Association of **MBL2** gene polymorphisms with sepsis in children and adult

Jun-Yi Yao1#, Zhi-Qian Luo1#, Wei Zhang1, Ying-Qing Li1, Yong-Yan Li1, Xiu-Ru Li1, Wei-Cheng Wang1, Zhi-Tao Liu1, Shao-Wen Cheng1,2, Chuan-Zhu Lyu1,3#

1Emergency and Trauma College, Hainan Medical University
2Trauma Center, The First Affiliated Hospital of Hainan Medical University
3Department of Emergency, The Second Affiliated Hospital of Hainan Medical University

ARTICLE INFO

**ABSTRACT**

Sepsis is a syndrome characterized by systemic inflammatory response caused by infection or toxin, with high morbidity and mortality. Different infection microflora and environment have great influence on the occurrence, development and prognosis of sepsis, but individual genetic factors also play an extremely important role. It was reported that the polymorphisms of *mannose-binding lectin* 2 gene were closely relevant to the occurrence and development of sepsis, but the conclusions in different studies were inconsistent. Therefore, we performed this reviews on the relevance of *mannose-binding lectin* 2 gene polymorphisms and sepsis.

1. Introduction

The incidence and mortality of sepsis are very high. Although the total mortality rate is decreasing, the total number of deaths is still increasing[1]. Therefore, more studies of risk factors of sepsis should be carried out to predict the occurrence and development of sepsis earlier, thus early intervention could be implemented to high-risk patients, which can effectively reduce the morbidity and mortality of sepsis. Studies have shown that genetic factors, especially single nucleotide polymorphisms, are able to affect individual susceptibility and severity of sepsis[2]. *Mannose-binding lectin* 2 (*MBL2*) gene mutation can lead to low serum *MBL* level and functional impairment, which is associated with the risk of...
sepsis[3,4]. The mutations at codon 54, 52 and 57 of exon 1 of MBL2 structure gene can block the formation of MBL2 peptide oligomer, reduce the binding force between MBL2 and ligand, then degrade MBL2 more easily, resulting in a low level of serum MBL2. However, current studies on the association between MBL2 gene polymorphism and risk of sepsis are still inconsistent. The relationship between MBL2 gene polymorphism and sepsis risk in adults and children is summarized as follows.

2. The association of MBL2 gene polymorphism with sepsis in children

2.1. A/O polymorphism

Compared with wild type, three alleles in MBL2 gene and one allele in promotor can cause the decrease of serum MBL2 level independently. If serum MBL2 is deficient, the host will mishandle apoptotic cells, which leads to sepsis. The further transformation of sepsis into severe sepsis or septic shock is also related to the mutation of MBL2 allele. Three single-point mutations in the MBL2 gene (B, C and D variant, together with the O variant) result in low MBL level. The wild-type is referred to as A variant. A/O or O/O individuals are often considered to be MBL2 deficient. In the study of Fidler KJ et al[5], the presence of MBL2 variant A/O allele significantly increased the severity of the systemic response to infection in 50 infected patients (local infections 2/15, sepsis 10/19, septic shock 12/16). The results showed that MBL2 level was closely correlated with genotypes, and MBL2 exon polymorphisms were associated with low MBL level, which significantly increased the risk of sepsis infection and septic shock to children treated in intensive care unit. In the year of 2008, Dzwonek AB et al[8] performed MBL phenotypic analysis on 120 newborn samples and their genotypes on the third day after birth (A/O, A/A and O/O). The results showed that MBL2 genotypes were not significantly associated with the risk of sepsis. Hartz A, et al[7] recently conducted a large-scale study, and 6878 infants with very low birth weight were collected and genotyped for MBL2, and classified plasma level as normal (A/A), low (A/O or O/O). But no association was found between genotypic MBL2 level and the risk of blood culture or clinically confirmed sepsis in the entire group with very low birth weight, but in the subgroup with infants born between 32 and 36 weeks of gestation. O/O MBL level appeared to be associated with the risk of gram-negative sepsis.

