

# Correlations between microRNA-146a and immunoglobulin and inflammatory factors in Guillain–Barré syndrome

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## Abstract

**Objective:** To study correlations between expression of microRNA-146a (miR-146a) and immunoglobulin and inflammatory cytokines in serum of patients with Guillain–Barré syndrome (GBS).

**Methods:** Eighty-four patients with GBS were selected as the experimental group and 50 healthy individuals as controls. Reverse transcription-PCR was used to detect expression of miR-146a in peripheral blood of all participants. Immunoturbidometric assay was used to detect immunoglobulins (Ig)G, IgA, and IgM in peripheral blood of all participants. The levels of interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) were measured by ELISA in peripheral blood. The expression of miR-146a was compared between the two groups and Pearson correlation analysis was used to analyze correlations between miR-146a and immunoglobulin and inflammatory factors.

**Results:** Expression of miR-146a was higher in the GBS group than in controls. Expression of IL-6, CRP, TNF- $\alpha$ , and IgG was significantly higher in the GBS group than in controls. miR-146a was significantly and positively correlated with IL-6, CRP, TNF- $\alpha$ , and IgG but not with IgA and IgM.

**Conclusion:** The expression profile of miR-146a in patients with GBS differs from that in healthy individuals. Thus, miR-146a may participate in the pathogenesis of GBS by regulating immune and inflammatory responses.

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## Keywords

Guillain–Barré syndrome, microRNA-146a, correlation, immunoglobulins, cytokines, inflammatory response

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## Introduction

Guillain–Barré syndrome (GBS) is a common neurological disease. Although its pathogenesis is not completely clear, involvement of the immune inflammatory response has been confirmed.<sup>1</sup> MicroRNAs (miRNA) are small, noncoding, single-stranded RNA molecules 17 to 22 nucleotides long; they are widely involved in the regulation of disease development and progression.<sup>2</sup> Recent studies have shown that microRNA-146a (miR-146a) regulates inflammation and immunity and serves as a biomarker for various diseases.<sup>3,4</sup> Because inflammation and immune responses are present in GBS, we speculated that expression of miR-146a might be altered in GBS patients, which was the basis for the design of this study. We analyzed correlations between miR-146a and related inflammatory markers and immunological markers, providing a new basis for understanding the pathogenesis and treatment of GBS.

## Methods

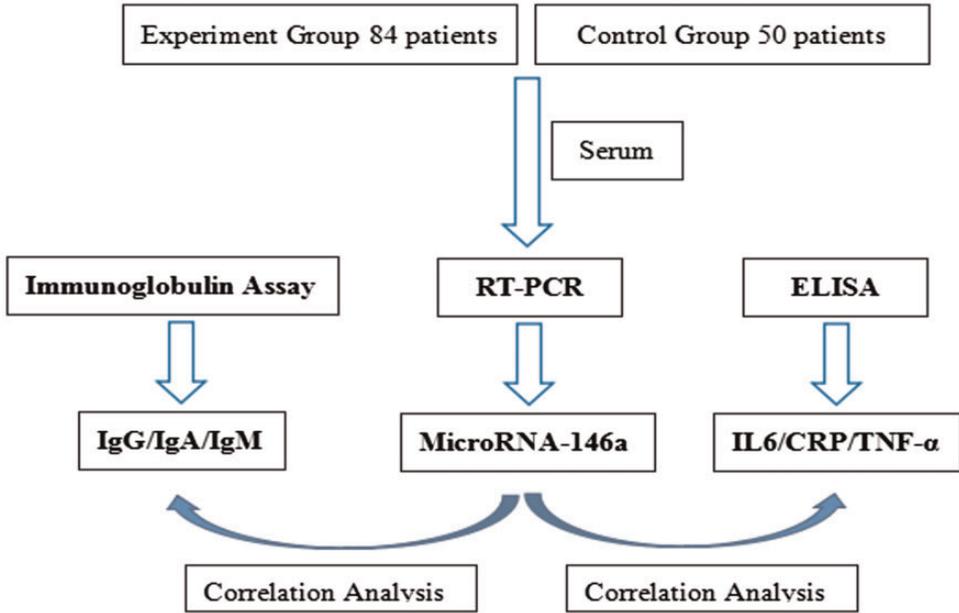
### *Clinical data*

This study included 84 patients with a first occurrence of GBS who were admitted to the Department of Neurology of the People's Hospital of Deyang City from January 2013 to January 2017, including 43 men and 41 women with an average age of  $35.46 \pm 4.25$  years (GBS group). Patients were included if their symptoms met the 2014 GBS international diagnostic criteria<sup>5</sup> and they consented to participate.

