ASSOCIATION BETWEEN MANNOSE-BINDING LECTIN GENE POLYMORPHISMS AND SUSCEPTIBILITY TO DENGUE VIRUS INFECTION: A PRELIMINARY REPORT

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Abstract. Mannose-binding lectin (MBL) can bind with a wide range of pathogens and can activate through lectin pathway or enhances opsonophagocytosis. MBL is encoded by the *MBL2* gene and single-nucleotide polymorphisms (SNPs) in the promoter and exon have functional effects on serum levels of MBL. MBL deficiency has been shown to predispose to infectious diseases. We assess whether or not, the variant MBL alleles are associated with susceptibility to dengue infection. Patients with confirmed dengue infection who were admitted to King Chulalongkorn Memorial Hospital during a calendar year were studied. Controls were patients without dengue infection. Deoxyribonucleic acid (DNA) was extracted from 50 µl of peripheral blood mononuclear cell (PBMC) using the DNA Blood Mini Kit. The SNPs in the promoter (-221 *X/Y*) and exon 1 (codon 54 *A/B*) of *MBL2* gene were genotyped by using 2 separate cycling reactions of the TaqMan allele discrimination system. Serum levels of MBL were determined by double-antibody sandwich ELISA. Chi-square was used for statistical analysis. Serum MBL levels and genotypes were determined in 110 dengue patients (mean age 18.1 years; 62 males and 48 females) and 42 controls (mean age 25.8 years; males: females = 1:1). Our study showed that *YB* haplotype is associated with low serum levels of MBL. There was no association between *MBL2* gene polymorphisms and susceptibility to dengue infection. The higher frequency of *YB* in dengue patients than in controls suggesting the likelihood of an association. Further studies are warranted.

Keywords: dengue, mannose-binding lectin, single-nucleotide polymorphism

INTRODUCTION

Dengue virus infections cause a spectrum of illness ranging from asymptomatic, mild undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (Hemungkorn *et al*, 2007). Individual susceptibility to dengue infection seems to be variable. For decades, two distinct hypotheses to explain the mechanism of DHF have been debated between secondary infection or immune enhancement and viral virulence (Prommalikit *et al*, 2004). Host genetic factors may also be relevant

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to this infection (Lan and Hirayama, 2011). Mannose-binding lectin (MBL) is an acute phase protein primarily produced by the liver which plays a critical role in the innate immune response before the production of antibodies, and it can bind with multiple carbohydrate recognition domains on microbial surfaces. MBL can activate the complement system via lectin pathway and promote opsonophagocytosis.

MBL is encoded by the *MBL2* gene on chromosome 10. *MBL2* has four exons. Single-nucleotide polymorphisms (SNPs) in the promoter and coding regions have functional effects on serum levels of MBL (Worthley *et al,* 2005). Three missense mutations of coding region in Exon 1 at codons 52 (allele *D*), 54 (allele *B*), and 57 (allele *C*) disrupt the collagen-like helical structure of the MBL peptides and interfere with oligomerization of MBL peptides, or assembly of MBL peptide triplets into mul-timers, resulting in low serum MBL levels (Madsen *et al,* 1994). An *MBL2*-coding region carrying any of the *B*, *C* or *D* mutant alleles is referred to as *O*, and the wide-type is referred to as *A*.

The MBL concentrations are also de-pendent on SNPs in the promoter region of *MBL2*. In particular, a SNP at position -221 (allele *Y* or *X*) has a significant ef-fect on serum MBL levels, with the allele *Y* and allele *X* being responsible for high and low MBL-expressing activity, respec-tively (Madsen *et al,* 1995). With the three structural SNPs in exon 1, the -221 alleles form the haplotypes *YA*, *XA*, *YB*, *YC,* and *YD*. The haplotypes *YB* are more frequent in individuals with low MBL levels than in those randomly selected or in those with high MBL levels (Crosdale *et al,* 2000). MBL deficiency has been shown to pre-

dispose to infectious diseases (Worthley *et al,* 2005).

The envelope of dengue virus has complete high-mannose glycans and non-structural proteins that may be opsonized by MBL (Chan, 1997). An activation of complement system is a constant finding in patients with DHF (Avirutnan *et al,* 2006). Thus, MBL may be a candidate gene for dengue infection. However, the data on MBL association with dengue infection is scarce in the literature and absent in Thai population. We assess whether or not, the variant MBL alleles are associated with susceptibility to dengue infection in an ethnically homogeneous population born in Thailand.

MATERIALS AND METHODS

Patients with serologically confirmed dengue infection by an enzyme-linked immunosorbent assay (ELISA) admitted to King Chulalongkorn Memorial Hospital during a calendar year were studied. Con-trols were patients with negative dengue serology. Informed consent was obtained from the subjects and controls recruited into the study or from their parents. Deoxy-ribonucleic acid (DNA) was extracted from 50 µl of peripheral blood mononuclear cell (PBMC) using the QIAamd DNA Blood Mini Kit® (Qiagen, Hilden, Germany) and stored at -20ºC until used for analysis.

