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## The clinical relevance of MBL2 gene polymorphism and sepsis

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### ABSTRACT

**Objective:** To detect the clinical relevance of mannose-binding lectin 2 (MBL2) gene polymorphism and sepsis in Chinese lived in Hainan island. **Methods:** Blood samples from 57 patients with sepsis and 69 patients without sepsis were collected in the ICU of several large hospitals in Hainan province. Genomic DNA was extracted from whole blood and then PCR purification product was sequenced and typed by 3730 sequencing analyzer. The concentration of MBL2 in serum was detected by ELISA. **Results:** We found that genotype and allele distributions in two groups were in accordance with the Hardy-Weinberg Equilibrium. The frequency of GA genotype was significantly higher than that in non-sepsis group ( $P=0.013$ ). A allele frequency in sepsis group was also much higher than that in non-sepsis group ( $P=0.028$ ). Logister regression analysis showed that the patients who carried A allele were more prone to get sepsis than G allele carrier ( $P=0.014$ ,  $OR=2.550$ ,  $95\%CI=1.207-5.386$ ). The MBL2 level in serum of sepsis patients with genotype GG and GA was significantly lower than that in non-sepsis group ( $P<0.05$ ). In sepsis group, the MBL2 serum level of patients with genotype GA was obviously lower than that in patients with genotype GG ( $P<0.05$ ). **Conclusions:** The variation of rs1800450 G→A increased the incidence of sepsis and decreased the level of MBL2 in serum.

## 1. Introduction

Sepsis is one of the most serious complications of clinical severe diseases, such as trauma, burn, shock or infection. It is also one of the leading causes of death in intensive care unit (ICU)[1]. Sepsis is rapidly changing, often resulting in poor prognosis due to lack of timely diagnosis and treatment. Early prediction of the occurrence

and development of sepsis and early intervention in high-risk patients can effectively reduce the incidence and mortality of sepsis.

We have done a systematic evaluation and meta-analysis on the genetic relevance between Mannose-binding lectin (MBL2) and sepsis[2], the results indicated that MBL2 gene polymorphism loci (rs5030737, rs1800450 and rs1800451) were closely related to the occurrence of sepsis. In this study, MBL2 gene polymorphism in patients with or without sepsis was analyzed to investigate the clinical relevance of MBL2 gene polymorphism and sepsis.

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## 2. Materials and methods

### 2.1. Clinical data

Blood samples from 57 patients with sepsis and 69 patients without sepsis were collected in the ICU of several large hospitals in Hainan province. The sepsis group included 36 males and 21 females with mean age of (48.3±11.6) years, the non-sepsis group included 41 males and 28 females, and the mean age was (46.8±10.8) years. All the participants were Chinese and lived in the Hainan island.

A sepsis diagnosis was made if patients met all of the following criteria<sup>[3]</sup>: (1) body temperature >38.3 °C or <36 °C, (2) heart rate >90 times/min or >normal heart rate at different age range +2 standard deviations, (3) breathing rate >20 times per minute or partial pressure of carbon dioxide in arterial blood <32 mmHg, (4) routine blood leukocytes count >(12 × 10<sup>9</sup>) /L or <(4 × 10<sup>9</sup>) /L or immature white blood cells during normal white blood cell count > 10%. Sepsis could be diagnosed by having the presence of two or more of the above signs and clinical evidence of infection. Patients were not eligible if they had following exclusive criteria: (1) Age is less than 14 years old, or more than 85 years old, (2) A sepsis is caused by non-infection, (3) Special groups such as pregnant women and lactating women, (4) In an immunosuppressive state: Autoimmune disease or acquired immunodeficiency disease, malignant tumor, long-term use of immunosuppressive agents, etc. The protocol for this study was approved by the Ethical and Protocol Review Committee of the Hainan Medical University.

### 2.2. Blood samples collection and DNA extraction

We took 5 mL peripheral venous blood from the patients with EDTA anticoagulant tubes. Also, Genomic DNA was extracted from whole blood using a TIANamp Blood DNA Kit (Tiangen Biotech, China) and stored in the -80 °C fridge to prepare.

