

## Original Article

# The mRNA expression levels of the Th22 specific transcription factor AHR in patients with vitiligo and its role in vitiligo disease progression

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**Abstract:** Objective: This study aimed to investigate the role of the mRNA expression levels of the auxiliary T lymphocyte 22 (Th22)-specific transcription factor aromatic hydrocarbon receptor (AHR) in vitiligo disease progression. Methods: A total of 90 patients with vitiligo were recruited as the study cohort, including 40 patients with progressive vitiligo (PV) and 50 patients with stable vitiligo (SV). Another 50 healthy individuals were recruited as the control group. The IL-22 mRNA and AHR mRNA expressions were measured using immunofluorescence PCR. An enzyme-linked immunosorbent assay (ELISA) was applied to measure the protein IL-22 and AHR expressions and to investigate the correlation between IL-22 and AHR and IL-22 mRNA and AHR mRNA. Results: The IL-22 and IL-22 mRNA expressions in the study group were significantly higher, and the ARH and ARH mRNA expressions were significantly lower than they were in the healthy control group ( $P < 0.05$ ). The patients with PV exhibited higher IL-22 and IL-22 mRNA expressions and lower ARH and ARH mRNA expression than the patients with SV ( $P < 0.05$ ). The IL-22 and IL-22 mRNA levels were negatively correlated with the ARH and ARH mRNA expression levels ( $P < 0.05$ ). Conclusion: The mRNA expression of AHR in vitiligo patients is significantly different from the mRNA expression of AHR in healthy individuals ( $P < 0.05$ ). The lower the ARH mRNA expression level, the more severe the patient's condition, a finding that provides a new theoretical basis and therapeutic direction for vitiligo treatment.

**Keywords:** Vitiligo, Th22, specific transcription factor AHR, mRNA, expression level, disease progression

## Introduction

Vitiligo is a long-term skin condition characterized by patches of the skin losing their pigment. The discolored areas usually become larger over time. The condition can affect the skin anywhere on the body. It can also affect the hair and the inside of the mouth. The edges of the patch may be smooth or irregular. They're sometimes red and inflamed or have a brownish discoloration [1, 2]. In non-segmental vitiligo (also called bilateral or generalized vitiligo), the symptoms often appear on both sides of the body as symmetrical white patches. In segmental vitiligo (also known as unilateral or localized vitiligo), the white patches affect only one part of the body. Based on its condition, vitiligo can also be classified as progressive vitiligo (PV) or stable vitiligo (SV). The global incidence of vitiligo is about 0.5%-1.0%. The prevalence of vitiligo is significantly higher among

people of color, the incidence rates among different age groups are significantly different, and about over 50% of patients develop vitiligo when they are under 20 years old. The older the age, the lower the incidence rate [3-5]. Vitiligo can have a major impact on an individual's daily life and work, with data showing that about 43% of patients with vitiligo suffer from anxiety, 20.3% suffer from depression, and some individuals even commit suicide [6, 7]. However, the pathogenesis of vitiligo remains unclear, and the disease is prone to recurrence, making it a challenge for dermatology. The development of molecular biology in recent years has provided new strategies for vitiligo treatment, and they are of great significance in guiding clinical interventions [8, 9].

T lymphocyte 22 (Th22) lymphocytes are a newly discovered subpopulation of CD4+ (helper) T cells, which exert biological effects mainly

through the secretion of IL-22, whose receptors are essentially distributed in the epithelial cells of the gastrointestinal tract, skin, and respiratory tract, especially in keratinocytes, and have been shown to be widely involved in the developmental process of inflammatory skin diseases [10, 11]. Aromatic hydrocarbon receptor (AHR) is a ligand-activated transcription factor belonging to the family of Th22-specific transcription factors. Studies have shown that AHR is associated with regulatory Th22 cell-induced differentiation and has a significant anti-oxidative stress effect, which is a key category among the many hypotheses of vitiligo pathogenesis [12, 13]. It has been pointed out that oxidative stress can induce an autoimmune response and cause basal cell degeneration in the epidermis, which in turn leads to melanocyte dysfunction and damage, accelerates the apoptosis process, and ultimately leads to the loss of melanocytes and the appearance of vitiligo [14, 15]. Thus, AHR may be used as an indicator for the diagnosis and treatment of vitiligo and could be used to guide the clinical intervention of vitiligo. This study aimed to investigate the role of this indicator to provide new strategies for vitiligo treatment.

