1) was detected on chromosome 10q22.1. It is composed of nine exons and eight introns that combine to form a protein consisting of 747 amino acid residues. This protein includes C-terminal and N-terminal domains, as well as a catalytic region [10]. Cytokines, which are critical for the development of autoimmune disorders, may also contribute to the loss of skin pigmentation. Interleukin (IL)-17 is increasingly implicated in the pathogenesis of several immune-mediated diseases.

In patients with vitiligo, keratinocytes have been shown to secrete IL33, which is transferred from the nucleus to the cytoplasm. Furthermore, IL-33 acts on keratinocyte modification through basic fibroblast growth factor (bFGF) and stem cell factor (SCF) expression, which are essential for melanocyte development and are suppressed by IL-33, while IL-6 and tumor necrosis factor α (TNF-α) expression can be increased [11]. IFNγ plays a crucial role in the worsening of inflammation and the collaboration between keratinocytes and lymphocytes. IFNγ enhances the movement of melanocyte-specific CD8 cytotoxic T lymphocytes (CTLs) toward the skin, stimulating keratinocytes to produce various chemokines, primarily CXCL10. The relationship between CXCL10 and its autoreactive T cell receptor CXCR3 plays a role in sustaining skin depigmentation in vitiligo [12]. Thus, the aim of the current study was to explore the possible correlation between the LncRNA and some immunological parameters with the expression of MCH and SIRT-1 and their role in vitiligo pathogenesis.

2. Methodology

2.1. Ethical Approval

This study was approved by the Council of AL-Anbar University. Written informed consent was obtained from each individual who participated in the study. The personal information for each patient and control was presented in a questionnaire form under the supervision of the consultant after obtaining approval for obtaining samples from patients and controls. All experimental procedures were conducted in accordance with the Declaration of Human Ethics Committee of the Ministry of Health of Iraq.

The study was conducted on 30 (GV), 30 (SV), and 25 age- and sex-matched healthy controls (HCs) during the time interval between **December** 2021 and **July** 2022. HCs did not have vitiligo or any other disease, had no history of smoking or alcohol consumption, did not have acute illness or infection at the time of sampling, and were not specific for any prescribed drugs, dietary restrictions, or other diseases that were excluded. All patients enrolled in this study were older than 11 years and below 40 years of age. In this study, verbal agreements of the volunteer patients were taken, and after clinical examination by the consultant physicians and after approval from the patients, we collected the samples. The history of treatment and lifestyle was taken into account, and the questionnaire was completed after clinical diagnosis by the consulting doctors, and the names of the patients were coded to preserve their confidential data.

2.2. Patients

Ethics From each patient 3 ml of blood was obtained, and blood was obtained by venipuncture, which was used for the analysis required to complete the research. The samples were divided into two parts.

The first part of the sample was placed in a gel tube, and serum was isolated for use in the estimation of serum concentrations of IL-17, IL-33, IFNγ, MCH, and SIRT-1 by ELISA. The second part of the sample (300 µl) was used for RNA isolation using TRIzol. All samples were transported to a freezer at -80 °C until

use.

The protocol supplied by Bioneer was used for RNA isolation, using a specific kit for genomic RNA purification. Quantitative real-time PCR (qRT–PCR) was used to assess the expression level in different groups of patients and controls for lncRNA (SIRT-1 and MCH), using qRT–PCR primers, which were designed to evaluate the fold change in each case. The primer sequences for the genes studied are listed in Table 1.

表 **1**

Table 1 Spearman's rank correlation coefficient between the parameters

Primer Name	Description	Sequence	Annealing	Amplicon size (bp)
lncSIRT1	Forward	GAGCAAAGGAGGCACAAAAC	159	
	Reverse	GCCCAGTGTCAATCTGGAAT		
lncMCH	Forward	GGGGATGAAGAAAACTCAGC	214	
	Reverse	AACGGAACTGCACTGATAACG		
GAPDH	Forward	TGCCACCCAGAAGACTGTGG	129	58
	Reverse	TTCAGCTCAGGGATGACCTT		

2.3. Statistical Analysis

Data analysis and presentation were performed using GraphPad Prism 8 (GraphPad Software, California, USA) for graphical representations, Jamovi 2.3.28 (The Jamovi Project 2023, Sydney, Australia) for correlation analyses, and MedCalc (MedCalc Software Ltd, Ostend, Belgium) for receiver operating characteristic (ROC) curve analysis. The normality of the data was assessed using the Shapiro-Wilk test. Continuous variables are presented as mean ± standard deviation (SD) for normally distributed data or median and interquartile range (IQR) for non-normally distributed data. Categorical variables are presented as frequencies and percentages. Comparisons among three or more groups were conducted using one-way analysis of variance (ANOVA) for normally distributed data or the Kruskal-Wallis test for non-normally distributed data. ROC curve analysis was used to evaluate the diagnostic accuracy of serum biomarkers in distinguishing patients with vitiligo from healthy controls. Gene expression data obtained from RT-PCR were analyzed using the 2-ΔΔCt method.