### 2.3. PCR amplification and genotyping

The upstream primer is 5/-CAGGCAGTTTCCTCTGGAAG-3 and the downstream primer is 5/- AGTCACGCAGTGTCAACAAGG-3. The reaction system contains 2× Power Taq PCR Master Mix 15 μL, DNA 1 μL, upstream and downstream primers each 1 μL, with double distilled water up to 30 μL. The PCR conditions were run at 95 °C for 5 min followed by 35 cycles of 30 s at 95 °C, 30 s at given annealing temperature, 30 s at 72 °C, and finally, 72 °C for 5 min (Takara Biotech, Dalian, China). PCR purification product was sequenced and typed by 3730 sequencing analyzer. Genotyping was performed in a blinded fashion without knowledge of the patients' clinical data, and approximately 10% of the samples were genotyped in duplicate to monitor genotyping quality.

### 2.4. Serum MBL2 measurement

The concentration of MBL2 in serum was detected by enzyme-linked immunosorbent assay (ELISA) by ELISA kit (American R&D Company) according to the manufacturer's instruction.

### 2.5. Statistical analysis

The genotype frequencies of the case group and the control group were evaluated by Hardy-Weinberg equilibrium (HWE). The clinical relevance of MBL2 gene polymorphism and sepsis was determined by *Chi*-square test. Logistic regression analysis was used to analyze the associations of MBL2 gene polymorphism with sepsis after the correction factors (such as age, gender) have been adjusted. We used *t* test to assess the difference of MBL serum level between two groups. Statistical significance was presented when *P*<0.05.

## 3. Results

### 3.1. Clinical data

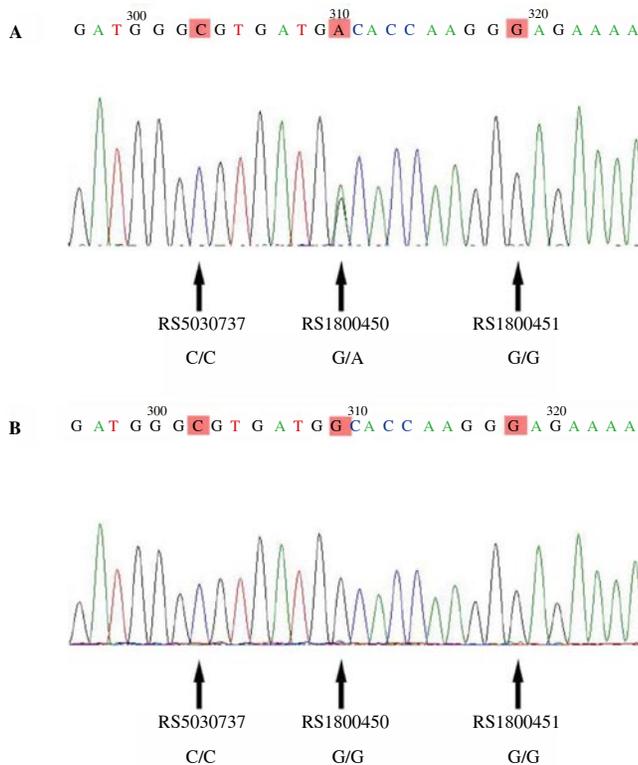
There was no significant difference in terms of sex ratio and age (*P*>0.05) between the two groups.

### 3.2. MBL2 gene polymorphisms and genotype frequency distribution

Only mutation site of rs1800450 G/A was found in the three sites of the MBL2 gene polymorphisms (rs5030737, rs1800450 and rs1800451). The sequencing peak was clear and has no obvious hybrid peaks. The results were so stable that we can easily distinguish GC, GA, CC, and GG genotypes (Figure 1). We found that genotype and allele distributions in two groups were in accordance with the Hardy-Weinberg test.

Frequency of rs1800450 genotypes GG, GA and AA was 52.6%, 47.4% and 0% in sepsis group, while 73.9%, 27.1% and 0% in non-sepsis group, respectively. Therefore, the frequency of GA genotype was significantly higher than that in non-sepsis group (*P*=0.013). Allele frequency analysis also showed A allele frequency in sepsis group was significant higher than that in non-sepsis group (*P*=0.028) (Table 1).

Logistic regression analysis showed that the patients who carried A allele were more prone to get sepsis than G allele carrier (*P*=0.014, *OR*=2.550, 95%*CI*=1.207-5.386), which means that the variation of rs1800450 G→A increased the incidence of sepsis.



**Figure 1.** Sequencing peak of rs5030737C/C, rs1800451G/G, rs1800450G/G and rs1800450G/A.

**Table 1**

Genotype distributions of rs1800450 polymorphism in different groups [n(%)].