Patients were excluded if they had (1) variant GBS; (2) polyneuritis caused by heavy metals, drugs, or other factors; (3) immunological and anti-inflammatory treatment before admission; (4) other infectious diseases; (5) GBS combined with immunological diseases; (6) lung, liver, or kidney dysfunction; or (7) tumor disease. Fifty healthy individuals with normal physical examinations were selected as the control group, including 27 men and 23 women with an average age of  $36.23 \pm 4.28$  years. Both groups were comparable in terms of age and sex (Figure 1). The study was approved by the Ethics Committee of Deyang People's Hospital (series number: 2019-06-002), and all participants signed an informed consent form.

### *Reagents and instruments*

The following reagents and instruments were used: Trizol reagent (Invitrogen, Carlsbad, CA, USA); trichloromethane, isopropanol, and absolute ethanol (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China); M-MLV reverse transcriptase kit and 10mM dNTP mix (Invitrogen); SYBR Premix Ex Taq (TaKaRa, Dalian, China); IgG/A/M kit (Shanghai Kehua Reagent Co. Ltd., Shanghai, China); 48T interleukin-6, C-reactive protein, and tumor necrosis factor- $\alpha$  kits (R&D Systems Inc., Minneapolis, MN, USA); TC-XR PCR amplification instrument (Hangzhou Bori Technology, Hangzhou, China); StepOne Real-Time PCR System quantitative PCR instrument (Life Technologies, Carlsbad, CA, USA); and a DNM-9602 microplate



**Figure 1.** Flow chart of the experimental process. Experiment group, patients with Guillain–Barré syndrome; RT-PCR, real-time quantitative PCR; Ig, immunoglobulin; IL6, interleukin-6; CRP, C-reactive protein; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

reader (Beijing Pulang New Technology Co. Ltd., Beijing, China).

### Sample collection

Peripheral venous blood samples (5 mL) were taken in the morning from fasting participants in both groups and were allowed to naturally coagulate for 10 to 20 minutes at room temperature. The coagulated blood samples were then centrifuged for 30 minutes at  $10,000 \times g$  and  $4^{\circ}\text{C}$ . The upper serum layer was carefully collected and stored in an ultra-low-temperature freezer at  $-80^{\circ}\text{C}$  until analysis.

### RT-PCR detection

Serum (200  $\mu\text{L}$ ) was prepared according to the operating instructions of the Trizol RNA extraction kits to extract total serum RNA. After purification, a nucleic acid protein analyzer was used to detect the total

RNA extracted. The optical density ratio at 260 and 280 nm was used to value determine purity; values of 1.8 to 2.0 indicated high purity. First-strand cDNA synthesis was performed according to the M-MLV reverse transcriptase kit instructions. For real-time fluorescent quantitative (RT)-PCR, each sample was set up with three replicate wells and tested according to the SYBR Premix ExTaq kit and analyzed with the StepOne Real-Time PCR machine. The following primers were used: miR-146a (forward: 5'-CCTGAGAAGTGAATTCC ATGGG-3', reverse: 5'-CTCAACTGGTG TCGTGGAGTC-3') and U6 as the internal reference (forward: 5'-CTCGCTTCGGCA GCACAT-3', reverse: 5'-AACGCTTCAC GAATTTGCGT-3').

### ELISA detection

The serum cytokines interleukin (IL)-6, C-reactive protein (CRP), and tumor necrosis

factor  $\alpha$  (TNF- $\alpha$ ) were detected by ELISA according to the operating instructions (R&D Systems Inc.).

### Immunoglobulin assay

Serum immunoglobulin levels for IgG, IgA, and IgM were determined by the immunoturbidimetric assay according to the ELISA kit instructions (Shanghai Kehua Reagent Co. Ltd.).

### Statistical methods

Because cycle threshold (Ct) values cannot be analyzed as raw data in statistical analysis, they need to be standardized. The calculation method was as follows:  $\Delta\text{Ct} = (\text{miR-146a Ct value} - \text{U6 Ct value})$ ;  $2^{-\Delta\text{Ct}}$  was used to represent the relative expression level of each target miRNA. All data were processed using SPSS 17.0 software (IBM Corp., Armonk, NY, USA) and expressed as mean  $\pm$  standard deviation. The *t*-test and *F*-test were used for comparisons between the two groups. Pearson correlation coefficients were used for correlation analysis. Count data were expressed as percentages or rate and comparisons were performed by  $\chi^2$  test.  $P < 0.05$  was considered statistically significant.

## Results

### Baseline information

Analysis of basic patient information demonstrated no significant differences regarding sex, age, or presence of diabetes, hypertension, hyperlipidemia, coronary disease, smoking, or drinking between the two groups (Table 1).

