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A case-control study on the relationship between miRNAs single nucleotide polymorphisms and sepsis risk

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Abstract

MicroRNAs (miRNAs) play an important role in the pathogenesis of sepsis, but the association of miRNAs single nucleotide polymorphisms (SNPs) and sepsis risk is not clear. We analyzed plasma levels of miR-187, miR-21, and miR-145 in 180 patients with sepsis and 180 healthy controls were analyzed, and the SNPs: rs12605436, rs13137, and rs353291 were detected by sequencing. Plasma levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured in all subjects by enzyme-linked immunosorbent assay (ELISA). The results showed that the levels of TNF-α and IL-6 in the plasma of patients with sepsis were significantly higher than those in patients of the control group ($P < .0001$). Plasma levels of miR-187 in patients with sepsis were significantly lower than those in the control group, while those of miR-21 and miR-145 were significantly higher than those in the control group ($P < .0001$). Plasma levels of miR-187 in sepsis patients were inversely correlated with those of TNF-α and IL-6 ($r = -0.2841, -0.2163$), and plasma levels of miR-21 and miR-145 were positively correlated with those of TNF-α and IL-6 ($r = 0.615, 0.3057, 0.4465, 0.2734$). The T allele of the miR-187 SNP rs12605436 was found to be a risk factor for sepsis (OR = 1.403, 95% CI = 1.205–1.612, $P < .001$). The T allele of the miR-21 SNP rs13137 and the T allele of the miR-145 SNP rs353291 (OR = 0.685, 95% CI = 0.566–0.820, $P < .001$) were found to be a protective factor for sepsis (OR = 0.755, 95% CI = 0.632–0.896, $P < .001$). From our results, we can see that the plasma levels of miRNAs containing the SNPs rs12605436, rs13137, and rs353291 are associated with the occurrence of sepsis.

Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II, CI = confidence interval, ELISA = enzyme linked immunosorbent assay, HIV = human immunodeficiency virus, HWE = Hardy–Weinberg equilibrium, IL = interleukin, LPS = lipopolysaccharide, MAF = minor allele frequency, miRNAs = microRNAs, NA = not applicable, OR = odds ratio, PDCD4 = programmed cell death 4, qRT-PCR = quantitative real-time PCR, SD = standard deviation, SNP = single nucleotide polymorphisms, SOFA = Sequential Organ Failure Assessment, TNF = tumor necrosis factor.

Keywords: IL-6, microRNAs, sepsis, TNF-α

1. Introduction

Sepsis is represented by a systemic inflammatory response syndrome caused by infection, which occurs rapidly and can progress to severe sepsis and septic shock.[1] At present, sepsis is still the main cause of death in hospitalized patients worldwide. Although there are good monitoring measures and techniques for diagnosis and treatment, the morbidity and mortality of sepsis are high, which is a major problem in the world of medicine.[2,3]

At present, patients with sepsis are mainly treated using antibiotics accompanied by fluid resuscitation. When necessary, one may choose to use vasopressor and supportive treatment of organ failure.[4] Mortality in patients with sepsis can be reduced by early-directed therapy and hemodynamic stabilization; still, mortality in sepsis patients remains high. For stratified critical sepsis, it may be useful to identify people with high mortality risk.[5,6] Traditional diagnostic markers for sepsis, such as C-reactive protein, procalcitonin, and interleukin-6 (IL-6), have a limited efficiency, and emerging diagnostic markers, such as microRNAs (miRNAs), are proving to be more efficient.[7,8] The potential role of circulating miRNAs in the diagnosis and staging of sepsis has become a hot topic in current research. It has been shown that a variety of miRNAs can be used as markers for the diagnosis of sepsis.[9] In this study, miR-187, miR-21, and miR-145 were selected, as they play an important role in the regulation of inflammatory response.[10–12] According to Benz et al,[9] miR-187 can inhibit the synthesis of proinflammatory cytokines, such as TNF-α, whereas IL-6, and miR-21 and miR-145 can promote their synthesis. In addition, TNF-α and IL-6 have significant clinical value in the diagnosis of neonatal sepsis.[13]

In addition, to study the effects of mutation on the regulation of miRNAs, this study investigated single nucleotide polymorphisms (SNPs) of miR-187 (rs12605436), miR-21 (rs13137), and miR-145 (rs353291) loci in the Chinese population. These 3 SNPs were highly mutated in the Chinese Han population.

According to the 1000 Genomes database, the minor allele frequency (MAF) of rs12605436 was 0.1333, the MAF of rs13137 was 0.4667, and the MAF of rs353291 was 0.4095. This study was aimed to study the associations of circulating miR-187, miR-21, and miR-145 during sepsis and the effects of single nucleotide polymorphisms in these miRNAs on the diagnosis and treatment of sepsis.

2. Materials and methods

2.1. Ethical review

The study passed the review of Juancheng People's Hospital ethics committee and all subjects signed the informed consent form.

