LincRNA-NR_024015 rs8506 TT genotype contributes to the risk of sepsis in a southern Chinese child population

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Abstract

Sepsis is a life-threatening heterogeneous syndrome due to a dysregulated immune response to infection. Studies have shown that genetic polymorphisms might have impact on the risk of sepsis. LincRNA-NR_024015, also known as testis development related 1(TDRG1), is a newly identified long non-coding RNA (lncRNA). It has been found to participate in vascular endothelial growth factor (VEGF) signaling in human diseases, but its relevance in the development of sepsis is still unclear. In the present study, we genotyped lncRNA TDRG1 rs8506 polymorphism in 474 patients and 678 healthy controls recruited from a southern Chinese child population using Taqman methodology. Overall, a significant association was found between rs8506 polymorphism and the risk of sepsis disease (TT vs. CC/CT: adjusted OR = 1.751, 95%CI = 1.024–2.993, P = 0.0406). In the stratified analysis, the results suggested that the carriers of TT genotypes had a significantly increased sepsis risk among the children aged 12–60 months, females, early-stage sepsis and survivors (TT vs. CC/CT: ORage = 2.413; ORfemale = 2.868; ORsepsis = 2.533; ORsurvivor = 1.822; adjusted for age and gender, $P < 0.05$, respectively). Our study indicated that lncRNA TDRG1 rs8506 TT genotype might contribute to the risk of sepsis in a southern Chinese child population. Future research is required to elucidate the possible immunoregulatory mechanisms of this association.

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Running title: TDRG1 rs8506 polymorphism and sepsis risk

Key words:

TDRG1 , genetic polymorphisms, risk, sepsis

Abbreviations

TDRG1, testis development related 1; lncRNA, long non-coding RNA; VEGF, vascular endothelial growth factor; HWE, Hardy–Weinberg equilibrium; OR, odd ratio; CI, confidence intervals; SD, standard deviation.

Summary

Sepsis is a life-threatening heterogeneous syndrome due to a dysregulated immune response to infection. Studies have shown that genetic polymorphisms might have impact on the risk of sepsis.*LincRNA-NR_-024015* , also known as testis development related 1(*TDRG1*), is a newly identified long non-coding RNA (lncRNA). It has been found to participate in vascular endothelial growth factor (VEGF) signaling in human diseases, but its relevance in the development of sepsis is still unclear. In the present study, we genotyped lncRNA *TDRG1* rs8506 polymorphism in 474 patients and 678 healthy controls recruited from a southern Chinese child population using Taqman methodology. Overall, a significant association was found between rs8506 polymorphism and the risk of sepsis disease (TT *vs* . CC/CT: adjusted OR = 1.751, 95%CI = 1.024–2.993, $P = 0.0406$). In the stratified analysis, the results suggested that the carriers of TT genotypes had a significantly increased sepsis risk among the children aged 12–60 months, females, early-stage sepsis and survivors (TT*vs.* CC/CT: $OR_{age} = 2.413$; $OR_{female} = 2.868$; $OR_{sepsis} = 2.533$; $OR_{survivor} = 1.822$; adjusted for age and gender,$P < 0.05$, respectively). Our study indicated that lncRNA *TDRG1* rs8506 TT genotype might contribute to the risk of sepsis in a southern Chinese child population. Future research is required to elucidate the possible immunoregulatory mechanisms of this association.

Introduction

Sepsis, a syndrome caused by a dysregulated immune response to infection, is a life-threatening worldwide health issue[1]. Globally, there were an estimated 48.9 million cases of sepsis and 11.0 million potential sepsis-related deaths in 2017[2]. According to the age, sepsis incidence peaked in early childhood who were younger than 5 years, representing 41.5% (estimated 20.3 million) of overall cases of sepsis in 2017[2]. At present, despite advances in the diagnosis and treatment of sepsis, it remains a clinical challenge for clinicians and researchers due to the fact that sepsis is still the main cause of mortality worldwide[2, 3].

Over the past decade, the development of genomics has substantially provided us with a better understanding of the biological pathway involved in various human diseases[4]. Emerging studies have demonstrated that polymorphisms of the genetic components encoding inflammation-associated mediators were markedly associated with the development of human sepsis[5-9]. Recently, non-coding RNAs, including microRNAs, long non-coding RNAs(lncRNAs) and circular RNAs, were also proven to mediate the pathogenesis of sepsis and could serve as biomarkers for septic diagnosis[10-12]. Currently, most studies predominantly focus on functions of microRNAs in sepsis, little is known about the relationship between lncRNAs and sepsis.