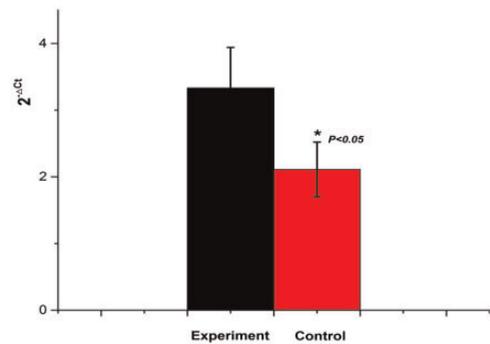
### Serum levels of miR-146a, IL-6, CRP, TNF- $\alpha$ , and immunoglobulins

The expression of miR-146a in the GBS group was higher ( $P < 0.01$ ) than that in the

**Table 1.** Comparison of baseline information between the two groups.

Item	GBS group (n = 84)	Control group (n = 50)
Age (years)	35.46 $\pm$ 4.25	36.23 $\pm$ 4.28
Sex (male/female)	43/41	27/23
Diabetes (no.)	3	1
Hypertension (no.)	5	2
Hyperlipidemia (no.)	9	5
Coronary disease (no.)	1	1
Smoker (no.)	32	18
Drinker (no.)	21	12

GBS group, patients with Guillain-Barré syndrome; control group, healthy individuals.



**Figure 2.** Comparison of miR-146a expression in the experimental (patients with Guillain-Barré syndrome) and control (healthy individuals) groups by  $2^{-\Delta\text{Ct}}$  method.

control group (Figure 2). Serum levels of IL-6, CRP, and TNF- $\alpha$  in the GBS group were higher ( $P < 0.05$ ) than those in the control group (Table 2). Serum levels of IgG in the GBS group were higher ( $P < 0.001$ ) than those in the control group. We found no significant difference in levels of IgM and IgA between the groups (Table 3).

### Correlation analysis

Pearson correlation analysis between miR-146a and inflammatory cytokines and

**Table 2.** Comparison of IL-6, CRP, and TNF- $\alpha$  in the two groups.

Item	GBS group	Control group	t-test	P-value
IL-6 (ng/L)	12.17 $\pm$ 1.42	11.34 $\pm$ 1.31	3.36	0.0009
CRP ( $\mu$ g/L)	1647.38 $\pm$ 272.95	1504.72 $\pm$ 212.23	3.16	0.001
TNF- $\alpha$ (pg/mL)	285.43 $\pm$ 32.09	260.27 $\pm$ 35.78	4.20	<0.001

GBS group, patients with Guillain-Barré syndrome; control group, healthy individuals; IL-6, interleukin-6; CRP, C-reactive protein; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

**Table 3.** Comparison of peripheral blood immunoglobulins in the two groups.

Item	GBS group	Control group	t-test	P-value
IgG (g/L)	15.43 $\pm$ 2.34	11.42 $\pm$ 2.12	9.92	<0.001
IgA (g/L)	1.85 $\pm$ 0.41	1.74 $\pm$ 0.42	1.48	0.14
IgM (g/L)	1.51 $\pm$ 0.33	1.43 $\pm$ 0.28	1.43	0.15

GBS group, patients with Guillain-Barré syndrome; control group, healthy individuals; IgG, IgA, and IgM, immunoglobulins G, A, and M.

**Table 4.** Pearson correlation analysis between microRNA-146a and inflammatory cytokines and immunoglobulins in the GBS group.

Item	microRNA-146a	
	r	P-value
IL-6	0.641	<0.05
CRP	0.306	<0.05
TNF- $\alpha$	0.594	<0.05
IgG	0.739	<0.05
IgA	0.096	0.385
IgM	0.114	0.301