2.2. Patient recruitment

One hundred eighty sepsis patients admitted to our hospital from January 2016 to January 2018 were randomly selected, including 102 patients (64 males and 38 females) with mild sepsis, and 78 severe sepsis patients (43 males and 35 females). The diagnostic criteria for sepsis were based on the Surviving Sepsis Campaign Guidelines for Management of Severe Sepsis and Septic Shock.[14] According to the condition of sepsis patients, standard hospitalization was done, as required, and prospective monitoring was performed based on the clinical outcome of the patient. The severity of sepsis patients was assessed using the Sequential Organ Failure Assessment.[15] The age, sex, source of infection, type of infectious pathogen, etc., of all subjects were also recorded. The subjects in the control group were recruited according to the age of the subject group (±5 years old), gender and other factors in a 1:1 ratio. Following patients were excluded: patients < 18 years of age, pregnant or lactating women, and subjects infected with human immunodeficiency virus (HIV). The basic characteristics of all participants in the study are shown in Table 1.

2.3. Plasma cytokine level detection

Five milliliters of venous blood was drawn from each subject and kept standing for 30 minutes. The supernatant was collected after centrifugation at 3000 rpm for 20 minutes, and the blood cells in the pellet were used for genome extraction. The extracted supernatant was stored at −80°C for serum cytokine detection by enzyme linked immunosorbent assay (ELISA). The TNF-α-detection kit used was E-EL-H0109c (Elabscience, Wuhan, China), and the IL-6-detection kit used was E-EL-H0102c (Elabscience, Wuhan, China).

2.4. Detection of levels of miRNAs in plasma

miRNAs were extracted from preserved plasma using Trizol (Invitrogen, CA). Plasma levels of miRNAs were detected using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). cDNA synthesis was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Using the synthesized cDNA as a template, miRNAs were then generated by qRT-PCR using the ABI 7900 HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). The miR-187 primer sequences used were: forward, 5′-AGT AAT GGG ATG GCT GGG TC-3′; reverse, 5′-ACT GTT GGT GGG ACT GTA AAC-3′. The probe sequence for miR-187 was 5′-CCG GCT GCA ACA CAA GAC ACG A-3′. The miR-21 primer sequences used were: forward, 5′-TCC CTC CAT ACT GCT GCA TT-3′; reverse, 5′-TCG ATG GGC TGT CTG ACA TT-3′. The probe sequence for miR-21 was 5′-CAA TTC AGT TGA GTC AAC ATC AGT C-3′. The miR-145 primer sequences were: forward, 5′-GGG CAA AAG GAA CCT CAG TG-3′; reverse, 5′-CAC ACA CAC ACA CAC TGA GG-3′. The miR-145 probe sequence was 5′-AGG GAU UCC UGG GAA AAC UGG ACG GGT CTG CTC GTG GGT-3′. The U6 primer sequences were: forward, 5′-CTG CTT CGG CAG CAC A-3′; reverse, 5′-AAC GCT TCA CGA ATT TGC GT-3′. The U6 probe sequence was 5′-CCA TGC TAA TCT CTG TAT CGT TCC A-3′. The total reaction volume for qRT-PCR was 20 μL, and the following kits were used: Taqman Gene Expression Master Mix and the TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA). The detailed RT-qPCR procedure is described as follows: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minutes. The expression levels of miRNAs, normalized to U6, were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1

Basic information of the participants.

Variables	Mild sepsis group (n=102)	Severe sepsis group (n=78)	Control group (n=180)	P
Age (y, mean±SD)	58.3±11.7	59.2±12.9	58.1±9.6	
Gender [male, n (%)]	64 (62.7%)	43 (55.1%)	121 (67.2%)	.187
SOFA score	4.9±2.9	8.5±3.2		<.001
Source of infection				
Respiratory infection	82 (80.4%)	59 (75.6%)		
Wound infection	10 (9.8%)	16 (20.5%)		
Urinary tract infection	3 (2.9%)	3 (3.8%)		
Primary bloodstream infection	41 (40.2%)	33 (42.3%)		
Abdominal infection	38 (37.3%)	27 (34.6%)		
Catheter-related infection	14 (13.7%)	12 (15.4%)		
Others	10 (9.8%)	5 (6.4%)		
Pathogen				
Gram-negative	37 (36.3%)	29 (37.2%)		
Gram-positive	18 (17.6%)	12 (15.4%)		
Gram-negative and gram-positive	35 (34.3%)	26 (33.3%)		
Fungus	12 (11.8%)	11 (14.1%)		