2.2. 54A/B polymorphism

Codon 54 and 57 are the two most widely studied loci for MBL2 gene polymorphisms, which can reduce the expression of MBL2[8,9]. Ozkan H et al[10] collected 93 full-term and premature infants, including 53 sepsis cases (3 with premature sepsis, 33 with delayed sepsis and 17 with very late sepsis), and compared them with normal genotype, and the results showed that AB and BB genotype infants were more likely to be diagnosed with neonatal sepsis. In addition, the presence of B allele was associated with an increased risk of neonatal sepsis. In the study of Koroglu OA et al[11], a total of 99 premature infants are collected in intensive care units, and codon 54 and 57 polymorphisms of the MBL2 gene were genotyped. The results showed that premature infants with MBL2 polymorphisms were more likely to develop early sepsis in the first week after birth. However, MBL1 gene polymorphism was not associated with late sepsis. The study did not measure serum MBL level, but speculated that MBL gene polymorphisms were associated with the early and late stages of sepsis, suggesting that MBL levels were associated with gestational age and increased with the growth of term infants and premature infants.

2.3. Other polymorphisms

In recent years, it has been found that there are six polymorphic loci in the MBL structure gene, as well as three mutation sites in the promotor region and in the exon of the MBL2 gene, respectively, which are H/L at 550 position of the promotor region, respectively. X/Y and P/Q at position 221 and 54, 52 and 57 codon of exon. Among these, the gene polymorphisms at the three loci of H/L, X/Y and P/Q have significantly reduced the serum level of functional MBL2[12,13]. In addition, heterozygous polymorphism reduced the number of functional MBL by 5-10 times[14]. In the study of Xue H[15], they sequenced H/L (rs11003125), X/Y (rs7096206) and P/Q (rs7095891) directly, and it was confirmed that the genotype frequencies of X/Y and P/Q polymorphisms were in line with the Hardy-Weinberg equilibrium, but the H/L genotype frequencies did not conform to the equilibrium, and the genotype distribution in infants was not calculated. In addition, in order to further understand the effect of the X/Y, P/Q genotype on the serum MBL2 level of Chinese newborns of Han nationality, the serum MBL2 concentration was measured. When compared with 4PQ genotype, no significant difference in median MBL level of 4PQ genotype was observed. However, it was not possible to compare the levels of MBL with other genotypes in infants with only one 4QQ genotype, but the 221Y/X genotype of MBL2 was positively correlated with neonatal sepsis (Table 1).

Table 1

| Ethnicity | Case | Control | Polymorphism | References |
|-----------|------|---------|--------------|------------|
| Caucasian | 2765 | 4113    | A/O          | [7]        |
| Caucasian | 87   | 313     | A/O          | [16]       |
| Caucasian | 87   | 47      | A/O          | [16]       |
| Caucasian | 42   | 85      | A/O (221Y/X) | [17]       |
| Caucasian | 41   | 145     | A/O (221Y/X) | [18]       |
| Caucasian | 38   | 82      | A/O          | [6]        |
| Caucasian | 10   | 38      | A/O          | [19]       |
| Caucasian | 35   | 15      | A/O          | [5]        |
| Caucasian | 50   | 306     | A/O          | [20]       |
| Caucasian | 53   | 40      | 54 A/B       | [10]       |
| Caucasian | 42   | 60      | 54 A/B       | [11]       |
| Asian     | 48   | 96      | 221 YX, P/Q  | [15]       |
3. The association of MBL2 gene polymorphism with sepsis in adult