2.4. Statistical Analysis

Data were analyzed using SPSS software version 25 and are displayed as percentages (%) and numbers (N). Chi-square tests were used for statistical analysis to examine the relationship between demographic characteristics and participants' knowledge and attitudes. Statistical significance was set at $P \leq 0.05$. Patients with incomplete questionnaires were excluded from the study.

3. Results

3.1. Clinicopathological Features

Demographic and clinical characteristics of the participants are presented in Table 2. The median age of the control group (n=25) and vitiligo group (n=63) was 25 years, with no significant difference between the groups (p>0.05). The sex distribution was similar in both groups, with 48% males and 52% females in the control group and 52.38% males and 47.62% females in the vitiligo group (p>0.05). Among the patients with vitiligo, 49.2% had GV and 50.8% had SV vitiligo. The majority of vitiligo patients (54%) had the disease for 1-5 years, while 22% had it for less than a year, and 24% for more than 5 years. A family history of vitiligo was present in 6.35% of the patients with vitiligo and in none of the controls, but the difference was not statistically significant (p>0.05). Approximately 46% of the patients with vitiligo were undergoing treatment at the time of the study.

表 **2**

Table 2 Demographic and clinical characteristics of the study participants

	Control group (n= 25)	Vitiligo group (n= 50)	*P*-value
Age (years)	25 (15.5, 33)	25 (16, 33)	0.721
Sex			
Male	12 (48)	33 (52.38)	0.797
Female	13 (52)	30 (47.62)	
Vitiligo types			
Generalized	–	31 (49.2)	NA
Segmental	–	32 (50.8)	
Period of disease			
<1 year	–	14 (22.2)	NA
1–5 years	–	34 (54)	
>5 years	–	15 (23.8)	
Family history			
Yes	0 (0)	4 (6.35)	0.574
No	25 (100)	59 (93.65)	
Treatment			
Yes	–	29 (46.03)	NA
No	–	34 (53.97)	

Note: Data are presented as median (interquartile range) for age and frequency (percentage) for the other parameters. *P*-values from the Mann–Whitney U test for age and Fisher's exact test for other parameters. NA: Non-applicable

3.2. Circulating Biomarkers

The circulatory levels of various biomarkers (SIRT-1, MCH, IL-17, IFNγ, and IL-33), as well as their ratios, were also investigated in individuals with vitiligo and healthy controls (**Table 3**). The ratios of these biomarkers were calculated to provide a comprehensive understanding of their interrelationships in the context of vitiligo. The plasma levels of SIRT-1 and MCH were significantly lower in the vitiligo group than in the control group (p<0.05). In contrast, IL-17, IFNγ, and IL-33 levels were significantly higher in the vitiligo group (p<0.05).

表 **3**

Table 3 Circulatory biomarker levels and their ratios in the control and vitiligo groups

	Control group	Vitiligo group	*P*-value

	(n= 25)	(n= 63)	
	Median (IQR)	Median (IQR)	
SIRT-1 (pg/mL)	1.19 (2.52)	0.56 (0.26)	0.001
MCH (pg/mL)	4.92 (3.95)	3.04 (1.08)	<0.001
IL-17 (pg/mL)	0.99 (0.39)	7.58 (2.97)	<0.001
IFNγ (pg/mL)	1.34 (0.78)	3.53 (1.2)	<0.001
IL-33 (pg/mL)	2.34 (2.95)	7.61 (3.24)	<0.001

Note: Data are presented as mean (95% confidence interval) and median (interquartile range) because the data were not normally distributed. P-values were calculated using the Mann-Whitney U test to compare the control and vitiligo groups.

According to vitiligo type, the circulatory levels of biomarkers SIRT-1, MCH, IL-17, IFNγ, and IL-33 were further analyzed to investigate potential differences based on the type of vitiligo, namely GV and SV, compared with the HCs group (Figure 1). SIRT-1 levels were significantly lower in the SV group (IQR: 0.4-0.69) compared in the HC group (IQR: 0.46-2.98) (p<0.05, Figure 1A). However, SIRT-1 levels did not differ significantly between the GV group (IQR: 0.51-0.73) and HC groups. In addition, MCH levels were significantly lower in both the GV (IQR: 2.42-3.51) and SV (IQR: 2.35-3.51) groups than in the HC group (IQR: 3.04-7.00) (p<0.05, Figure 1B). Moreover, IL-17 levels were significantly higher in both the GV (IQR: 5.91-9.99) and SV (IQR: 6.22-8.42) groups than in the HC group (IQR: 0.85-1.31) (p<0.05, Figure 1C). Similarly, IFNγ levels were significantly elevated in both the GV (IQR: 3.02-4.23) and SV (IQR: 2.73-4.37) groups than in the HCs group (IQR: 0.93-1.87) (p<0.05, Figure 1D). Lastly, IL-33 levels were significantly higher in both GV (IQR: 6.41-9.32) and SV (IQR: 5.44-8.58) groups than in the HC group (IQR: 1.45-4.74) (p<0.05, Figure 1E).