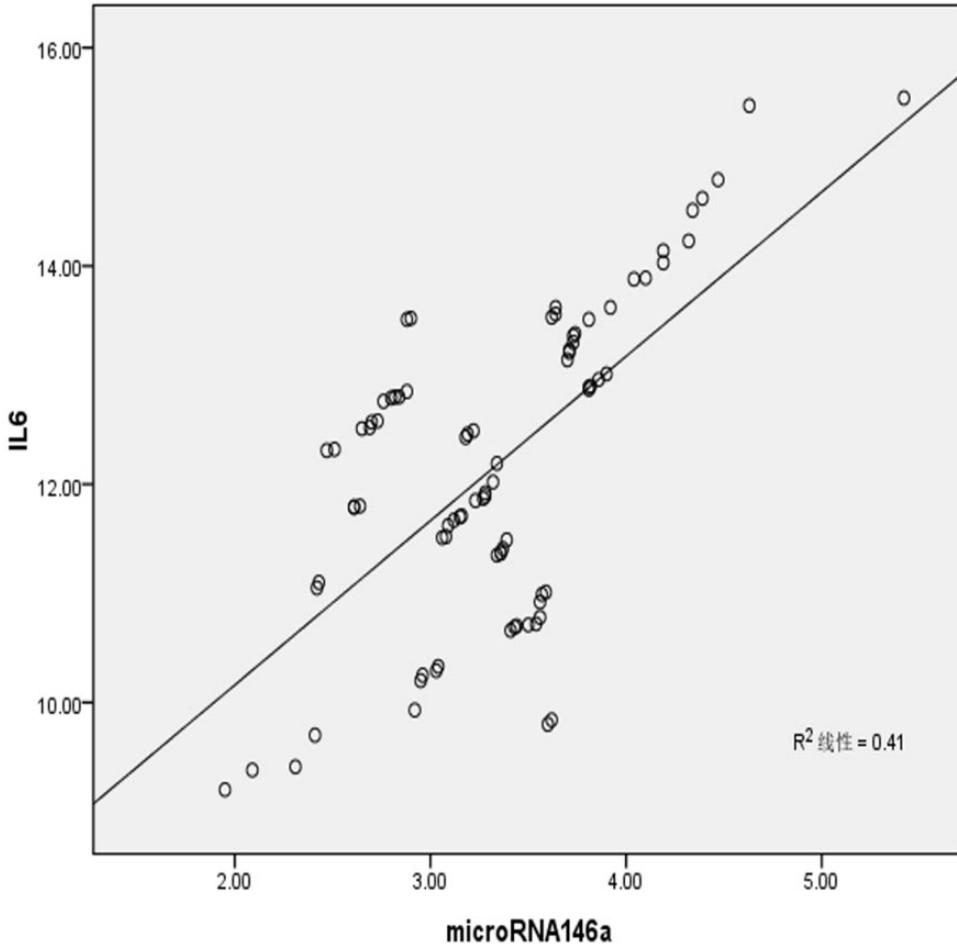
GBS group, patients with Guillain-Barré syndrome; IL-6, interleukin-6; CRP, C-reactive protein; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; IgG, IgA, and IgM, immunoglobulins G, A, and M.

immunoglobulins demonstrated positive correlations ( $P < 0.05$ ) between miR-146a in peripheral blood of GBS patients and levels of IL-6, CRP, TNF- $\alpha$ , and IgG (Table 4 and Figures 3–8).

## Discussion

GBS, also known as acute inflammatory demyelinating polyneuropathy, is a peripheral

nervous system disease. Its pathological features include demyelination of peripheral nerve roots, and its clinical manifestations include progressive ascending symmetrical paralysis, soft limbs, and varying degrees of sensory disturbances. The pathogenesis of GBS is not completely clear, but for most patients, their history can be traced back to a respiratory or digestive system infection occurring a few weeks before onset, suggesting that GBS may be associated with the earlier inflammatory response.<sup>6</sup> Studies have shown that, in GBS, related effector cells can be attracted to the peripheral nerve roots by inflammatory cytokines, which can induce the production of nitric oxide, oxygen free radicals, and other substances that lead to demyelination of nerve roots.<sup>7</sup> IL-6 and TNF- $\alpha$  in peripheral blood can be secreted by various cells and liver cells, and these are commonly used indicators of an inflammatory response. Serum levels of IL-6 and TNF- $\alpha$  are higher than normal in patients with GBS, suggesting that an inflammatory reaction occurs; in contrast, CRP is mainly induced by an inflammatory reaction following activation of the complement system. CRP enhances