LncRNA is a type of RNA with more than 200 nucleotides in length[10, 13]. Although lacking of protein-coding capacity, lncRNAs have been found to be related to so many pathological processes of human diseases[10]. *LincRNA-NR_024015* , also known as testis development related 1(*TDRG1*), is a newly identified tumor-associated lncRNA. Studies have shown that lncRNA *TDRG1*could serve as a proto-oncogene in multiple tumor types[14-17]. Chen and colleagues revealed that lncRNA *TDRG1* might promote endometrial carcinoma cell proliferation and invasion by targeting vascular endothelial growth factor (VEGF)[16]. It has long been documented that VEGF serves as a critical regulator of vascular permeability and inflammation[18-20]. Furthermore, growing evidences have indicated that the level of plasma VEGF was markedly associated with the development of human sepsis[21-25]. Based on these findings, we considered that *TDRG1* might have critical effects on the pathogenesis of sepsis by interacting with VEGF. Although significant correlations were recently found between the polymorphism of lncRNA *TDRG1* rs8506 and cancer susceptibility[26, 27], its association with sepsis susceptibility and clinical outcomes has not been addressed yet.

In terms of the potential immunopathological roles of lncRNA *TDRG1* in sepsis, the present study aimed to investigate the association between the lncRNA *TDRG1* rs8506 polymorphism and sepsis susceptibility in the current hospital-based case–control study with 474 cases and 678 healthy controls in a southern Chinese child population.

Materials and Methods

Study population

The study included 474 patients with sepsis, who were randomly selected from January 2016 to December 2018 in our hospital. The diagnostic criterial for sepsis, severe sepsis and septic shock were based on the Surviving Sepsis Campaign Guidelines for Management of Severe Sepsis and Septic Shock[28]. The 678 subjects in the control group were recruited from our hospital according to the age and gender of the patients in an approximate 1:1 ratio. The characteristics of all subjects in the present study were shown in Table 1. This study had received the ethics approval of the Institutional Review Board of Guangzhou Women and Children's Medical Center (2015042202). Written informed consent was obtained from each participant guardian.

DNA extraction and SNP genotyping

The SNP and associated information were adopted in this study according to the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/, http://snpinfo.niehs.nih.gov/). Genomic DNA samples were extracted from peripheral blood samples by the phenol–chloroform method using the TIANamp Blood DNA Kit (TianGen Biotech, Beijing, China) as described previously[29]. Genotyping for the *LincRNA-NR_024015* (namely, lncRNA *TDRG1*) rs8506 C>T was performed in the 384-well plate using Taqman PCR method[30]. Genotyping was performed blindly to the status of the case or control.

Statistical Analysis

The differences in the genotype frequencies of lncRNA *TDRG1* rs8506 and the demographic variables between sepsis cases and healthy controls were compared applying the χ^2 test. Hardy–Weinberg equilibrium (HWE) of the healthy controls for rs8506 was tested using the goodness-of-fit χ^2 test. Multivariate logistic regression analyses were carried out to calculate the odd ratios (ORs) and their 95% confidence intervals (CIs) for risk of sepsis, which were also stratified by the age, gender, sepsis subtype, prognosis and the number of organs with dysfunction.

Results

Population Characteristics

In order to describe whether SNP in the lncRNA *TDRG1* rs8506 was associated with susceptibility to sepsis in a southern Chinese child population, we carried out a case–control study with a cohort of 474 Chinese patients with sepsis and 678 healthy controls. The general and clinical characteristics of the study population were summarized in Table 1. On average, the patients were 24 months old (range: 1–180; standard deviation, SD: ±35) and the healthy controls were 26 months old (range: 1–168; SD: ±31). Of the 474 patients, 63% were male individuals and 37% were female; while 59% of the healthy controls were male and 41% were female. No statistically significant differences were found between cases and control groups with respect to age ($P = 0.272$) and gender ($P = 0.111$). During the observation period, 61.2% of the patients were in severe sepsis and 17.9% were in septic shock. Considering the prognosis of the patients, the patient group was subdivided into survivors (394; 83.1%) and non-survivors (80;16.9%). Moreover, 58.2% of all the enrolled patients developed 1-2 organs with dysfunction; 3 or more organs with dysfunction occurred in about 20% of all the patients.

Association Analysis

The genotypes of lncRNA *TDRG1* rs8506 were successfully evaluated in the present study. As shown in Table 2, the distribution of the genotypes agreed with the Hardy–Weinberg equilibrium in the healthy controls ($P = 0.234$). We observed that the population who carried rs8506 TT genotype had a 1.755-fold higher risk of sepsis than those did not carry (TT *vs* . CC/CT: OR = 1.755, 95%CI = 1.028–2.996,$P = 0.0394$). Furthermore, after adjustments for age and gender, significantly elevated risk of sepsis was also found in the rs8506 TT genotype, as compared with rs8506 CC/CT genotype (TT *vs* . CC/CT: adjusted OR = 1.751, 95%CI = 1.024–2.993, $P = 0.0406$).