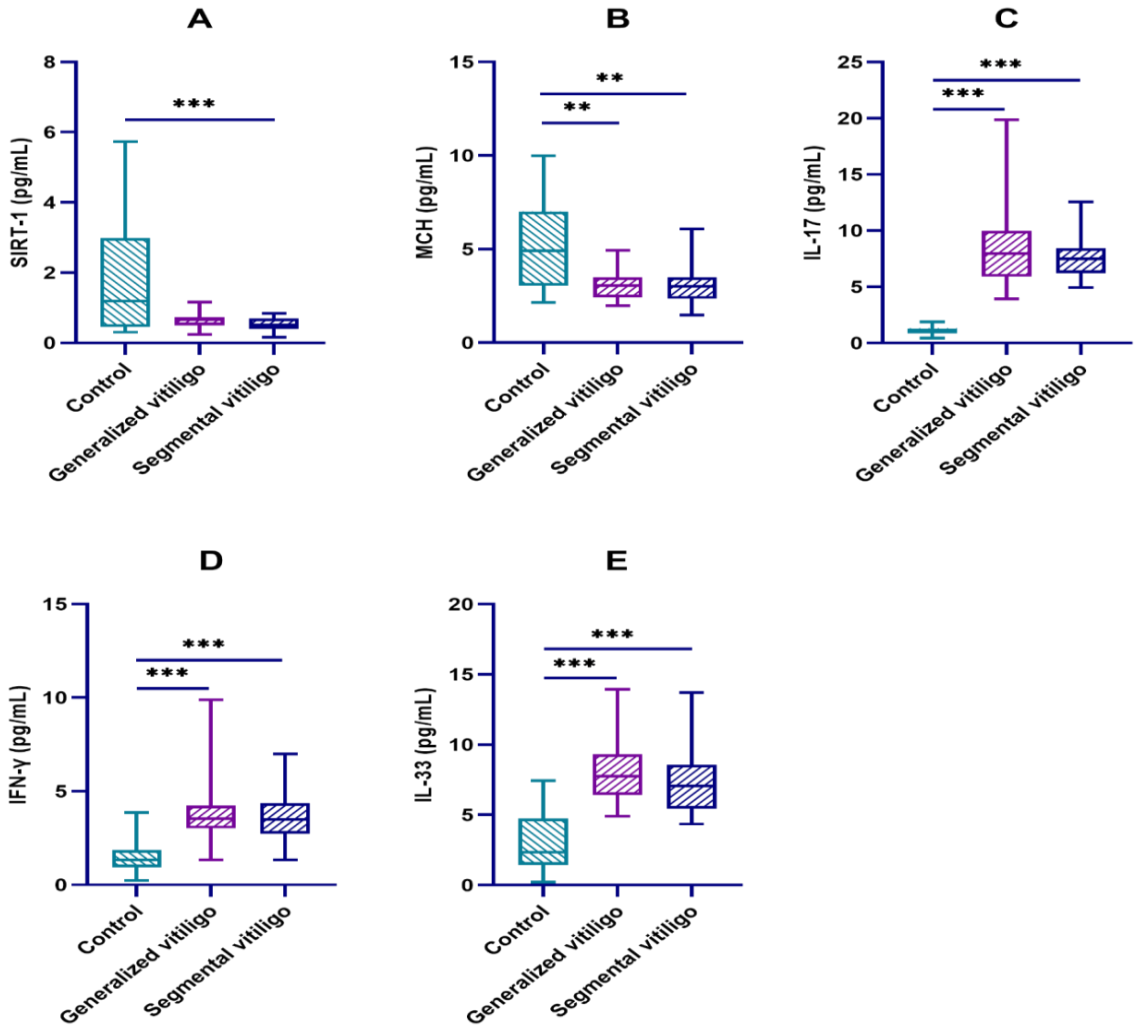

图 1

Fig.1 Comparison of serum levels of SIRT-1, MCH, IL-17, IFNγ, and IL-33 among healthy controls and patients with generalized and segmental vitiligo. (A) SIRT-1 levels, (B) MCH levels, (C) IL-17 levels, (D) IFNγ levels, and (E) IL-33 levels. Data presented in the box plots are presented as median and interquartile range (with minimum and maximum values). Statistical significance was determined using Dunn's multiple comparison test (* p<0.05, ** p<0.01, ***p< 0.001, ns: non-significant).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of the studied biomarkers (SIRT-1, MCH, IL-17, IFNγ, and IL-33) in differentiating patients with