## Materials and methods

### General information

A total of 90 patients with vitiligo and admitted to our hospital were recruited as the study cohort, including the PV group (n = 40) and the SV group (n = 50) according to each patient's disease stage, and the non-segmental type group (n = 45) and the segmental type group (n = 45) according to the type of white patches each patient has. Another 50 healthy individuals who underwent physical examinations during the same period at our hospital were placed in the control group.

Inclusion criteria: (1) patients aged  $\geq 18$  years, (2) patients who met the diagnostic criteria for vitiligo and who had the corresponding clinical symptoms [16], and (3) patients with a clear consciousness and good compliance.

The study procedures were reported to the hospital ethics committee for approval. The study was conducted after the informed consent forms were signed by all the patients.

Exclusion criteria: (1) patients with comorbid psychiatric disorders, (2) patients who used

glucocorticosteroids or immunosuppressants within the three months prior to the intervention, (3) women who were pregnant or breast-feeding, (4) patients with severe hepatic or renal dysfunction; (5) patients with acute, chronic, primary or psychiatric disorders, (6) patients who had undergone an autologous epidermal cell transplantation within the last month, (7) patients with coagulation disorders.

### Intervention methods

3 ml of fasting peripheral venous blood samples were collected from the two groups using a vacuum tube containing procoagulant, then centrifuged at 3000 r/min for 5 min and stored at  $-80^{\circ}\text{C}$  for testing.

### Observation indicators

**IL-22 and AHR protein expressions:** The serum IL-22 and AHR protein expression levels in the two groups were measured using ELISA with a reagent kit purchased from the Shanghai Fusheng Biotechnology Co. The measurements were carried out in strict accordance with the kit instructions. Each index was tested three times, and the average value was taken as the final result. The IL-22 and AHR protein expressions were compared between the study and control groups, between the patients with PV and SV, and between the patients with the segmental type and the non-segmental type.

**The relative expressions of IL-22 mRNA and AHR mRNA:** The IL-22 mRNA and AHR mRNA expression levels were determined using PCR, and the differences in the relative expression levels of the IL-22 mRNA and the AHR mRNA were compared between the study and the control groups, between the patients with PV and SV, and between the patients with the segmental type and the non-segmental type. The mRNA primer sequences were as follows:

Assay	Gene	Primer sequences
ChIP	AHR	F: CCCTCAAGGAAGACGGAATGG
		R: CCGGCTGAATAGCAGGAGCA
RT-PCR	AHR	F: ACATCACCTACGCCAGTCGC
		R: TCTATGCCGCTTGGAAGGAT
	SPI	F: ATGATGACACAGCAGGTGGA
		R: TTGCCATACACTTTCCACA

### Statistical analysis

The collected data were entered into an EXCEL table. SPSS 22.0 was used to analyze the data.

**Table 1.** Baseline data

Baseline data		PV (n = 40)	SV (n = 50)	t/X <sup>2</sup>	P
Gender	Male	23	30	0.057	0.811
	Female	17	20		
Average age (years)		33.39±4.33	33.41±4.29	0.022	0.982
Average weight (kg)		62.32±4.18	62.34±4.09	1.119	0.266
Average course		2.91±0.32	3.01±0.29	1.552	0.124
Education level	University and above	20	25	0.981	0.334
	Junior middle school	18	20		
	Lower secondary	2	5		
Monthly income	< 1000 Yuan	18	12	0.889	0.359
	1000-5000 yuan	23	28		
	Over 5,000 yuan	9	10		
Baseline data		Non-segmental (n = 45)	Segmental (n = 45)	t/X <sup>2</sup>	P
Gender	Male	29	24	1.147	0.284
	Female	16	21		
Average age		63.10±3.22	62.98±3.55	0.168	0.867
Average weight (kg)		63.01±3.98	62.88±4.11	0.152	0.88
Average course		3.02±0.23	2.98±0.41	0.571	0.569
Education level	University and above	19	26	1.001	0.334
	Junior middle school	19	19		
	Lower secondary	7	0		
Monthly income	< 1000 Yuan	17	13	1.029	0.312
	1000-500 yuan	20	21		
	Over 5,000 yuan	8	11		