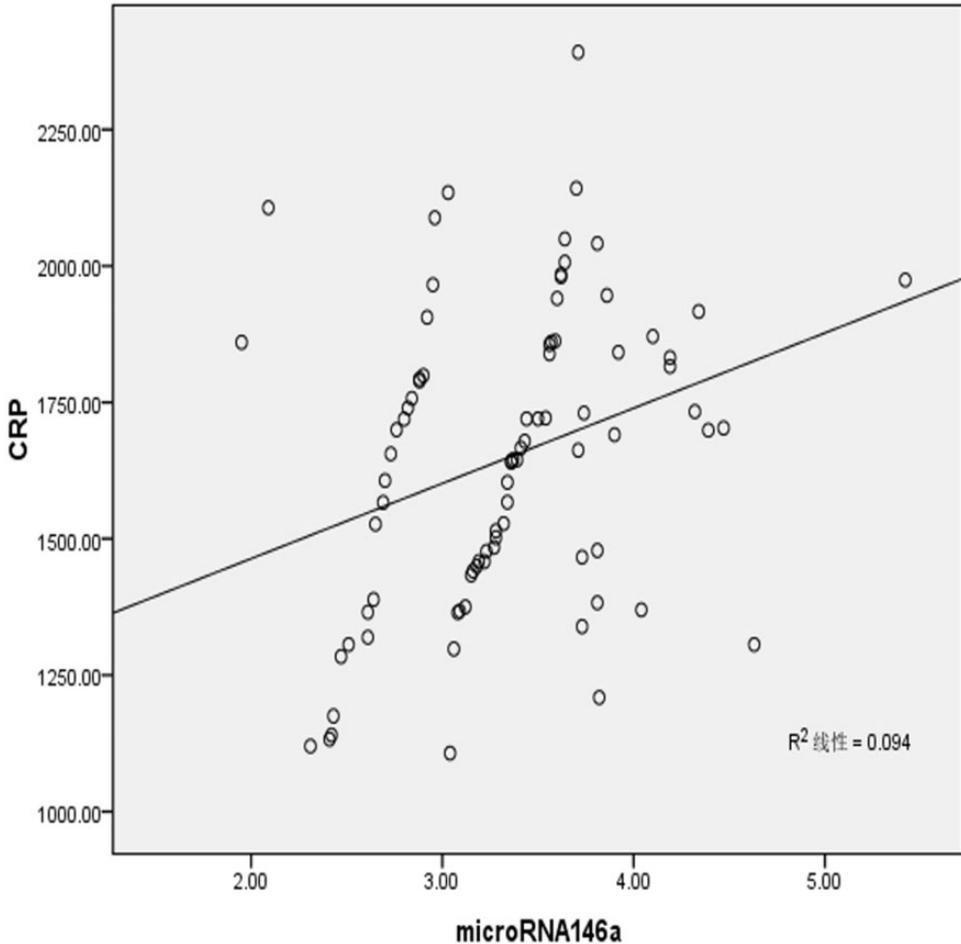


**Figure 3.** Correlation analysis between microRNA-146a and interleukin (IL)-6.

phagocytosis of cells and clears the DNA produced by apoptotic cells. The inflammatory reaction can then initiate GBS by activating the immune pathway.<sup>8</sup> In this study, we found that expression levels of IL-6, CRP, and TNF- $\alpha$  in the peripheral blood of patients with GBS were significantly higher than those in the healthy group, indicating that inflammatory cytokines participate in GBS, a conclusion similar to that found by Liu.<sup>9</sup>

GBS is widely recognized as an immunoreactive disease. Its underlying mechanism

is thought to be related to B cells in the immune system that can produce a class of immunoglobulins within peripheral nerves that are stimulated by exogenous antigens. Cross-immune reactions cause damage to peripheral nerve roots. In this study, we found that IgG levels were significantly higher in the peripheral blood of patients with GBS than in that of the control group, whereas levels of IgA and IgM were not elevated. At present, IgG is believed to play a role in the pathogenesis of GBS. Injecting serum from GBS patients