vitiligo from healthy controls. The ROC analysis results are shown in Figure 2. SIRT-1 demonstrated a high sensitivity of 98.4% (95% CI: 91.5 - 100.0) and moderate specificity of 64% (95% CI: 42.5 - 82.0) at a cut-off value of ≤0.986 (Figure 2A). The area under the curve (AUC) was 0.721 (p=0.006), indicating fair diagnostic accuracy. The positive and negative likelihood ratios (LR+ and LR-) were 2.73 and 0.025, respectively. In addition, MCH exhibited a sensitivity of 95.2% (95% CI: 86.7 - 99.0) and specificity of 56% (95% CI: 34.9 - 75.6) at a cut-off value of ≤4.663 (Figure 2B). The AUC was 0.77 (p<0.001), suggesting fair diagnostic performance. The LR+ and LR values were 2.16 and 0.085, respectively.

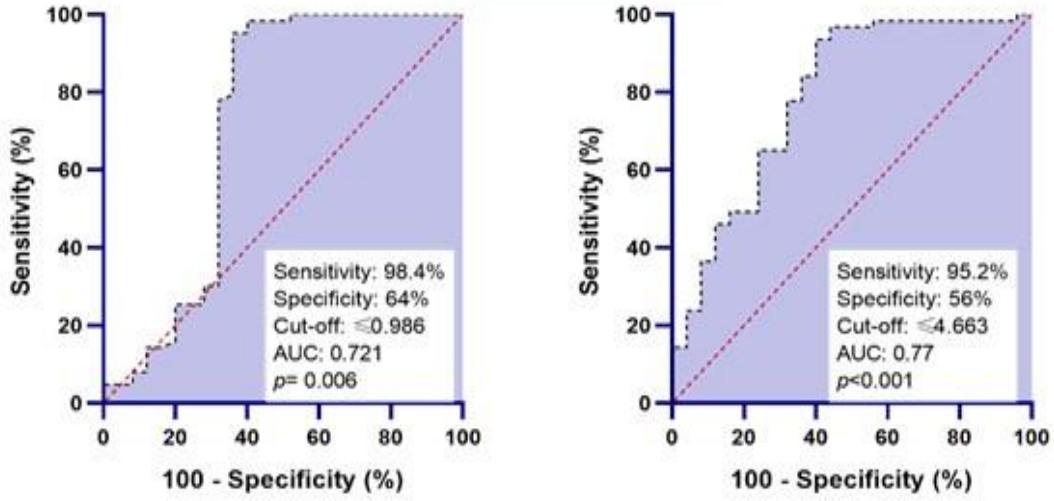

图 2

Fig.2 Receiver operating characteristic (ROC) curves of biomarkers in vitiligo and healthy controls. (A) SIRT-1 and (B) MCH. The area under the curve (AUC), sensitivity, specificity, cutoff values, and *p*-values are shown for each biomarker.

3.3. Gene Expression Analysis

Gene expression analysis of SIRT-1 and PMCH was performed in patients with GV and SV to elucidate their potential roles in the pathogenesis of vitiligo. *LncRNA* SIRT-1 expression was significantly higher in patients with GV than in those with SV (p=0.030, Mann-Whitney test), with mean expression levels of 2.851 (SE: 1.052) and 0.507 (SE: 0.134), respectively (Figure 3A). In contrast, no significant difference in *LncRNA* PMCH expression was observed between the two vitiligo types (p=0.695, Mann-Whitney test), with mean expression levels of 1.750 (SE: 0.870) in GV patients and 1.883 (SE: 0.962) in SV patients (Figure 3B). However, to further elucidate the potential impact of treatment on gene expression in different vitiligo types, we stratified the data based on the treatment status (treated or untreated) within each vitiligo type (generalized or segmental).

Interestingly, no significant differences in LncRNA SIRT-1 or PMCH expression were found among the four subgroups (untreated GV, treated GV, untreated SV, and treated SV) (p=0.142 and p=0.178, respectively, Kruskal-Wallis test).