2.5. Genotype detection

Genomic DNA was extracted using a QIAamp DNA Blood Mini kit (Qiagen Inc, CA), eluted in a $50\,\mu L$ volume and stored at $-80°C$. Primer sequences used for PCR amplification were as follows: rs12605436 site forward: 5′-CCA GCT TCA TCC ATG TCC CT-3′, reverse: 5′-GAC CCA GCC ATC CCA TTA CT-3′; rs13137 site forward: 5′- ACT CTA AGT GCC ACC AGA CA-3′, reverse: 5′- TGC CTA CCA TCG TGA CAT CT-3′; miR-145 rs353291 site forward: 5′-CTC TGA GCC TCA CTG ATC CC-3′, reverse: 5′-GCT CTT CCC CTA CAC ATG GT-3′. The PCR amplification reaction volume was $20\,\mu L$, including 100 ng of genomic DNA, $2\,\mu L$ of $10\times$ PCR buffer, $1.5\,\mu L$ of $25\,mmol/L$ Mg^{2+}, $1\,\mu L$ of $10\,\mu mol/L$ dNTP, $1\,\mu L$ of $10\,pmol/L$ forward and reverse primers, and $0.25\,\mu L$ of DNA polymerase. Less than $20\,\mu L$ volume was supplemented with sterile water. The PCR reaction conditions are: $95°C$ for 5 minutes, $94°C$ for 20 seconds, $60°C$ for 30 seconds, followed by 30 cycles of $72°C$ for 30 seconds, $75°C$ for 5 minutes, and $4°C$ storage. After the PCR, the amplification product was purified and sequencing was outsourced to Shanghai Shen Gong Industrial Co Ltd. Fifty percent of the sequencing results were taken for repeated detection to ensure the accuracy of the sequencing results.

2.6. Statistical analysis

Continuous variables were expressed as mean $\pm$ SD and statistical analysis was performed using independent sample t test and one-way ANOVA. The categorical variables were expressed as a percentage [n (%)] and statistical analysis was performed using χ^2 test. Whether the genotype frequency was consistent with the Hardy–Weinberg equilibrium (HWE) was assessed by the χ^2 test. The association of SNPs with occurrence of sepsis was determined based on the distribution of allele frequencies and genetic models (additive, dominant, and recessive models). The odds ratio (OR) and 95% confidence interval (CI) were used in unconditional logistic regression analysis, and the age, gender, and other factors were corrected.

3. Results

3.1. Plasma levels of TNF-α and IL-6

The plasma levels of TNF-α and IL-6 were detected by ELISA. The results showed that the levels of TNF-α and IL-6 in the plasma of sepsis patients were significantly higher than those in the control group ($P < .0001$) (Fig. 1A and B).

3.2. Correlation between plasma TNF-α and IL-6 levels and severity of sepsis

The results of the analysis showed that the plasma levels of TNF-α and IL-6 in patients with severe sepsis were significantly higher than those in patients with mild sepsis, whereas the plasma levels of TNF-α and IL-6 in patients with mild sepsis were significantly higher than those in the control group. One-way analysis of variance showed that the difference between the plasma levels of TNF-α and IL-6 in the 3 groups was statistically significant ($P < .0001$) (Fig. 2A and B).

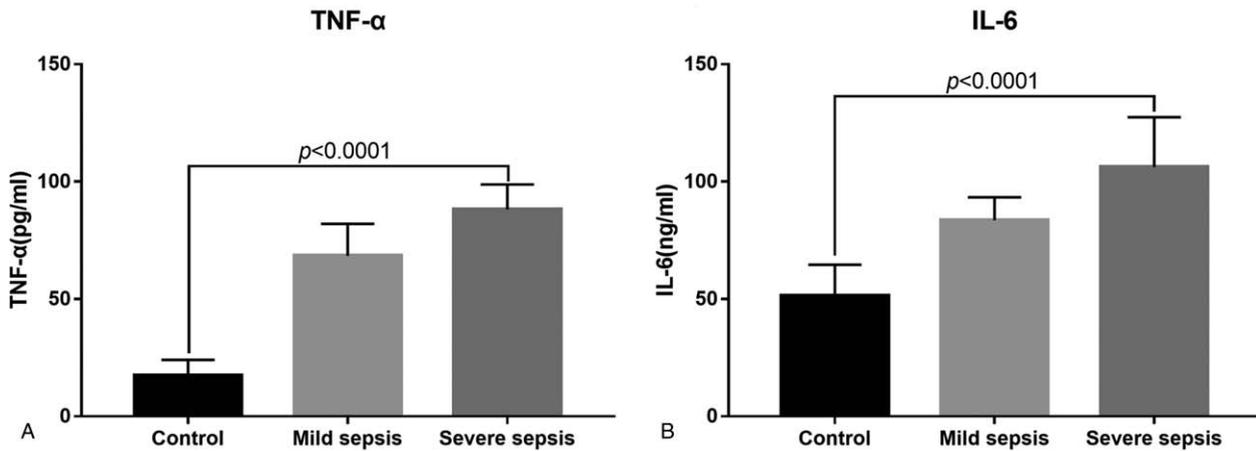### 3.3. Comparison of plasma levels of miRNAs

The plasma levels of miR-187 in sepsis patients were significantly lower than those in the control group, while the plasma levels of miR-21 and miR-145 in sepsis patients were significantly higher than those in the control group. The difference among the groups was statistically significant ($P < .0001$) (Fig. 3A–C).