3.1. A/O polymorphism

Molle I et al[21] performed a retrospective study to investigate the association between MBL2 gene mutation (AO/OO, AA) and the risk of severe infection in multiple myeloma patients receiving autologous transplantation. The study found that patients with variant MBL2 were at higher risk of infection than those with homozygote MBL2. The risk of sepsis in wild type MBL2 homozygotes was significantly reduced. Moreto A et al[22] found significant higher number of fungal infections in patients with MBL2 variants. And there was no difference in the incidence of gram-negative bacteria in patients with wild-type MBL2 genotypes compared to those with variant MBL2. Bronkhorst MW et al[23] collected 219 patients with severe trauma, and there were 139 cases with systemic inflammatory response syndrome, 79 cases with sepsis and 37 cases with septic shock. The results showed that the genotype of exon 1 AO of MBL2 was related to the increased positive rate of wound culture. In addition, the incidence of systemic inflammatory response syndrome, sepsis or septic shock in MBL2 AO or OO genotype patients was higher than that in wild type AA genotype patients, but the difference was not statistically significant. Other studies have found no significant association between A/O polymorphism and the risk of sepsis[24].

3.2. 54A/B polymorphism

The mutation frequency of MBL2 gene was significantly different among different ethnic groups, except the frequency of Cys52 point mutation which was lower in the study population. The frequency of Asp54 mutation was 0.19 in England and 0.11 in Han nationality in Hong Kong. The frequency of Asp54 mutation was rare among Africans. The frequency of Glu57 mutation was 0.29 in African Gambians and 0.02 in Caucasians[25]. The MBL2 gene rs1800450 polymorphism (codon 54A/B, G230A) was genotype (GG, GA, AA, G, A) in Chinese Han patients with sepsis, and the HWE test was performed, the results showed that the allele could significantly increase the risk of sepsis, the GA genotype was closely related to the pathogenesis of sepsis, while the AA genotype had no significant correlation with the occurrence of sepsis according to the studies of Liu L et al[26]. A total of 266 patients with sepsis and 398 healthy subjects were included, the association of three single nucleotide polymorphisms of MBL2 gene (54, 550, 54) with sepsis in Korean was detected, the results showed that single nucleotide polymorphism was not associated with the occurrence of sepsis, but the homozygosity of promoter 54 (A/A) and promoter 550 (H/H) was related to the severity of sepsis, but not to the outcome of sepsis, Huh JW et al[27].

3.3. Other polymorphisms

Based on the MBL2 gene polymorphism in Korean patients, 41 patients with persistent Staphylococcus aureus bacteremia and 46 patients with a bacteremia recovery were compared. Six mononucleotide polymorphic loci of MBL2 were selected (2550G/C, 2221G/C, 4C/T, 54GGC/GAC, 57GGA/GAA), including alleles A/B, A/C and A/D of exon 1, and alleles H/L, X/Y and PQ of promoter region, respectively. In addition, the level of MBL2 in serum was measured. The high MBL genotype group was HYPA/HYPA, HYPA/LXPA, HYPA/LYPA, HYPA/LYQA, LYPA/LXPA, LYPA/LYQA, LYQA/LXPA, and the median of serum MBL concentration was 1 773 ng/mL. The median of serum MBL concentration was 686 ng/mL with LXPA/LYPB, LYPB/LYP, and the median of serum MBL concentration was 286 ng/mL in low yield group with HYPA/LYPB, HYPA/HYPB, LXPA/LXPA, LXPA/LYPB, LYPB/LYPB. Low MBL genotype was significantly higher in patients with persistent bacteremia than in normal controls, and that was an important risk factor for persistent bacteremia[28]. In the latest comprehensive Meta-analysis, MBL’s A/O polymorphism was significantly associated with sepsis, but there was no association between 221Y/X and 550H/L13[1](Table 2).