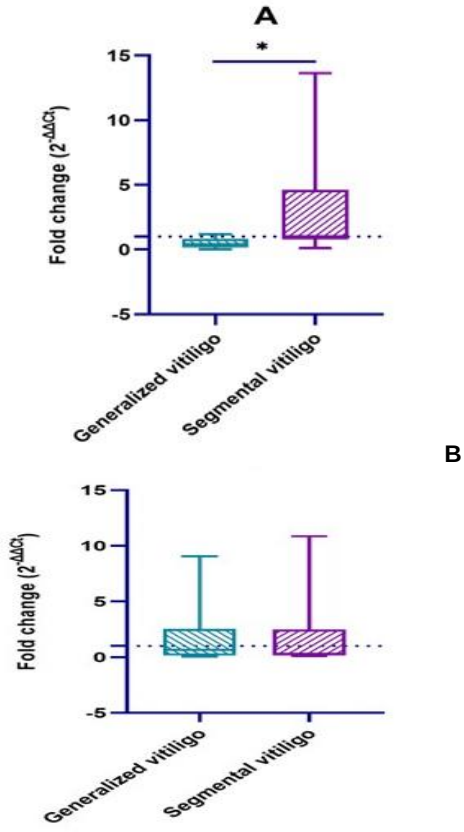

图 2

Fig.2 Gene expression levels of (A) LncRNA SIRT-1 and (B) PMCH in patients with GV and SV

For SIRT-1, the mean expression levels were3.838 (SE: 1.770) in untreated SV, 1.700 (SE: 0.936) in

treated SV, 0.508 (SE: 0.208) in untreated GV, and 0.507 (SE: 0.196) in treated GV. For PMCH, the mean expression levels were 0.319 (SE: 0.136) in untreated SV, 3.419 (SE: 1.695) in treated SV, 0.702 (SE: 0.399) in untreated GV, and 2.828 (SE: 1.654) in treated GV.

3.3. Correlation Analysis

The correlations between immunological cytokines and circulatory levels of SIRT-1 and MCH were investigated using Spearman's rank correlation coefficient. the relationships between SIRT-1 and MCH (r = 0.224, p < 0.01), In the overall sample, significant correlations were observed between IL-17 and SIRT-1 (r = -0.324, p < 0.01), IL-17 and MCH (r= -0.211, p < 0.05), IL-17 and IFNγ (r= 0.379, p< 0.01), and IL-17 and IL-33 (r = 0.253, p < 0.05). Among patients, a significant negative correlation was observed between IFNγ and SIRT-1 (r = - 0.205, p< 0.05) and IFNγ and MCH (r = -0.121, p< 0.05), and a significant positive correlation was observed between IFNγ and IL-33 (r = 0.425, p< 0.05). A weak negative correlation between SIRT-1, MCH, and IL-33 is shown in Table 4.

Table 4 Spearman's rank correlation coefficient between the parameters

		SIRT-1	MCH	IL-17	IFNγ	IL-33
Overall (all participants)	SIRT-1	—				
	MCH	0.224	—			
	IL-17	-0.324**	-0.211	—		
	IFNγ	-0.133	-0.305**	0.379**	—	
	IL-33	-0.163	-0.236*	0.253*	0.425***	—
Patients	SIRT-1	—				
	MCH	-0.121	—			
	IL-17	-0.201	0.097	—		
	IFNγ	-0.205	-0.008	-0.171	—	
	IL-33	-0.270*	-0.161	-0.230	0.425	—

We also investigated the association between the lncRNA expression of *SIRT1* and *MCH* genes and plasma concentrations in patients with vitiligo. The results presented in Figure 4 demonstrate varying degrees of correlation between lncRNA expression and protein levels depending on the patient subgroups analyzed.