3.4. Correlation between plasma levels of miRNAs and levels of TNF-α and IL-6

Correlation between plasma levels of miR-187, miR-21, and miR-145 and plasma levels of TNF-α and IL-6 was investigated in patients with sepsis. The results showed that miR-187 levels were negatively correlated with levels of TNF-α and IL-6 (r = − 2841, −0.2163; Fig. 4A and D). However, plasma levels of miR-21 and miR-145 were positively correlated with plasma levels of TNF-α and IL-6 (r = 0.615, 0.3057, 0.4465, 0.2734; Fig. 4B, C,
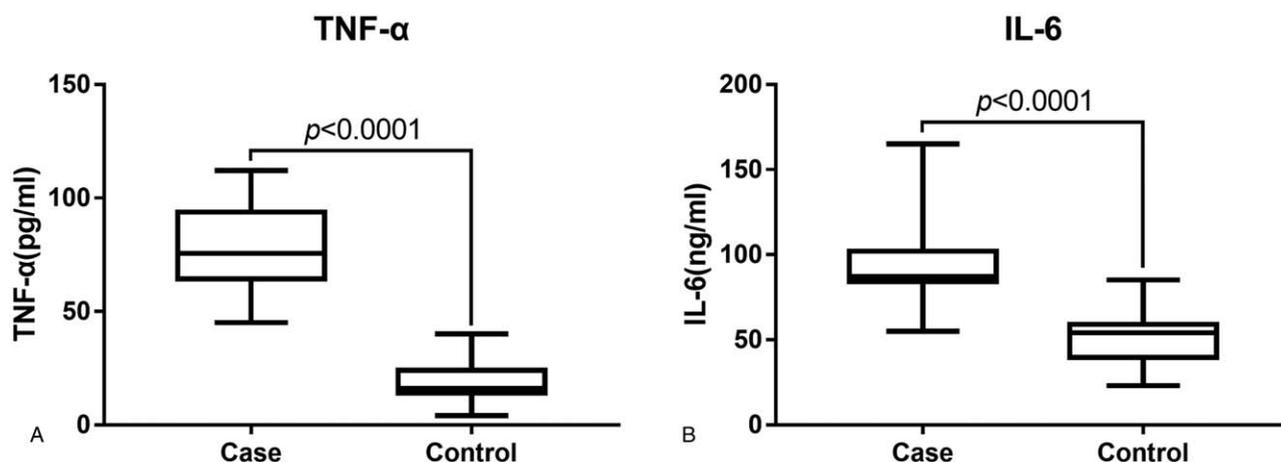
Figure 1. Comparison of plasma TNF-α and IL-6 levels in sepsis patients and in the control group. A, Plasma TNF-α level in sepsis patients compared with that in the control group. B, Plasma IL-6 level in sepsis patients compared with the control group. TNF = tumor necrosis factor, IL = interleukin.

Figure 2. Plasma levels of TNF-α and IL-6 in patients with sepsis of different severity. A, Comparison of plasma TNF-α levels in patients with sepsis of different severity. B, Comparison of plasma IL-6 levels in patients with sepsis of different severity. TNF = tumor necrosis factor, IL = interleukin.

E, F). However, in the control group, plasma miR-187 levels were negatively correlated with plasma TNF-α and IL-6 levels ($P > 0.05$; Fig. 5A and D). Plasma levels of miR-21 and miR-145 were not correlated with plasma TNF-α, IL-6 levels ($P > .05$; Fig. 5B, C, E, F).

3.5. miRNA gene polymorphism and sepsis risk

The frequency distributions of rs12605436, rs13137, and rs353291 were consistent with the Hardy–Weinberg equilibrium ($P > .05$). Taking the CC genotype of the rs12605436 as a reference, homozygous (TT), dominant model, and recessive model all had higher risk of sepsis (OR = 1.748, 95% CI = 1.320–2.131, $P < 0.001$; OR = 1.413, 95% CI = 1.138–1.744, $P = .001$; OR = 1.590, 95% CI = 1.225–1.893, $P = .001$; respectively). Using C allele of the rs12605436 locus as a reference, T allele was revealed to be a risk factor for sepsis (OR = 1.403, 95% CI = 1.205–1.612, $P < .001$). With reference to the AA genotype of the rs13137 locus, the heterozygous (AT) dominant model and the homozygous (TT) recessive model were found to be protective factors for sepsis (OR = 0.781, 95% CI = 0.620–0.981, $P = .032$; OR = 0.583, 95% CI = 0.380–0.845, $P = .002$; OR = 0.724, 95% CI = 0.586–0.898, $P = .003$; OR = 0.651, 95% CI = 0.425–0.932, $P = .015$; respectively). With reference to the rs13137, A allele and the T allele were found to be a protective factor for sepsis