Table 2

| Ethnicity | Case | Control | Polymorphism | References |
|-----------|------|---------|--------------|------------|
| Caucasian | 140  | 250     | A/O (221Y/X) | [24]       |
| Caucasian | 197  | 75      | A/O (221Y/X) | [29]       |
| Caucasian | 170  | 236     | A/O(221Y/X, 550H/L) | [30]       |
| Caucasian | 174  | 353     | A/O          | [4]        |
| Caucasian | 11   | 102     | A/O          | [21]       |
| Caucasian | 376  | 689     | A/O (221Y/X) | [31]       |
| Caucasian | 145  | 400     | A/O (221Y/X) | [32]       |
| Caucasian | 39   | 28      | A/O          | [33]       |
| Caucasian | 57   | 114     | A/O (221Y/X) | [34]       |
| Caucasian | 59   | 84      | A/O (221Y/X) | [35]       |
| Caucasian | 143  | 47      | A/O (221Y/X) | [36]       |
| Caucasian | 164  | 52      | A/O (221Y/X) | [37]       |
| Caucasian | 116  | 103     | A/O (221Y/X) | [23]       |
| Caucasian | 152  | 196     | A/O (221Y/X) | [38]       |
| Caucasian | 31   | 41      | A/O          | [22]       |
| Caucasian | 496  | 477     | A/O (221Y/X) | [39]       |
| Asian     | 7    | 106     | 54A/B        | [40]       |
| Asian     | 266  | 396     | 54A/B (550H/L) | [27]       |
| Caucasian | 28   | 53      | 54A/B(221Y/X, 550H/L) | [41]       |
| Asian     | 41   | 46      | 54A/B(221Y/X, 550H/L) | [28]       |

In recent years, the researches on MBL2 gene polymorphisms and sepsis have been increasing in the world. However, at present, there are some limitations in this field, such as the small number of samples increased the possibility of false positive and false negative association, and the subjects were from different geographical regions and races, the composition of the control population was different, the basic diseases were different, the research methods were not consistent, and the binding degree of MBL2 level to various pathogenic microorganisms was different. In particular, children with different age groups have different factors. Sepsis is an extremely
complex disease affected by a variety of genetic and environmental factors. Therefore, further research is needed to achieve early diagnosis and accurate treatment of sepsis.

Conflict of interest statement
The authors report no conflict of interest.

Foundation project
This study was supported by National Natural Science Foundation of China (81860347); Hainan Provincial Natural Science Foundation of China (818MS140); Young Talents’ Science and Technology Innovation Project of Hainan Association for Science and Technology (QCXZM201816); Hainan Provincial Health and Family Planning Commission Project (18A200178); Undergraduate Innovative Experiment Project of Hainan Medical University (HYCX2018122).