表 **4**

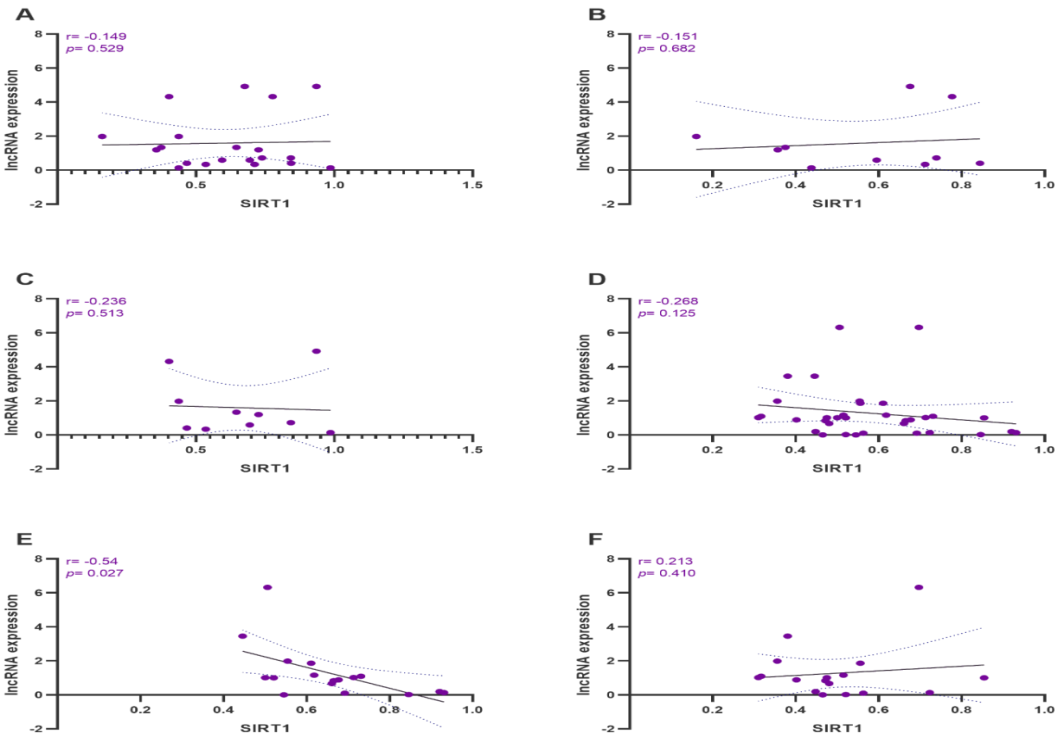

图 **4**

Fig.4 Scatter plots illustrating the association between lncRNA expression of SIRT1 and SIRT1 plasma concentration in segmental and generalized vitiligo patients. (A-C) Correlation analysis in segmental vitiligo patients: (A) all patients (n=30), (B) treated patients (n=20), and (C) non-treated patients (n=10). (D-F) Correlation analysis in generalized vitiligo patients: (D) all patients (n=30), (E) treated patients (n=18), and (F) non-treated patients (n=12). The x-axis represents SIRT1 plasma concentration (pg/ml), whereas the y-axis represents lncRNA expression of *SIRT1* (fold change). Each data point corresponds to an individual patient, with the solid line indicating the best fit and the shaded area representing the 95% confidence interval.

When patients were divided into subgroups based on treatment status, a moderate, statistically significant negative correlation (r= -0.396, p= 0.04) was observed in treated patients, while non-treated patients exhibited

a weak significant negative correlation (r= -0.154, *p*= 0.444). Figure 4 presents the association between lncRNA expression of *SIRT1* and SIRT1 concentration in patients with segmental and generalized vitiligo.

Among patients with segmental vitiligo, a weak, significant negative correlation was observed in the overall group (Figure 4A; r= -0.149, *p*= 0.529), treated patients (Figure 4B; r= -0.151, *p*= 0.682), and non-treated patients (Figure 4C; r= -0.236, *p*= 0.513). Conversely, in patients with generalized vitiligo, a weak, significant negative correlation was found in the overall group (Figure 4D; r= -0.268, *p*= 0.125), whereas treated patients displayed a strong, statistically significant negative correlation (Figure 4E; r= -0.54, *p*= 0.027). Intriguingly, untreated patients with generalized vitiligo exhibited a weak, non-significant positive correlation (Figure 4F; r= 0.213, *p*= 0.410).

Expanding on these findings, Figure 5 presents the association between lncRNA expression of MCH and the concentration of MCH in segmental and generalized vitiligo patients. In patients with segmental vitiligo, a weak, non-significant negative correlation was observed in the overall group (Figure 5A; r= -0.075, *p*= 0.548), whereas treated patients displayed a strong, statistically significant negative correlation (Figure 5B; r= -0.83, *p*= 0.005). Non-treated patients with segmental vitiligo exhibited a weak, significant negative correlation (Figure 5C; r= -0.139, *p*= 0.707). In contrast, patients with generalized vitiligo showed very weak, non-significant negative correlations in the overall group (Figure 4D; r= -0.04, p= 0.747) and non-treated patients (Figure 5F; r= -0.066, *p*= 0.802), whereas treated patients demonstrated a very weak, non-significant positive correlation (Figure 5E; r= 0.102, *p*= 0.694).