(OR = 0.755, 95% CI = 0.632–0.896, $P < .001$). With reference to the CC genotype of the rs353291 locus, heterozygous (CT) dominant model and the homozygous (TT) recessive model were revealed to be protective factors for sepsis (OR = 0.778, 95% CI = 0.619–0.974, $P = .027$; OR = 0.455, 95% CI = 0.255–0.710, $P < .001$; OR = 0.692, 95% CI = 0.557–0.861, $P < .001$; OR = 0.494, 95% CI = 0.284–0.783, $P = .001$; respectively). Based on the C allele of the rs353291 locus, the T allele was found to be a protective factor for sepsis (OR = 0.685, 95% CI = 0.566–0.820, $P < .001$), as shown in Table 2.

### 3.6. miRNA gene polymorphisms and miRNA levels

Plasma levels of miR-187, miR-21, and miR-145 were compared among individuals with different genotypes. Plasma levels of miR-187, miR-21, and miR-145 levels were significantly higher in wild-type individuals than in mutant individuals, and the difference was statistically significant ($P < .05$) (Fig. 6A–C).

4. Discussion

This study investigated the relationship between plasma levels of miR-187, miR-21, and miR-145 levels and their SNPs in association with the risk of sepsis. The results showed that SNPs
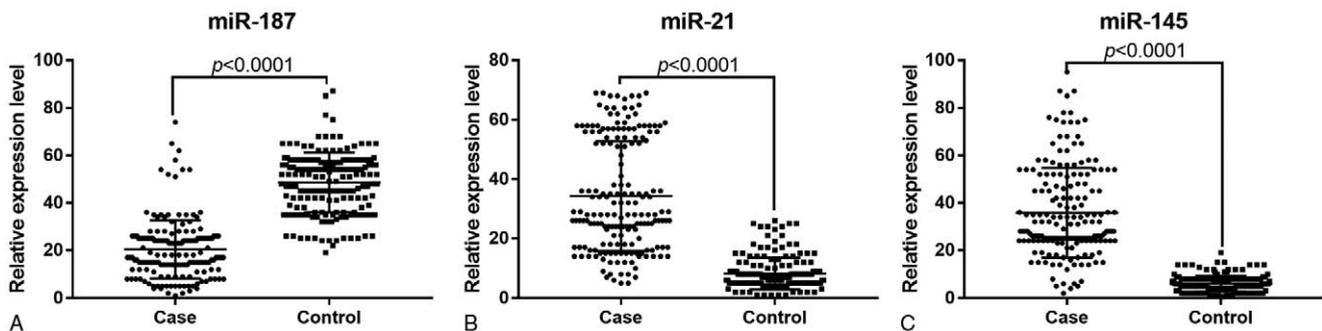

Figure 3. Comparison of plasma levels of miR-187, miR-21, and miR-145. A, Comparison of the plasma level of miR-187 between sepsis patients and the control group. B, Comparison of the plasma level of miR-21 between sepsis patients and the control group. C, Comparison of plasma level of miR-145 between sepsis patients and the control group.
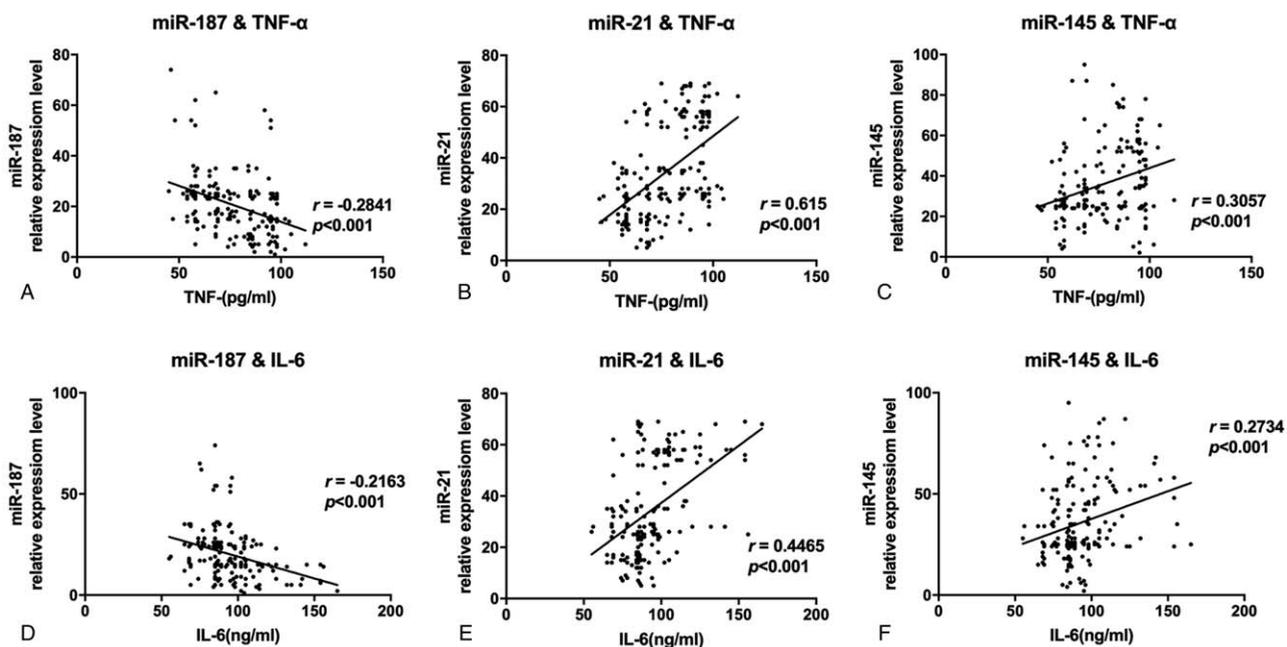
Figure 4. Correlation of plasma levels of miR-187, miR-21, and miR-145 with levels of TNF-α and IL-6 in patients with sepsis. A, Correlation between miR-187 and TNF-α levels. B, Correlation between miR-21 and TNF-α levels. C, Correlation between miR-145 levels and TNF-α levels. D, Correlation between miR-187 levels and IL-6 levels. E, Correlation between miR-21 and IL-6 levels. F, Correlation between miR-145 and IL-6 levels. The lines in the figure represent the trend line. TNF = tumor necrosis factor, IL = interleukin.