Group	n	GG	GA	AA	G	A
Sepsis group	57	30 (52.6)	27 (47.4)	0 (0)	87 (76.3)	27 (23.7)
Non-sepsis group	69	51 (73.9)	18 (27.1)	0 (0)	120 (87.0)	18 (13.0)
$\chi^2$			6.157			4.819
P			0.013			0.028

### 3.3. MBL2 level in serum

The MBL2 level in serum in sepsis patients with genotype GG and GA was significantly lower than that in non-sepsis group ( $P < 0.05$ ). In sepsis group, the MBL2 serum level of patients with genotype GA was obviously lower than that in patients with genotype GG ( $P < 0.05$ ) (Table 2).

**Table 2**

MBL2 serum level in different groups.

Group	GG	GA	AA
Sepsis group	1.44±0.68	1.03±0.61 <sup>#</sup>	—
Non-sepsis group	2.17±0.92 <sup>*</sup>	1.86±0.77 <sup>*</sup>	—

\*: sepsis group vs. non-sepsis group,  $P < 0.05$ ; #: in sepsis group GG vs. GA,  $P < 0.05$ .

## 4. Discussion

Sepsis is mutually influenced by infectious bacteria, environmental factors and genetic factors from the body itself. Different individuals show different susceptibility and prognosis to sepsis. Studies have shown that among infection and the pathogenic high-risk factors of sepsis, genetic factors played a more important role on sepsis than environmental factors[4,5].

It is indicated that MBL2 has a close connection with sepsis and the potential effects of MBL2 on sepsis gets more and more attention. As an important member of complement system, MBL2 plays a potential role in innate immunity and is the first line anti-infection immune molecule in host non-specific immunity. The single nucleotide polymorphisms of MBL2 can influence the concentration of serum MBL2 protein. Three loci are located on exons 1, 52, 54 and 57 codons are corresponding to type D, B and C structural gene mutations[6]. The polymorphism of MBL2 might get changed as a result of insufficiency of MBL2. This change affects its opsonic action and has susceptibility with infectious diseases to some extent[7,8].

Garred[9] investigated the association of MBL polymorphisms with sepsis in 272 patients with SIRS in intensive care units, and found that the MBL variant alleles have close relationship to the occurrence of sepsis. Other studies found that the levels of MBL in the blood of newborn and preterm infants are significantly lower than those without sepsis, which will increase the risk of neonatal inflammatory response syndrome to sepsis, and premature infants with MBL gene polymorphisms are more likely to develop neonatal sepsis and pulmonary infection[10-12]. Therefore, MBL2 expression level, activity change and polymorphic loci are valuable for predicting the prognosis of sepsis. It is an important candidate gene for early diagnosis of sepsis.

Our study found that only mutation site of rs1800450G/A was found in the three sites of the MBL2 gene polymorphisms (rs5030737, rs1800450 and rs1800451). Allele frequency analysis shown A allele frequency in sepsis group was significantly higher than that in non-sepsis group. Logistic regression analysis showed that the patients who carried A allele were easier to get sepsis than G allele carrier, which means that the variation of rs1800450 G→A increased the incidence of sepsis and was closely related to the occurrence of sepsis.

The protective function of MBL is closely related to the level of MBL in plasma. In other words, maintaining a certain concentration of the circulating MBL level is the foundation for its physiological function. The lower the level of MBL is, the higher the susceptibility to pathogenic microorganism is. The expression level of MBL in the serum is closely related to the gene polymorphism of MBL, that is,

the MBL serum level is mainly determined by MBL gene. Being influenced by structural gene mutation and activity of promoter region, MBL gene mutation can cause lower level of MBL in serum and affect the complement activation system[13,14].

As shown in the results of this study, the MBL2 level in serum in sepsis patients with genotype GG and GA was significantly lower than that in non-sepsis group. In sepsis group, the MBL2 serum level of patients with genotype GA was obviously lower than that in patients with genotype GG. These mutations make less MBL2 polymer forming and weaken its ability to combine with ligand. Meanwhile, it will make MBL2 polymer easier to be degraded by metalloprotease and influence the concentration of MBL2 in plasma[15].

To sum up, MBL2 rs1800450G/A is closely related to the occurrence of sepsis. It is absolutely necessary to further explore the molecular mechanism on that, which can provide effective targets for early diagnosis and individualized treatment of sepsis and reduce the incidence and mortality of patients with sepsis.

### Conflict of interest statement

The authors declare that there is no conflict of interest.

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