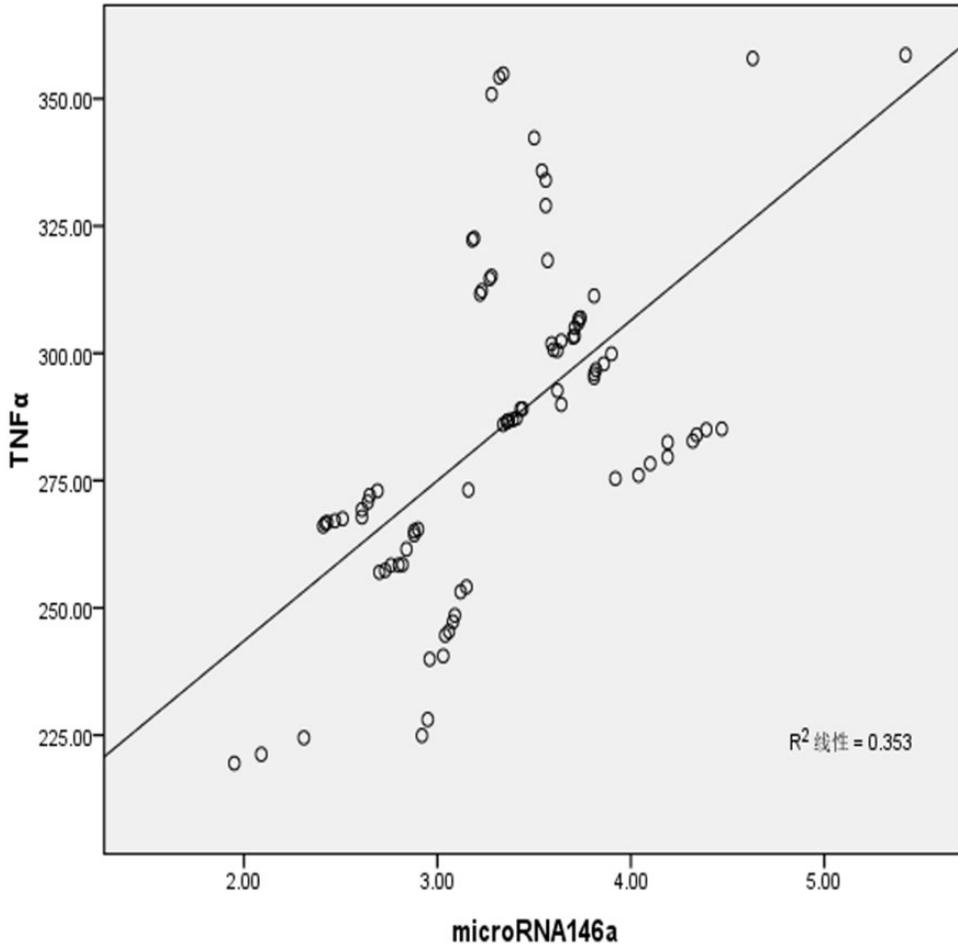


**Figure 4.** Correlation analysis between microRNA-146a and C-reactive protein (CRP).

into experimental animals can cause demyelinating changes in the peripheral nerves. Further analysis found that IgG antibodies can cause nodes of Ranvier—gaps in the myelin sheath of nerves. Loss of glial protein clusters at the nodes reduces the anchoring ability between myelin and axons and thus leads to demyelination of the nerve roots.<sup>10–13</sup> Yang et al.<sup>14</sup> found that levels of IgG (but not of IgA and IgM) differed in the peripheral blood of patients with GBS and healthy individuals,

similar to our results. This indicates that IgG plays an important role in the pathogenesis of GBS.

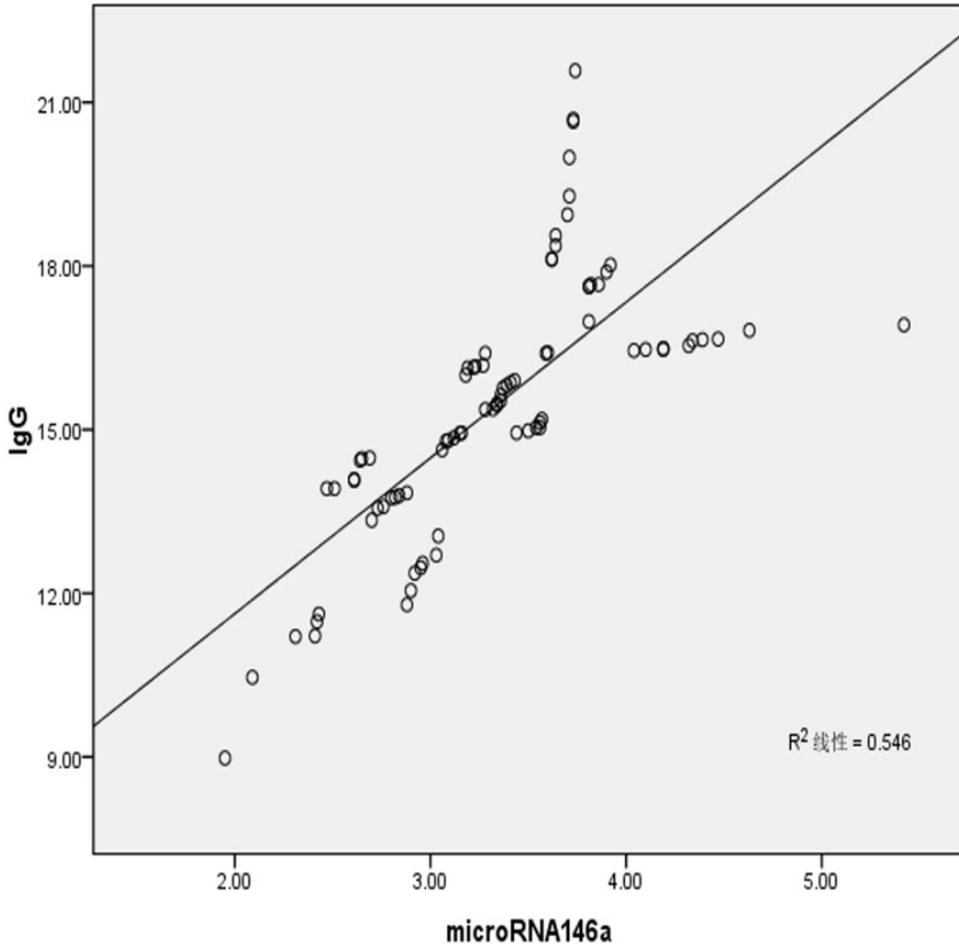
miRNAs can regulate immune or inflammatory responses by binding to target genes via complete or incomplete base pairing.<sup>15</sup> The regulation of inflammation by miRNAs is primarily through altered expression of specific miRNAs in stimulated immune or bystander cells.<sup>16</sup> There is also evidence that biogenesis of miRNAs is regulated as part of the inflammatory