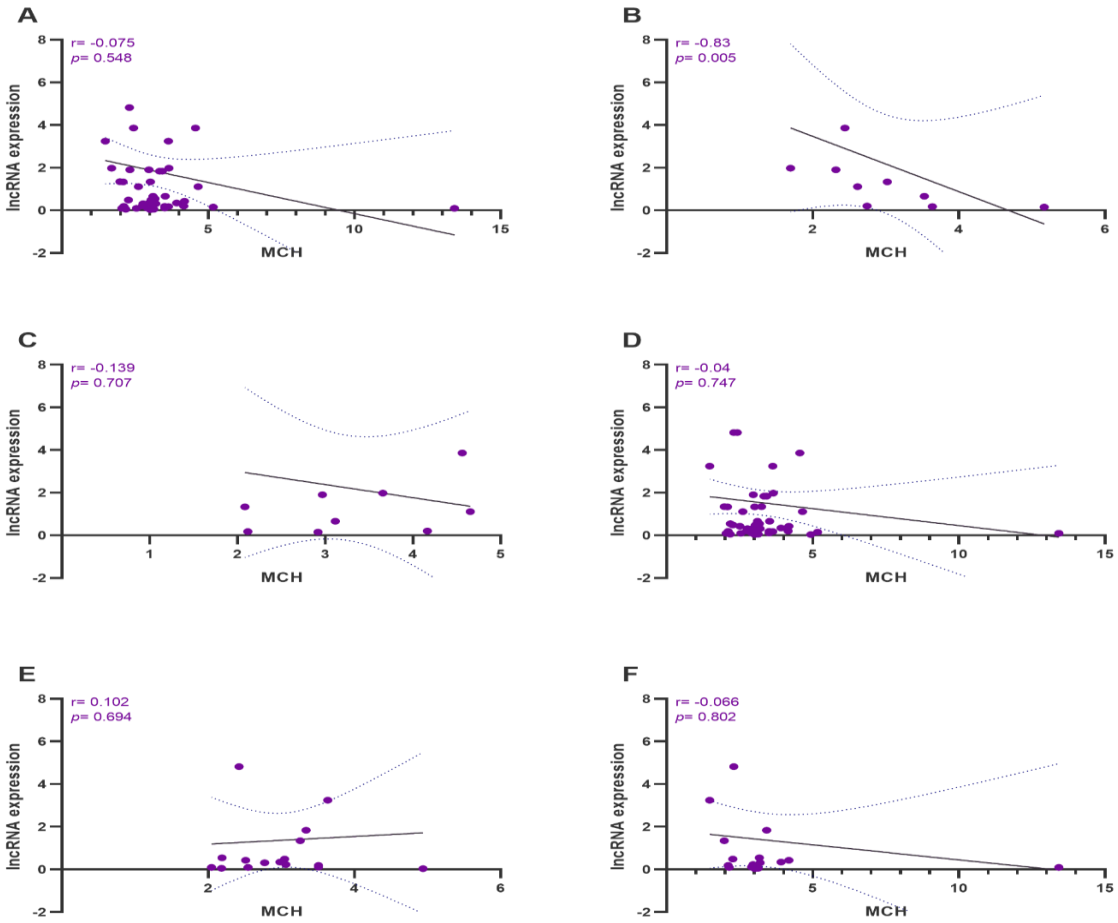

图 **5**

Fig.5 Scatter plots illustrating the association between lncRNA expression of *MCH* and MCH plasma concentration in segmental and generalized vitiligo patients. (A-C) Correlation analysis in segmental vitiligo patients: (A) all patients (n=30), (B) treated patients (n=20), and (C) non-treated patients (n=10), showing a weak, significant negative correlation, a strong, statistically significant negative correlation, and a weak, significant negative correlation, respectively. (D-F) Correlation analysis in generalized vitiligo patients: (D) all patients (n=30), (E) treated patients (n=18), and (F) non-treated patients (n=12). The x-axis represents MCH plasma concentration (pg/ml), while the y-axis represents lncRNA expression of *MCH* (fold change). Each data point corresponds to an individual patient, with the solid line indicating the best fit and the shaded area representing the 95% confidence interval.

4. Discussion

The pathogenesis of vitiligo is largely unknown; however, changes in the cytokine profiles,

autoimmunity, and genetic factors can contribute to the initiation of vitiligo. Melanocyte abnormality and destruction of MCH receptor autoantibodies, the overexpression of MCH, a high level of homocysteine, an increase in catecholamine, free oxygen radicals, cytomegalovirus, and stress may be related to the pathogenesis of vitiligo [13]**.** The present study assessed the expression of LncRNA-SIRT-1, LncRNA-MCH, IL-17, IL-33, and IFNγ in vitiligo patients in an attempt to reveal their roles in vitiligo pathogenesis and the possibility of using them as possible therapeutic targets to find a statistical correlation between variables and lncRNA. The present study was conducted on 30 (GV) and 30 (SV) patients, in addition to 25 HCs, matched for age and gender; the current study indicates that among lncRNAs, the expression of lncRNA SIRT-1 and lncRNA MCH was elevated in patients with vitiligo and participated in the immunopathogenesis of vitiligo via the decline in serum SIRT-1, MCH, and elevated immunological cytokines. This study highlights the crucial role of IL-17, IL-33, and IFNγ in disease progression. IL-17 is a proinflammatory cytokine that plays an important role in the development of autoimmune diseases [14]. Previous studies have indicated that IL-17 increases in patients with vitiligo, is also associated with the participation area of the disease, and is important in the development of vitiligo [15]**,** [16]. IL-17 synergizes with these local inflammatory mediators, which may cause further inhibition of melanocyte proliferation. IL-17 is overexpressed in various other chronic autoimmune inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, multiple sclerosis, systemic sclerosis, and chronic inflammatory bowel disease [17].