of miR-187 (rs12605436), miR-21 (rs13137), and miR-145 (rs353291), and their plasma levels were associated with the development of sepsis. The presence of T allele at rs12605436 locus is a risk factor for sepsis, whereas the presence of T allele at rs13137 locus and at rs353291 locus is a protective factor for sepsis. The mechanism may be related to the expression level of miRNAs and miRNA-mediated regulation of the expression of inflammatory cytokines, such as TNF-α and IL-6.
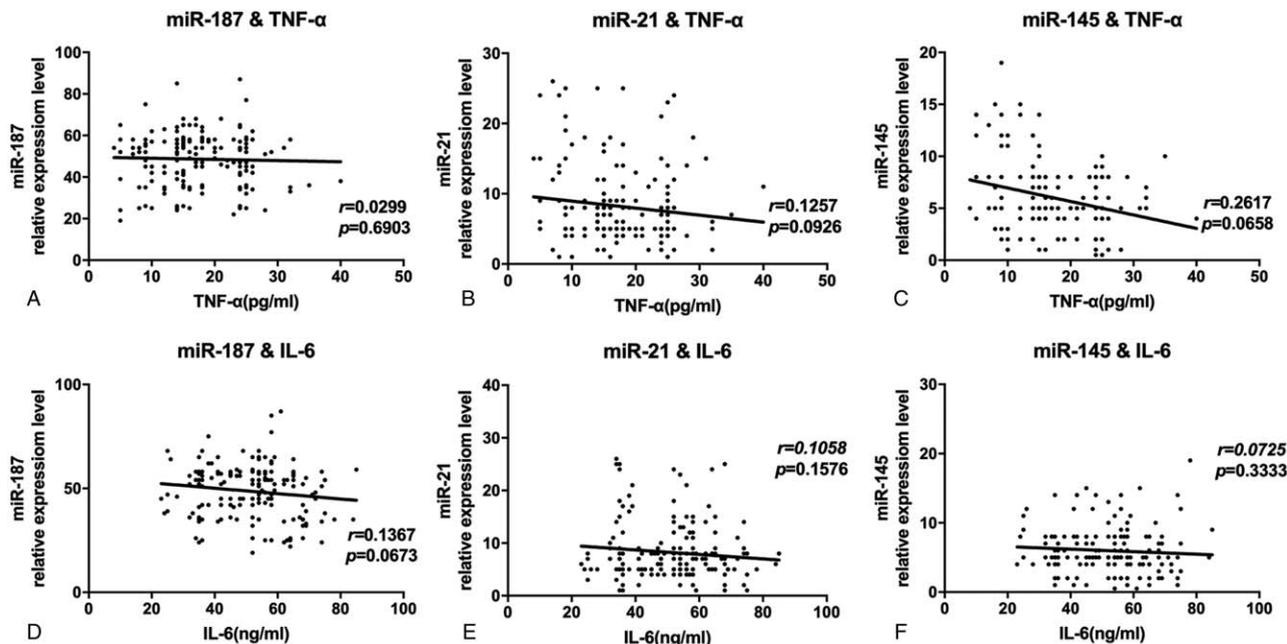
Figure 5. Correlation between plasma miR-187, miR-21, miR-145 levels, and TNF-α and IL-6 levels in the control group. A, Correlation between plasma miR-187 and TNF-α levels. B, Correlation between plasma miR-21 and TNF-α levels. C, Correlation between plasma miR-145 and TNF-α levels. D, Correlation between miR-187 and IL-6 levels. E, Correlation between miR-21 and IL-6 levels. F, Correlation between miR-145 and IL-6 levels. The lines in the figure represent the trend line.