References
[1] Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348(16): 1546-1554.
[2] Namath A, Patterson AJ. Genetic polymorphisms in sepsis. *Crit Care Nurs Clin North Am* 2009; 21(4): 835-856.
[3] Gordon AC, Waheed U, Hansen TK, Hitman GA, Garrard CS, Turner MW, et al. Mannose-binding lectin: biology and clinical implications. *Intern Med J* 2010; 35(9): 548-555.
[4] Worthley DL, Bardy PG, Mullighan CG. Mannose-binding lectin: relationship to levels, incidence and outcome. *Shock* 2006; 25(1): 88-93.
[5] Fidler KJ, Wilson P, Davies JC, Turner MW, Peters MJ, Klein NJ. Increased incidence and severity of the systemic inflammatory response syndrome in patients deficient in mannose-binding lectin. *Intensive Care Med* 2004; 30(7): 1438-1445.
[6] Dzwonek AB, Neth OW, Thielbaut R, Gulczynska E, Chilton M, Hellwig T, et al. The role of mannose-binding lectin in susceptibility to infection in preterm neonates. *Pediatr Res* 2008; 63(6): 680-685.
[7] Hartz A, Pelgel J, Humberg A, Preuss M, Schreiter L, Rupp J, et al. The association of mannose-binding lectin 2 polymorphisms with outcome in very low birth weight infants. *PLoS One* 2017; 12(5): e0178032.
[8] Madsen HO, Garred P, Thiel S, Møller I, Peterslund NA, Thiel S, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J Immunol* 1995; 155(6): 3013-3020.
[9] Garred P, Larsen F, Madsen HO, Koch C. Mannose--binding lectin deficiency--revisited. *Mol Immunol* 2003; 40(2): 73-84.
[10] Ozkan H, Koksal N, Cetinkaya M, Kiliç Ş, Celebi S, Oral B, et al. Serum mannose–binding lectin (MBL) gene polymorphism and low MBL levels are associated with neonatal sepsis and pneumonia. *J Perinatol* 2012; 32(3): 210-217.
[11] Koroglu OA, Onay H, Erdemir G, Yalaz M, Cakmak B, Akisu M, et al. Mannose–binding lectin gene polymorphism and early neonatal outcome in preterm infants. *Euronatol* 2010; 98(4): 305-312.
[12] Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. *Biochimica Biophys Acta* 2002; 1572(2-3): 401-413.
[13] Zhang AQ, Yue CL, Pan W, Gao JW, Zeng L, Gu W, et al. Mannose-binding lectin polymorphisms and the risk of sepsis: evidence from a meta-analysis. *Epidemiol Infect* 2014; 142(10): 2195-2206.
[14] Selander B, Mårtensson U, Weintraub A, Holmström E, Matsushita M, Thiel S, et al. Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2. *J Clin Invest* 2006; 116(5): 1425-1434.
[15] Xue H, Xue XG, Yang CY, Chen QQ, Lin N, Chen M, et al. Low serum mannan binding lectin (MBL) levels and 221 yx genotype of MBL2 gene are susceptible to neonatal sepsis in the chinese han population. *Iran J Pediatr* 2017; 27(3): e9448.
[16] zierceko AS, Szała-Po dziej A, Kilpatrick DC, Soboci ski M, Chojnacka K, Sokolowska A, et al. Components of the lectin pathway of complement activation in paediatric patients of intensive care units. *Immunobiology* 2016; 221(5): 657-669.
[17] Auriti C, Prencipe G, Inglese R, Azzari C, Ronchetti MP, Tocci A, et al. Role of mannose-binding lectin in nosocomial sepsis in critically ill neonates. *Hum Immunol* 2010; 71(11): 1084-1088.
[18] van der Zwet WC, Catsburg A, van Elburg RM, Savelkoul PH, Vandenvroucke-Grauls CM. Mannose-binding lectin (MBL) genotype in relation to risk of nosocomial infection in pre-term neonates in the neonatal intensive care unit. *Clin Microbiol Infect* 2010; 14(2): 130-135.
[19] Frakking FN, Brouwer N, van Eijkelenburg NK, Merkus MP, Kuijpers TW, Offringa M, et al. Low mannose-binding lectin (MBL) levels in neonates with pneumonia and sepsis. *Clin Exp Immunol* 2007; 44(1): 171-171.
[20] Ahrens P, Kattner E, Köhler B, Härtel C, Seidenberg H, et al. Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. *Pediatr Res* 2004; 55(4): 652-656.
[21] Mollé I, Peterslund NA, Thiel S, Steffensen R. MBL2 polymorphism and risk of severe infections in multiple myeloma patients receiving high-dose melphalan and autologous stem cell transplantation. *Bone Marrow Transplant* 2006; 38(8): 555-560.
[22] Moreto A, Fariñas-Alvarez C, Puente M, Ocejo-Vinyals JG, Sánchez-Velasco P, Hocajeda JP, et al. Mannose-binding lectin gene variants and infections in patients receiving autologous stem cell transplantation. *BMC Immunol* 2014; 15(1): 17.
[23] Bronkhorst MW, Lomax MA, Vossen RH, Bakker J, Patka P, van Lieshout EM. Risk of infection and sepsis in severely injured patients related to single nucleotide polymorphisms in the lectin pathway. *Br J Surg* 2013; 100(13): 1818-1826.
[24] Kronborg G, Weis N, Madsen HO, Pedersen SS,  Wejse C, Nielsen H, et al. Low levels of serum mannan-binding protein. *Mol Immunol* 2003; 39(7): 1438-1445.
[25] Madsen HO, Garred P, Kurtzhals JA, Lammi LU, Ryder LP, Thiel S, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994; 40(1): 37-44.

[26] Liu L, Ning B. The role of MBL2 gene polymorphism in sepsis incidence. Int J Clin Exp Pathol 2015; 8(11): 15123-15127.

[27] Huh JW, Song K, Yum JS, Hong SP, Lim CM, Koh Y. Association of mannan-binding lectin-2 genotype and serum levels with prognosis of sepsis. Crit Care 2009; 13(6): R176.