IL-33 plays an important role in vitiligo, and its effective role lies in its secretion from apoptotic keratinocytes as well as inhibition of bFGF and SCF [18]. The results of these studies showed elevated levels of IFNγ in patients with vitiligo, which enhances the movement of melanocyte-specific CD8 cytotoxic T lymphocytes (CTLs) toward the skin, stimulating keratinocytes to produce various chemokines, primarily CXCL10. The relationship between CXCL10 and its autoreactive T cell receptor CXCR3 plays a role in sustaining skin depigmentation in patients with vitiligo [12]**.**

SIRT1, a protein present in the body, regulates the MAPK pathway by communicating with other proteins, such as Akt-apoptosis signal-regulating kinase-1. This communication helps reduce the levels of molecules that promote cell death, ultimately reducing oxidative stress and preventing cells from dying in areas of perilesional vitiligo keratinocytes [19]. SIRT1 was suggested to increase the level of differentiated keratinocytes and protect them from UVB-induced DNA damage, which may explain why patients with skin cancer are insusceptible to vitiligo [20].

Furthermore, recent studies have highlighted the potential involvement of lncRNAs in the regulation of vitiligo. Interestingly, one study revealed that the expression of lncRNA TUG1 is notably reduced in the serum of individuals with vitiligo. However, different investigations have suggested that lncRNA MALAT1 is increased in the skin lesions of patients with vitiligo and may provide protection against DNA damage caused by UV radiation through its role as an miR-211 inhibitor. Another study reported no significant alterations in the levels of lncRNA TUG1 and lncRNA MALAT1 in peripheral blood mononuclear cells (PBMC) of individuals with vitiligo [21].

The results of the current study showed a decrease in expression of serum MCH and SIRT-1 and an increase in gene expression of lncRNA in the GV group compared with HCs, indicating a regulatory role for lncRNA in vitiligo patients,according to what thus study indicated. The elevated expression of lncRNAs could potentially be involved in the disappearance of melanocytes by decreasing the activity of crucial genes that play a role in the growth and survival of these cells.

Recently, people with vitiligo have been shown to produce autoantibodies against their own melanocyte proteins, including melanogenic enzymes and a receptor called MCH-R1[22]. Researchers have suggested that the melanocortin system and MCH have opposing effects on pigmentation, with MCH expression promoting lighter pigmentation and melanocortin activity promoting darker pigmentation [9].

lncRNAs can influence the expression of neighboring protein-coding genes, ultimately affecting the overall levels of mRNA and proteins within a cell. The upregulation of lncRNA is possibly related to the pathogenesis of vitiligo,where a study conducted by [23] indicated that excessive expression of lncRNA H19 is associated with the pathogenesis of vitiligo through dysregulation of genes regulating the immune response. SIRT1 related interaction and their possible contribution to and involvement in dermatological diseases have not been sufficiently investigated [24].

5. Conclusion

Sirtuin – 1 (SIRT-1) type of Sirtuins family (deacetylase enzyme family SIRT 1-7 activity) with NAD+ dependent activity, SIRT-1 gene located on chromosome 10q22.1 melanin-concentrating hormone (MCH): pitted first discovered in the pituitary of chum salmon, the name comes from its ability to control pigmentation in skin.There are insufficient studies supporting the functions of MCH in mammalian skin physiology including skin pigmentation. cytokines implicated in several immunological diseases, including some cytokines involved in the pathogenesis of vitiligo. This study demonstrated the deregulated expression of lncRNA SIRT-1 and lncRNA PMCH in patients with vitiligo, suggesting that both contribute to the pathogenesis of vitiligo through serum SIRT-1,MCH downregulation, and IL-17 upregulation.

Declarations

Author Contributions

Safa Sadeq Fayez, Ahmed AbdulJabbar Suleiman contributed equally to the research conceptualization, methodology, data collection, investigation, analysis, validation, visualization, and writing.

Ethical Approval and Consent to participate

The Research Ethics Council of the AL-Anbar University, Ramadi, Anbar, Iraq, granted all necessary clearances and ethical approval.

Conflict of interest

The authors declare that they have no conflict of interest.

Abbreviations

LncRNAs – Long non-coding RNAs
SIRT-1 gene – Sirtuin – 1 gene,
MCH: Melanin-concentrating hormone
GV: generalized vitiligo.
SV – Segmented Vitiligo
HCs: healthy controls
IL-17, interleukin-17
IFNγ: interferon gamma
IL-33 – interleukin-33;
CI, confidence interval.
IQR, interquartile range

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