The count data were expressed as [n (%)] were examined using chi-squared tests. The measurement data (mean ± standard deviation) were compared using t-tests. Spearman was used for the correlation analysis.  $P < 0.05$  indicated significant difference.

## Results

### Comparison of the general clinical data

There were no significant differences between the groups of patients with PV and SV or between the groups with segmental vitiligo and non-segmental vitiligo in terms of sex, age, disease duration, education level, or family income ( $P > 0.05$ ), indicating that they were comparable (Table 1).

### Comparison of the individual IL-22 and AHR protein levels and the IL-22 mRNA and AHR mRNA levels between the and the control groups

The IL-22, IL-22 mRNA expressions in the study group were significantly higher than they were

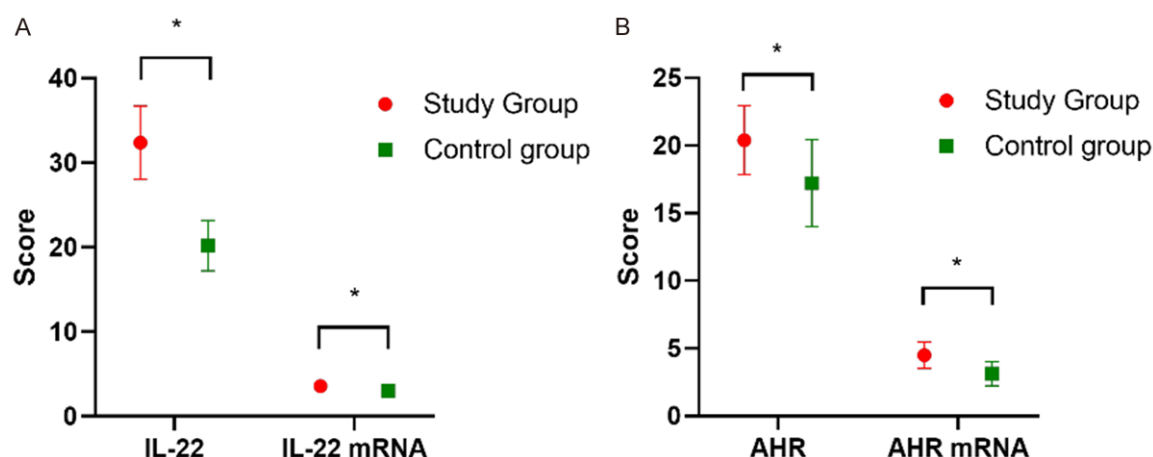
in the healthy controls, and the ARH, ARH mRNA expressions in the study group were significantly lower than they were in the healthy controls ( $P < 0.05$ ) (Figure 1).