**Figure 5.** Correlation analysis between microRNA-146a and tumor necrosis factor (TNF)- $\alpha$ .

response via alterations in transcription, processing, or stabilization of mature or precursor miRNA transcripts.<sup>17</sup> The initiation, spread, and resolution of inflammation are subject to both positive and negative regulatory events that depend on miRNAs.<sup>18</sup> The positive feedback initiates a cascade of molecular events that serve to combat the invasion of microbial pathogens and repair tissue damage. The negative feedback, which is activated only during severe inflammation, is vital for preventing

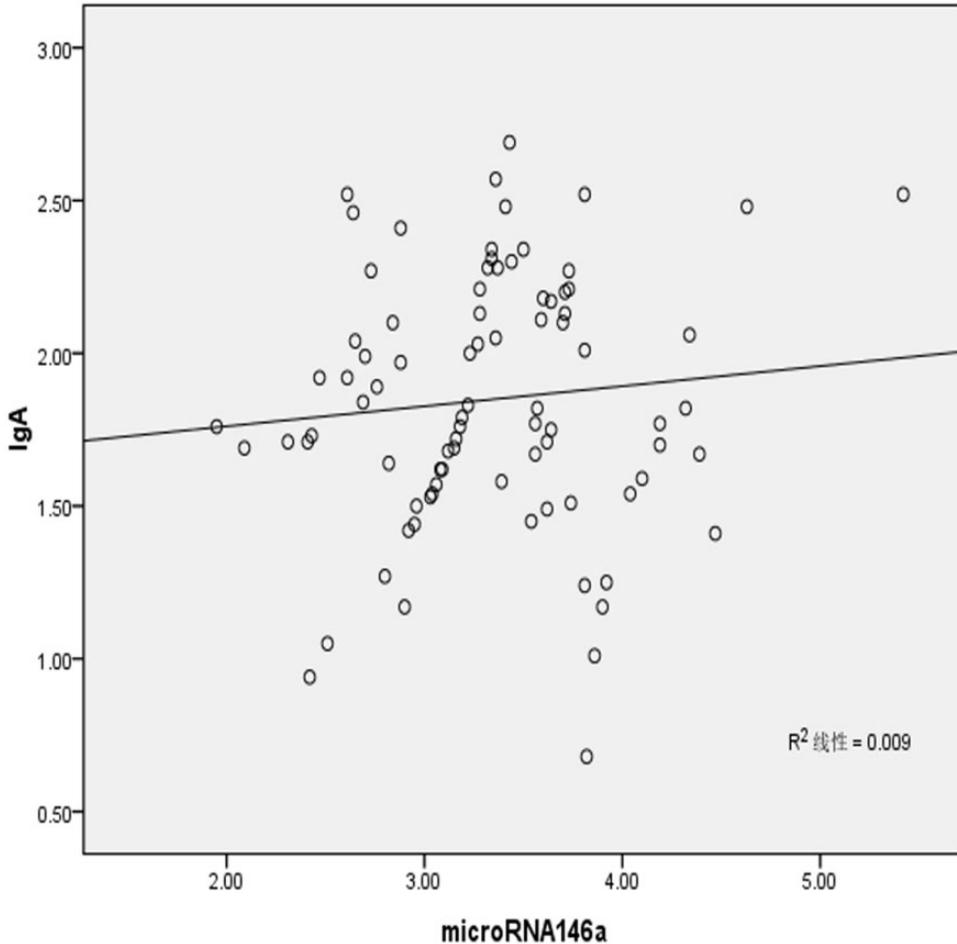
potentially damaging end-stage processes and maintaining tissue homeostasis. Studies have shown that Epstein–Barr virus can encode the latent membrane protein LMP1, which can upregulate miR-146a expression by activating the nuclear factor- $\kappa$ B signaling pathway in the host, resulting in a decrease in the body's ability to resist Epstein–Barr virus and thus preventing its elimination. This suggests that miR-146a exerts a regulatory effect on the body's innate immunity.<sup>19,20</sup> In the present study,



**Figure 6.** Correlation analysis between microRNA-146a and immunoglobulin (Ig)G.

we found that miR-146a expression levels in the peripheral blood of patients with GBS were significantly different from those in the healthy individuals, suggesting that miR-146a may be involved in the regulation of GBS, perhaps indicating a potential use of miRNA in clinical practice. miR-146a can be used as an auxiliary indicator for diagnosing GBS. In addition, we found that miR-146a was positively correlated with levels of IL-6, CRP, TNF- $\alpha$ , and IgG but was not correlated with IgA or IgM.