Table 2

rs12605436, rs13137, and rs353291 genotypes and allele frequencies.

	Case (n = 180)	Control (n = 180)	P	Crude OR (95% CI)	P	Adjusted OR (95% CI)
rs12605436						
CC	85 (47.22%)	116 (64.44%)		1.000 (reference)		
CT	61 (33.89%)	52 (28.89%)	.046	1.601 (0.981–2.616)	.061	1.277 (0.990–1.621)
TT	34 (18.89%)	12 (6.67%)	<.001	3.867 (1.800–8.434)	<.001	1.748 (1.320–2.131)
Dominant model			<.001	2.026 (1.298–3.163)	.001	1.413 (1.138–1.744)
Recessive model			<.001	3.260 (1.557–6.938)	.001	1.590 (1.225–1.893)
C	23164.17 (%)	284 (78.89%)		1.000 (reference)		
T	129 (35.83%)	76 (21.11%)	<.001	2.087 (1.477–2.951)	<.001	1.403 (1.205–1.612)
rs13137						
AA	94 (52.22%)	65 (36.11%)		1.000 (reference)		
AT	66 (36.67%)	77 (42.78%)	.024	0.593 (0.366–0.960)	.032	0.781 (0.620–0.981)
TT	20 (11.11%)	38 (21.11%)	.001	0.364 (0.185–0.712)	.002	0.583 (0.380–0.845)
Dominant model			.002	0.517 (0.331–0.806)	.003	0.724 (0.586–0.898)
Recessive model			.010	0.467 (0.249–0.871)	.015	0.651 (0.425–0.932)
A	254 (70.56%)	207 (57.50%)		1.000 (reference)		
T	106 (29.44%)	153 (42.50%)	<.001	0.585 (0.424–0.807)	<.001	0.755 (0.632–0.896)
rs353291						
CC	102 (56.67%)	69 (38.33%)		1.000 (reference)		
CT	65 (36.11%)	75 (41.67%)	.020	0.586 (0.364–0.944)	.027	0.778 (0.619–0.974)
TT	13 (7.22%)	36 (20.00%)	<.001	0.244 (0.113–0.519)	<.001	0.455 (0.255–0.710)
Dominant model			<.001	0.475 (0.305–0.740)	<.001	0.692 (0.557–0.861)
Recessive model			<.001	0.311 (0.150–0.637)	.001	0.494 (0.284–0.783)
C	269 (74.72%)	213 (59.17%)		1.000 (reference)		
T	91 (25.28%)	147 (40.83%)	<.001	0.490 (0.352–0.682)	<.001	0.685 (0.566–0.820)

OR was calculated using unconditional logistic regression analysis. OR>1.0 indicates that the factor is a risk factor, and OR<1.0 indicates that the factor is a protective factor.

Sepsis is a systemic inflammatory response, mainly caused by a host's dysregulation of infection.[2] Previous studies have shown that there are approximately 10 sepsis patients per 1000 hospitalized patients, of which approximately 30% of the sepsis patients have multiple organ dysfunction syndrome, 20% of sepsis patients, and 60% to 80% of patients suffer from septic shock resulting in patient death.[16,17] Due to the high mortality rate caused by sepsis, early diagnosis and treatment are very important for improving the prognosis.