### Comparison of the IL-22 and AHR protein expression levels of IL-22 mRNA and AHR mRNA in the PV and SV

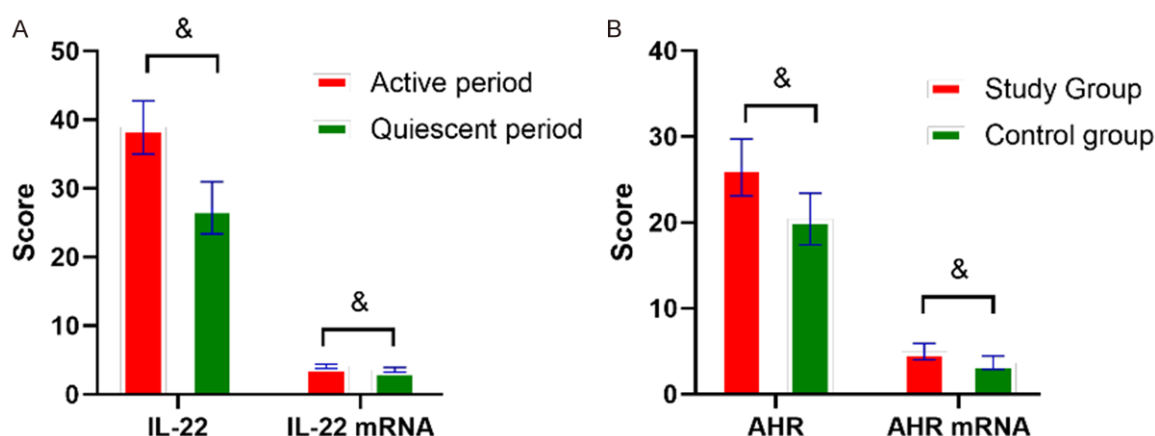
The IL-22, IL-22 mRNA expressions in the patients with PV were significantly higher than they were in patients with SV, and the ARH, ARH mRNA expressions in the patients with PV were significantly lower than they were in the patients with SV ( $P < 0.05$ ) (Figure 2).

### Comparison of the mRNA and protein expression levels of IL-22 and AHR in the patients with non-segmental versus segmental vitiligo

The patients with segmental vitiligo had significantly higher IL-22 and IL-22 mRNA expressions and lower ARH and ARH mRNA expressions than the patients with non-segmental vitiligo ( $P < 0.05$ ) (Figure 3).



**Figure 1.** Differential comparison of the IL-22 and AHR protein expressions in the study group and the control group. A. IL-22 and IL-22 mRNA expression; B. AHR and AHR mRNA expression \* $P < 0.05$ .



**Figure 2.** Comparison of the differences in the IL-22 and AHR protein levels and the IL-22 and AHR mRNA levels in the patients with SV and the patients with PV. A. IL-22 and IL-22 mRNA expression; B. AHR and AHR mRNA expression; & $P < 0.05$ .

#### Correlation analysis between the IL-22 levels and the AHR protein expressions in patients with vitiligo

There was a significant negative correlation between the serum IL-22 levels and the serum AHR levels in the vitiligo patients ( $r = -0.7971$ ,  $P < 0.05$ ), and with an increase in the serum IL-22 levels in the vitiligo patients, the patients' AHR levels also showed a gradual increasing trend (Figure 4).

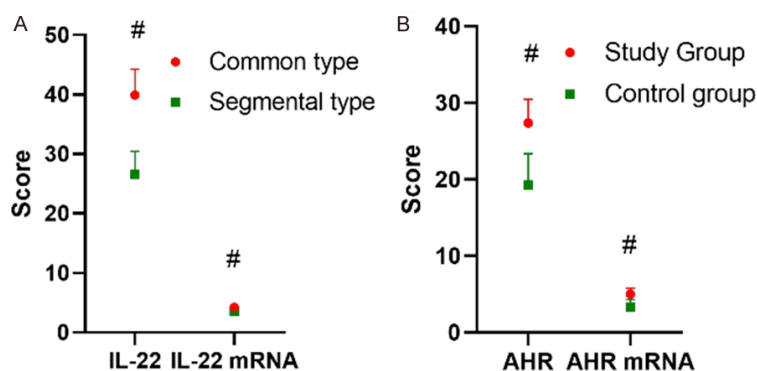
#### Correlation analysis between the IL-22 mRNA and AHR mRNA levels in vitiligo patients

The correlation analysis showed that the IL-22 mRNA and AHR mRNA levels in the vitiligo patients showed a significant negative correla-