miR-146a may participate in the pathogenesis of GBS by regulating the immune response and inflammation. The miR-146 family contains two miRNAs, miR-146a and miR-146b, which are expressed in a negative feedback process to control excessive inflammation in response to pro-inflammatory stimuli.<sup>21</sup> Both miR-146a and miR-146b regulate inflammatory processes by directly targeting toll-like receptor and its downstream effectors IRAK1 and TRAF6. Importantly, miR-146a negatively

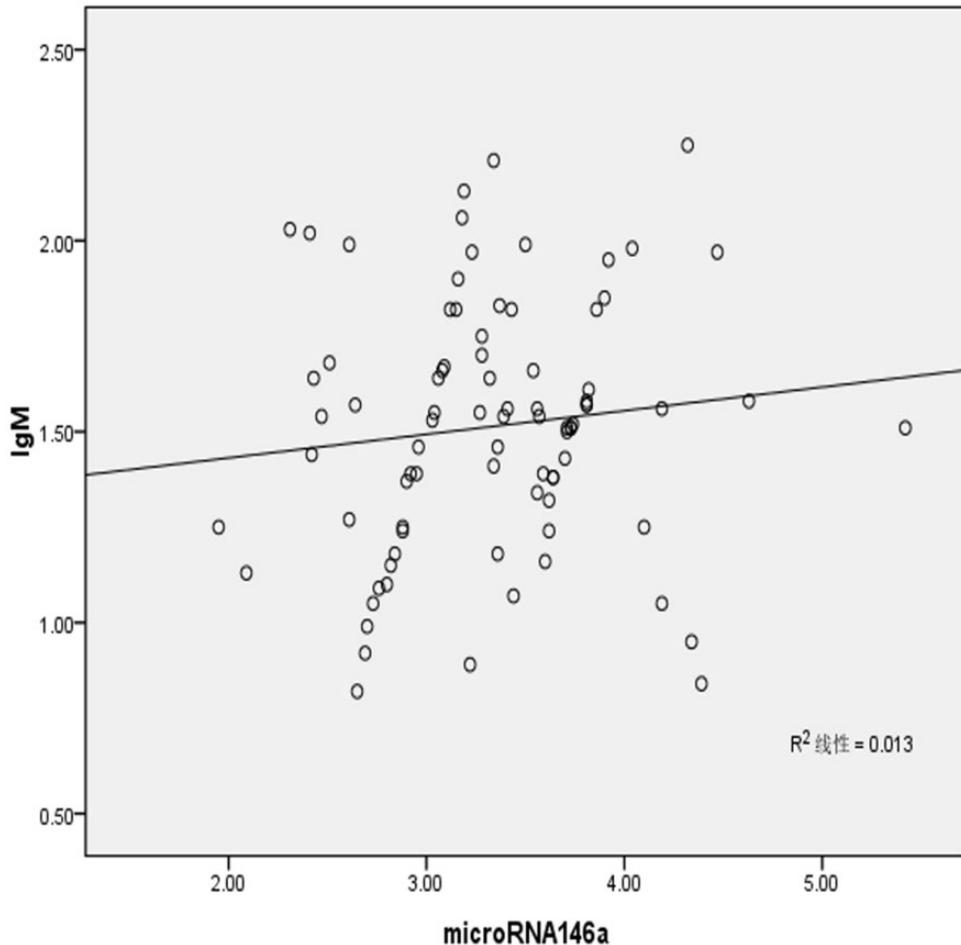


**Figure 7.** Correlation analysis between microRNA-146a and immunoglobulin (IgA).

regulates the interferon (IFN) response and adaptive immunity by targeting adaptor protein (AP)-1 activity and IL-2 expression, as well as immune cell activation and cytokine production.<sup>22</sup> miR-146b regulates diabetes-associated retinal inflammation by inhibiting adenosine deaminase 2.<sup>23</sup>

In summary, we demonstrated differential expression of miR-146a in the peripheral blood of patients with GBS and of healthy individuals. miR-146a may affect GBS by regulating immune and inflammatory

responses. However, because of the limitations of our experiment, we only correlated miR-146a expression of immunological and inflammatory response-related indicators and did not conduct molecular and pathological studies on the specific underlying regulatory mechanism. Furthermore, we conducted this study in a single center with a relatively small sample size. The small sample may lead to a certain degree of bias in the research results; thus, additional research is needed to verify our results.



**Figure 8.** Correlation analysis between microRNA-146a and immunoglobulin (IgM).

### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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