miRNAs are short noncoding RNAs involved in post-transcriptional gene regulation and are critical in several life processes such as cell growth and development.[18,19] According to previous studies, miR-187 plays different roles in different types of human cancers, such as hepatocellular carcinoma and colorectal cancer. It has been found that miR-187 can inhibit the metastasis and epithelial–mesenchymal transition of liver cancer and colorectal cancer. Studies on colorectal cancer have shown that miR-187 also inhibits the growth of colorectal cancer cells.[20–22] There is no research to confirm that miR-187 is associated with sepsis. Some studies have shown that when miR-187 is silenced, there is a significant increase in the production of LPS-induced TNF-α and IL-6. miR-187 directly targets the stability and translation of TNF-α mRNA and indirectly reduces the expression of IL-6 by downregulating IκBζ.[10] The results of this study showed that the plasma level of miR-187 in sepsis patients was significantly lower than that in the control group, and the levels of TNF-α and IL-6 were significantly higher than those in the control group, which was consistent with the results of Rossato et al.[10] miR-21 regulates the expression of programmed cell death 4 (PDCD4) after lipopolysaccharide (LPS) stimulation. PDCD4 is essential for LPS-mediated lethality. Studies have shown that PDCD4-deficient mice are immune to
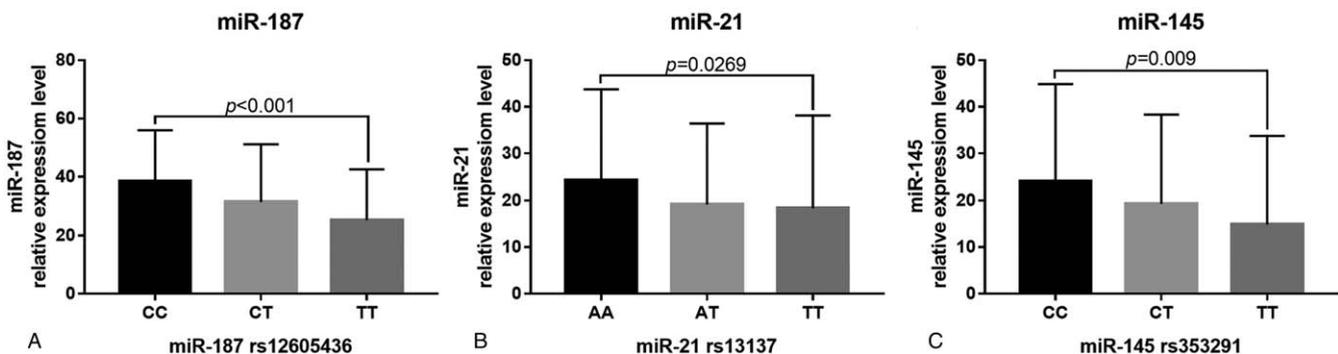
Figure 6. Comparison of plasma levels of miR-187, miR-21, miR-145 in individuals with different genotype. A, Shows miR-187 levels in individuals with different genotypes at rs12605436 locus. B, Shows miR-21 levels in individuals with different genotypes at rs13137 locus. C, Shows miR-145 levels in individuals with different genotypes at rs353291 locus.

LPS-induced death, and that LPS-mediated induction of IL-6 requires PDCD4.[23–25] miR-145 promotes TNF-α expression,[9] and overexpression of miR-145 reduces IL-6 levels in the vascular smooth muscle cell supernatant.[26] However, the results of this study showed that the levels of miR-21 and miR-145 in the plasma of patients with sepsis were positively correlated with IL-6 and TNF-α levels. Such correlation may arise due to the interaction of different regulatory pathways. In this study, plasma miR-21 was positively correlated with the expressions of IL-6 and TNF-α in patients with sepsis. Therefore, it was concluded that the expression of IL-6 and TNF-α may be regulated by various miRNAs, as was previously shown by Benz et al.[9] However, plasma miR-187 levels were not associated with plasma TNF-α and IL-6 levels in the control group, and plasma miR-21 and miR-145 levels were not associated with plasma TNF-α and IL-6 levels. Analysis of the reasons I believe that the regulation of the expression of cytokines, such as IL-6 and TNF-α, is not mediated by a single miRNA, but is affected by several miRNAs. Smaller sample sizes may also be influencing factors, and further expansion of sample size is needed for research.

This study also examined the effects of SNPs in miRNAs involved in the development of sepsis. The MAFs of SNP loci selected in this study were all lower than 0.05. Previously, no research has been conducted to confirm that rs12605436, rs13137, and rs353291 are associated with the expression and regulation of miR-187, miR-21, and miR-145. The selection of these SNP loci is based on the high frequency of mutations in these SNP loci in the Chinese population. Studying these SNP loci may be of interest to elucidate the regulation of miRNA expression. The results of the study showed that the risk of sepsis in the rs12605436 T allele carrier was 1.403 times higher than that in the C allele carrier. The rs13137 T allele carrier was 0.755 times less likely to be infected with sepsis compared to the A allele carrier. The rs353291 T allele carrier was 0.685 times less likely to develop sepsis compared with the C allele carrier. This indicates that the genetic variation in miRNAs has a certain influence on their expression and regulation, and this result is of great significance for providing a more comprehensive understanding of the regulation of expression of miRNAs.

However, this study had some limitations. First, the number of cases was small, and the number of homozygous samples was also low. Therefore, the correlation between SNP and sepsis of different severity could not be analyzed. Second, it was not possible to design an in vitro model to assess the regulation of IL-6 and TNF-α expression by miRNAs. In addition, it is necessary to analyze the effects of more SNPs and SNPs interactions on the risk of sepsis in large samples, and to explore the effects of miRNAs.

5. Conclusion

rs12605436, rs13137, and rs353291 SNPs affect miR-187, miP-21, and miR-145 levels, respectively. Plasma levels of miRNA containing these SNPs are associated with sepsis and its severity. The possible cause of this association is the involvement of miRNAs in the regulation of the expression of inflammatory cytokines, such as TNF-α and IL-6.

Author contributions

Conceptualization: Yan Zhang.
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Formal analysis: Min Li, Pengfei Hu.
Investigation: Lanying Bao.
Supervision: Yan Zhang.
Writing – review & editing: Yan Zhang.

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