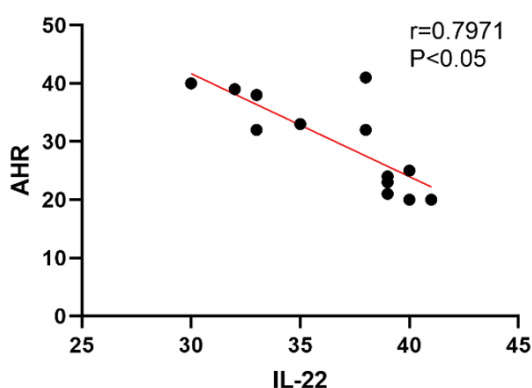
tion ( $r = -0.9028$ ,  $P < 0.05$ ), and with the decrease in the IL-22 mRNA levels in vitiligo patients, there was also a significant decrease in the AHR mRNA levels (Figure 5).

#### Discussion

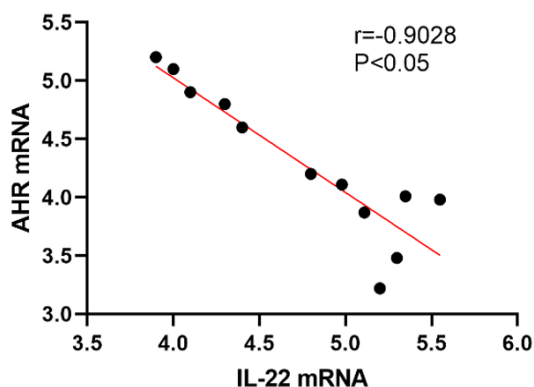
Vitiligo is a skin condition that causes white patches to appear on the skin. Therefore, the normal life and work and appearance of the patients is significantly affected. It is clinically recommended to diagnose and treat the condition as early as possible. The pathogenesis of vitiligo remains unclear, and scholars have proposed genetic, autoimmune, and oxidative stress hypotheses, and many other pathogenic hypotheses [17]. The typical feature of vitiligo is



**Figure 3.** Comparison of the differences in the IL-22 and AHR protein levels and the IL-22 and AHR mRNA levels in the patients with non-segmental and segmental vitiligo. A. IL-22 and IL-22 mRNA expressions; B. AHR and AHR mRNA expressions; # $P < 0.05$ .



**Figure 4.** Correlation analyses between the IL-22 levels and the AHR levels in patients with vitiligo. A significant negative association between the serum IL-22 levels and the serum AHR protein levels in the vitiligo patients ( $r = -0.7971$ ,  $P < 0.05$ ).



**Figure 5.** Correlation analysis of the IL-22 mRNA and AHR mRNA levels in the vitiligo patients. A significant negative correlation between the IL-22 mRNA and the AHR mRNA levels in the vitiligo patients ( $r = -0.9028$ ,  $P < 0.05$ ).

the destruction of melanocytes in the skin, and the melanocytes in the skin lesions are often significantly reduced or absent. So far, there is still a lack of effective treatment options. Data show that vitiligo is one of the three most persistent skin diseases in the world. There are more than 50 million patients with vitiligo worldwide, and the incidence is about 0.5%-1.0%. There are currently about 30 million patients with vitiligo in China, of which 19 million developed symptoms before the age of 20 [18]. In

recent years, molecular mechanisms have become a research hotspots for vitiligo.

Th22 cells belong to a subpopulation of CD4+ helper T lymphocytes. They are differentiated from cell lines such as Langerhans cells, and the Th22 cells play a physiological role mainly through the secretion of IL-22, which combines with IL-22R receptors to produce biological effects [19]. The IL-22R receptor is widely distributed in the respiratory tract, skin, and gastrointestinal tract, especially in keratinocytes. Studies have found that AHR is one of the specific transcription factors targeted by Th22. Studies on the AHR signaling pathway mainly focus on environmental pollutants and PAH toxic activation, but in recent years, there has been an increasing number of studies on the role of AHR regulation. The AHR signaling pathway can also target and regulate processes such as autoimmunity, the formation of mucosal barriers, and reduction-oxidation reactions, and in particular, the AHR signaling pathway can influence individual autoimmunity by regulating the proliferation and differentiation of innate immune cells and the secretions of the corresponding cytokines [20-22].