Stratified Analysis

We further performed a stratified analysis of the relationship between lncRNA *TDRG1* rs8506 polymorphism and sepsis susceptibility by clinical features (Table 3). The rs8506 TT genotype was found to be markedly associated with an increased sepsis risk among the children aged 12–60 months (TT *vs.* CC/CT: OR = 2.377, 95%CI = 1.068–5.286, $P = 0.0338$; adjusted OR = 2.413, 95%CI = 1.083–5.376, $P = 0.0311$), females (TT *vs.* CC/CT: OR = 2.848, 95%CI = 1.218–6.658, $P = 0.0157$; adjusted OR = 2.868, 95%CI = 1.226–6.714, $P = 0.0152$), sepsis (TT *vs.* CC/CT: OR = 2.508, 95%CI = 1.139–5.522, $P = 0.0224$; adjusted OR = 2.533, 95%CI = 1.149–5.586, $P = 0.0213$), and survivors (TT*vs.* CC/CT: OR = 1.845, 95%CI = 1.061–3.209, $P = 0.0301$; adjusted OR = 1.822, 95%CI = 1.046–3.175, $P = 0.0343$). However, no significant associations were found in other stratified analyses.

Discussion

In the present study, we enrolled a cohort of 474 cases with sepsis and 678 controls to evaluate the association between*LincRNA-NR_024015* rs8506 polymorphism and the sepsis susceptibility among southern Chinese children. The result showed that the carriers of rs8506 TT genotype had a significantly increased risk of sepsis when compared with that carrying CC/CT genotypes. Interestingly, the stratified analysis revealed that the increased risk level of rs8506 TT variant appeared more obvious in the children of 12–60 months old and female. Moreover, we also found that the rs8506 TT variant showed significantly elevated risk of sepsis in the subgroup of the patients who were in the early stage of sepsis or alive. Therefore, our findings provided evidences that *LincRNA-NR_024015* rs8506 TT genotype might be associated with the susceptibility of sepsis in a southern Chinese child population.

Long non-coding RNAs (lncRNAs) are a type of non-protein-coding RNAs which exceed 200 nucleotides in length[10, 13]. It has been suggested that lncRNAs play important roles in the pathogenesis of various diseases through chromatin rearrangement, transcriptional control as well as post-transcriptional processing[10, 13]. However, little is known about the relationship between lncRNAs and the sepsis susceptibility[11, 31]. In the present study, we found that*LincRNA-NR_024015* rs8506 TT genotype was notably associated with an increased risk of sepsis. To our knowledge, this is the first study to evaluate the relationship of the rs8506 polymorphism with sepsis risk in a southern Chinese child population.

LincRNA-NR_024015 (gene ID: 732253) is also known as testis developmental related gene 1 (*TDRG1*). *TDRG1* was initially identified as a novel human testis-specific gene which served as a regulator in sperm motility and the development of testicular germ cell tumors[32, 33]. Recent studies have suggested that lncRNA *TDRG1* might play important roles in tumor progression in several cancer types including cervical[14], esophageal[26], ovarian[17] and endometrial carcinoma[16]. Chen *et al*provided evidences that lncRNA TDRG1 might directly bound to VEGF-A protein and upregulated its expression, thus promoting the progression of endometrial carcinoma[16]. Moreover, lncRNA TDRG1 and VEGF were found to be co-expressed and remarkably upregulated in fibrovascular membranes from diabetic retinopathy patients than those from epiretinal membrane[34]. These reported data indicated that lncRNA TDRG1 might be beneficial to stimulate the VEGF pathway[34]. VEGF is a potent mediator that not only increases the vascular permeability, but also promotes leukocytes adhesion by eliciting the expression of adhesive molecules[18-20]. Actually, emerging data have suggested that circulating VEGF levels were elevated during the development of sepsis[21-25] and the levels of this factor were associated with sepsis severity and mortality[21-23]. Blockade of VEGF signaling in a mouse model might have beneficial effects on the survival of sepsis by decreasing inflammatory responses and endothelial permeability[35]. Importantly, the expression level of lncRNA *TDRG1* in esophageal tumor tissues with rs8506 CT and TT genotype was significantly higher than those with rs8506 CC genotype[26]. Therefore, in combination with the findings in our study, we speculated that rs8506 TT genotype might increase the risk of sepsis via upregulating the levels of lncRNA *TDRG1* and VEGF. Further study is needed to confirmed this possibility in the future.