[28] Chong YP, Park KH, Kim ES, Kim MN, Kim SH, Lee SO, et al. Association of mannose-binding lectin 2 gene polymorphisms with persistent staphylococcus aureus bacteremia. PLoS ONE 2014; 9(3): e89139.

[29] Garred P, Strøm J, Taaning E, Madsen HO. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome in patients with systemic inflammatory response syndrome. J Infect Dis 2003; 188(9): 1394-1403.

[30] Eisen DP, Dean MM, Thomas P, Marshall P, Gerns N, Heatley S, et al. Low mannose-binding lectin function is associated with sepsis in adult patients. FEMS Immunol Med Microbiol 2006; 48(2): 274-282.

[31] Hellemann D, Larsson A, Madsen HO, Bonde J, Jarløv JO, Wiis J, et al. Heterozygosity of mannose-binding lectin (MBL) genotype predicts advantage (heterosis) in relation to fatal outcome in intensive care patients. Hum Mol Genet 2007; 16(24): 3071-3080.

[32] Huttunen R, Aittoniemi J, Laine J, Vuento R, Karjalainen J, Rovio AT, et al. Gene-environment interaction between MBL2 genotype and smoking, and the risk of gram-positive bacteremia. Scand J Immunol 2008; 68(4): 438-444.

[33] Cervera C, Balderramo D, Belén Suárez, Prieto J, Fuster F, Linares L, et al. Donor mannose-binding lectin gene polymorphisms influence the outcome of liver transplantation. Liver Transpl 2010; 15(10): 1217-1224.

[34] Horcajada JP, Lozano F, Muñoz Ana, Suarez B, Fariñas-Alvarez C, Almela M, et al. Polymorphic receptors of the innate immune system (MBL/MASP-2 and TLR2/4) and susceptibility to pneumococcal bacteremia in HIV-infected patients: a case-control study. Curr HIV Res 2009; 7(2): 218-223.

[35] de Rooij BJ, van Hoek B, ten Hove WR, Roos A, Bouwman LH, Schaapberder AF, et al. Lectin complement pathway gene profile of donor and recipient determine the risk of bacterial infections after orthotopic liver transplantation. Hepatology 2010; 52(3): 1100-1110.

[36] Klostergaard A, Steffensen R, Møller JK, Peterslund N, Juhl-Christensen C, Mølle I. Sepsis in acute myeloid leukaemia patients receiving high-dose chemotherapy: No impact of chitotriosidase and mannose-binding lectin polymorphisms. Eur J Haematol 2010; 85(1): 58-64.

[37] Smithson A, Perello R, Aibar J, Espinosa G, Tassies D, Freire C, et al. Genotypes coding for low serum levels of mannose-binding lectin are underrepresented among individuals suffering from noninfectious systemic inflammatory response syndrome. Clin Vaccine Immunol 2010; 17(3): 447-453.

[38] Garcia-Laorden MI, Rodriguez de Castro F, Solé-Violán J, Payeras A, Briones ML, Borderías L, et al. The role of mannose-binding lectin in pneumococcal infection. Eur Respir J 2013; 41(1): 131-139.

[39] Mills TC, Chapman S, Hutton P, Gordon AC, Bion J, Chiche Jean-Daniel, et al. Variants in the mannose-binding lectin gene, MBL2, do not associate with sepsis susceptibility or survival in a large european cohort. Clin Infect Dis 2015; 61(5): 695-703.

[40] Horiuchi T, Gondo H, Miyagawa H, Otsuka J, Inaba S, Nagafuji K, et al. Association of MBL gene polymorphisms with major bacterial infection in patients treated with high-dose chemotherapy and autologous PBSC. Genes Immun 2005; 6(2): 162-166.

[41] Davis SM, Clark EAS, Nelson LT, Silver RM. The association of innate immune response gene polymorphisms and puerperal group A streptococcal sepsis. Am J Obstet Gynecol 2010; 202(3): 308.e1-8.