In this study, we investigated the correlation between the expression level of AHR mRNA, a Th22-specific transcription factor, and the disease progression in vitiligo patients by setting up different subgroups, and the results showed that the expression of IL-22, IL-22 mRNA was significantly higher and the expression of AHR and AHR mRNA was significantly lower in vitiligo patients compared with the healthy controls ( $P$



< 0.05), suggesting that the secretions of IL-22 are more vigorous and the secretions of AHR are lower in vitiligo patients. A clinical comparative study of 80 vitiligo patients showed that vitiligo patients have significantly higher levels of IL-22, IL-17 and other cellular inflammatory factors compared to normal individuals, with significant differences between the groups [23]. We believe that the pathogenesis of vitiligo is complex, among which the oxidative stress and autoimmune hypotheses are more plausible. Th22 cells are a subtype of CD4<sup>+</sup> T lymphocytes, and they regulate the biological processes through the secretion of IL-22, TNF- $\alpha$ , and other cytokines. IL-22 has been proven to have a physiological role in promoting the inherent immunity of the tissue, and it can participate in the autoimmune process through the activation of the STAR system. Previous studies have indicated that AHR also plays a key part in regulating the physiological processes of T helper cells and dendritic cells. It has been confirmed that AHR is associated with various inflammatory and energetic metabolic processes. Therefore, vitiligo patients have higher levels of IL-22, lower levels of AHR, and altered mRNA expressions of these indices compared to healthy individuals [24].

The correlation between AHR expression and vitiligo progression was also explored. The results showed that patients with SV and segmental vitiligo had higher levels of IL-22 and IL-22 mRNA and lower levels of AHR and ARH mRNA. A retrospective analysis of vitiligo patients indicated that Th22 and Th17 cells play a crucial role in the pathological mechanism of vitiligo. After the 60 vitiligo patients were divided by disease stage and type, the researchers found that the IL-22 and IL-17 levels in patients with PV were significantly higher than they were in the patients with SV, and the peripheral blood levels of IL-22 mRNA and IL-17 mRNA in patients with segmental vitiligo were significantly higher than they were in patients with non-segmental vitiligo, which is similar to the results in this study [25]. We believe that Th22 participates in the intrinsic immune response of the body through the secretion of IL-22, and the mechanism may be related to the fact that elevated IL-22 levels cause melanocyte destruction and inflammatory responses. IL-22 mRNA is an intermediate product of IL-22 expression, and higher levels of IL-22 tend to

represent an increase in IL-22 mRNA levels. A survey found that the role of AHR was less explored in the pathological process of vitiligo, but because the skin is susceptible to exogenous and endogenous AHR ligands that regulate the AHR signaling pathways, the association of AHR with vitiligo pathology has a physiological basis, so there is also a close link between AHR and immune regulation, skin homeostasis, pigmentation, and antioxidant activity [26]. It was mentioned above that oxidative stress triggers vitiligo, and this process destroys melanocytes, so this process is closely related to the development of vitiligo. Since AHR can affect the apoptosis of melanocytes by regulating oxidative stress, it is hypothesized that AHR is closely related to the onset and development of vitiligo. We confirmed that the AHR levels in vitiligo patients decreased significantly as the IL-22 level increased, suggesting that AHR may regulate the development of vitiligo by inhibiting IL-22 expression.

In summary, the expression of the Th22-specific transcription factor AHR mRNA did show some changes in patients with vitiligo compared to healthy individuals. Meanwhile, the expression level of ARH mRNA was closely related to the development of vitiligo. The lower the expression level of ARH mRNA, the more severe the patient's condition, a finding that provides a new theoretical basis and direction for the treatment of vitiligo patients. We will carry out in vitro cell experiments to clarify how AHR regulates the expression of Th22 cells and influences the occurrence and development of vitiligo to provide a new theoretical basis for the clinical treatment of vitiligo.

### Disclosure of conflict of interest

None.

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