Epidemiological studies have showed that global sepsis apparently occurred in females and young children below 5 years old[2]. Similar to the present study, the increased risk of the rs8506 TT variant genotype was

more evident in the children of 12–60 months old and in females, as compared with the CC/CT genotypes. Furthermore, it is surprising in our study, the rs8506 TT genotype was markedly associated with an increased sepsis risk among sepsis and survivor subgroup of the patient cohort, but not severe sepsis, septic shock or non-survivor. Owing to the robustly low frequency of rs8506 TT genotype in the enrolled cohort, we considered that the sample size was not enough to test the power of analysis. The current study is only an investigation that focus on the relationship between gene polymorphism and disease susceptibility. Therefore, more mechanistic studies are needed to confirm the roles of lncRNA *TDRG1* rs8506 TT in the progression of sepsis in children.

Although this is the first study to evaluate the association between lncRNA *TDRG1* rs8506 polymorphism and sepsis risk in southern Chinese children, several possible limitations should be addressed in present study. First, there are only 474 sepsis patients and 678 controls included. Therefore, the sample size in the current study might have impact on the test power of statistical analysis. Second, only rs8506 T allele was under investigation in the present study, other lncRNA *TDRG1* gene polymorphisms with potential function remain to be took into consideration. Third, it has been shown that many factors (i.e., living environment, social-economic factor, population education) have impact on the incidence of sepsis[2, 36]; however, we could only collect frequency-matched cases and controls by age and gender due to lack of these information.

In summary, we verified a significant association between lncRNA *TDRG1* rs8506 TT genotype and increased sepsis susceptibility in southern Chinese children, especially for children aged 12–60 months, females, and those with early stage of sepsis. Future studies with larger sample size and mechanistic experiments should be conducted to strengthen our findings.

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Disclosures

The authors declare that they have no conflict of interest.

Author contributions

Li JQ and Li HH designed the experiments; Li JQ, Li HH and Wei B performed the experiments; Che D and Xu YF analyzed the data; Pi L, Fu LY and Zhou HZ collected the samples and clinical data; Li JQ wrote the manuscript; Gu XQ revised and finalized the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1 Frequency of selected characteristics in sepsis cases and healthy controls

Variables	**Cases (n = 474)**
	No.
Age (range, month)	1-180
Mean ± SD	23.78 ±35.16
<12	233
12-60	194
>60	47
Gender	
Male	301
Female	173
Sepsis subtypes	**Sepsis subtypes**
sepsis	99
severe sepsis	290
septic shock	85
Prognosis	**Prognosis**
Survivors	394
Non-survivors	80
Number of organs with dysfunction, n (%)	**Number of organs with dysfunc**
1-2	276
3 or more	95
Note: [a] Two-sided χ^2 test for differences between Sepsis patients cases and controls.	**Note:** [a] Two-sided χ^2 test for diffe
Abbreviations: SD, standard deviation.	**Abbreviations:** SD, standard devi

Table 2 Genotype frequency of lncRNA TDRG1 rs8506 in sepsis cases and healthy controls

Genotype	**Cases (N =474)**	**Controls (N =678)**	*P*-**value** [a]	**OR (95% CI)**	*P*-**value**	**Adjusted OR (95% CI)** [b]	*P*-**value** [b]
LncRNA TDRG1 rs8506 C>T (HWE =0.234)	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**	**LncRNA TDRG1 rs8506 C>T (HWE =0.234)**
CC	299(63.08)	413(60.91)	**0.0436**	1.000		1.000	
CT	144(30.38)	239(35.25)		0.832(0.645-1.074)	0.1577	0.832(0.645-1.075)	0.1592
TT	31(6.54)	26(3.83)		1.647(0.958-2.832)	0.0712	1.644(0.955-2.830)	0.0727
Dominant	175(36.92)	265(39.09)	0.4563	0.912(0.716-1.162)	0.4566	0.912(0.716-1.163)	0.459

Recessive	443(93.46)	652(96.17)	**0.039**	**1.755(1.028-2.996)**	**0.0394**	**1.751(1.024-2.993)**	**0.0406**
Note: [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviations:** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.	**Note:** [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the healthy controls. [b] Adjusted for age and gender. The values are shown in bold if $P < 0.05$. **Abbreviatio** CI, confidence interval; OR, odds ratio; HWE, Hardy–Weinberg equilibrium.

Table 3 Stratification analysis of susceptibility in sepsis patients

Variables
Age, months
<12
12-60
>60
Gender
Male
Female
Sepsis subtypes

sepsis
severe sepsis
septic shock
Prognosis
Survivors
Non-survivors
Number of organs with dysfunction, n (%)
1-2
3 or more
Note: [a] χ^2 tests were used to determine differences in genotype distributions between the patients with sepsis and the heal
Abbreviations: CI, confidence interval; OR, odds ratio.