The SNPs in the promoter (-221 *X/Y*) and exon 1 (codon 54 *A/B*) of *MBL2* gene were genotyped by using two separate cycling reactions of the TaqMan® allelic dis-crimination system (Applied Biosystems, Foster City, CA) as described in previous studies (Ip *et al,* 2004, 2005). The *MBL2* mutant structural allele *B*; however, is in

Table 1

Serum MBL levels and MBL genotypes with or without the mutant allele B in dengue patients and controls.

Variable	Mean levels of serum MBL in dengue patients (ng/ml)	Mean levels of serum MBL in controls (ng/ml)
MBL genotypes		
YA/YA, YA/XA, XA/XA	2,738.97 (673.89-7,446.40)	2,521.24 (717.71-6,160.89)
YA/YB, XA/YB, YB/YB	327.52 (undetectable-723.73)	960.79 (216.58-3,346.33)

Table 2

Frequencies of MBL genotypes and YB haplotype in dengue patients and controls.

Variable	Dengue patients (%)	Controls (%)	*p*-value
MBL genotypes			0.783
YA/YA	37 (33.6)	19 (45.2)	
YA/XA	36 (32.7)	12 (28.6)	
XA/XA	8 (7.3)	3 (7.1)	
YA/YB	24 (21.8)	7 (16.7)	
XA/YB	3 (2.7)	1 (2.4)	
YB/YB	2 (1.8)	-	
YB carrier	29 (26.4)	8 (19.1)	0.466

linkage disequilibrium with the promoter polymorphism *X/Y*, so that *B* only occurs with *Y* (Madsen *et al,* 1995). The data from the two separate TaqMan polymerase chain reactions in the present study were combined to give three haplotypes: *YA*, *YB* and *XA*. *YB* is commonly referred as a mutant haplotype *O* (Neth *et al,* 2001).

Serum levels of MBL were determined by double-antibody sandwich ELISA in which a mouse monoclonal anti-human MBL antibody (HYB 131-01; Antibody Shop, Copenhagen, Denmark), either un-labelled or labelled with biotin, was used as the primary or secondary antibody, re-spectively. Horseradish-peroxidase (HRP)-conjugated streptavidin (R&D Systems, Minneapolis, MN) and substrate solution containing tetramethylbenzidine (Substrate Reagent Pack; R&D Systems, Minneapolis, MN) were used for detection of bound secondary antibody. Chi-square was used for statistical analysis. A *p*-value <0.05 was considered to be significant.

RESULTS

Serum MBL levels and genotypes were determined in 110 dengue patients (mean age 18.1 years; 62 males and 48 females) and 42 controls (mean age 25.8 years; males: females = 1:1). The SNPs in the promoter (-221 *X/Y*) and exon 1 (codon 54 *A/B*) of the MBL gene were genotyped and were identified as six genotypes in this study: *YA/YA, YA/XA, XA/XA, YA/YB, XA/YB* and *YB/YB*. *YB* haplotype is associated with low serum levels of MBL (Table 1 and Fig 1). There was no association
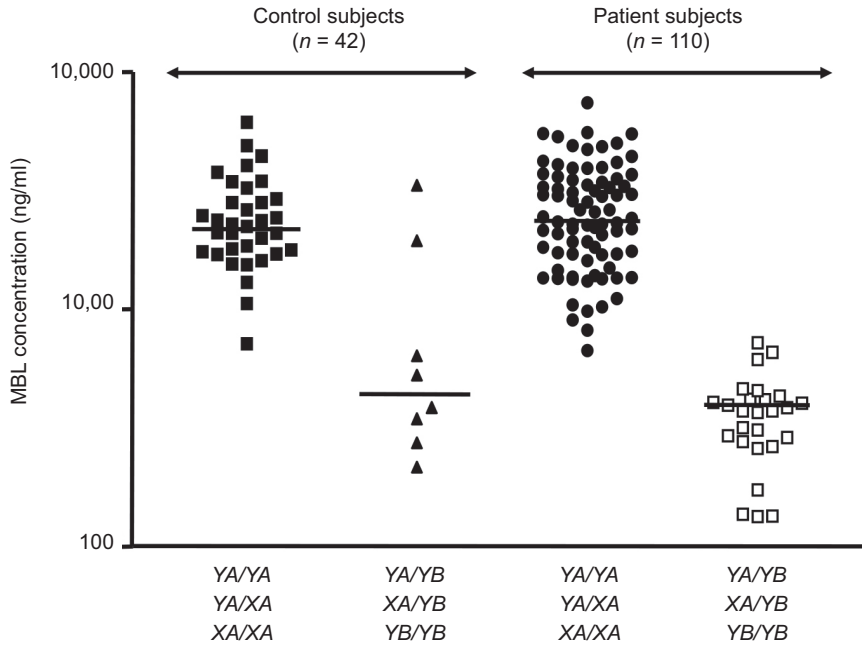
Fig 1–Serum MBL levels and MBL genotypes with or without the mutant allele B in dengue patients and controls.

between *MBL2* gene polymorphisms and susceptibility to dengue infection but the frequency of *YB* was higher in patients than in controls. Frequencies of MBL genotypes and *YB* haplotype in dengue patients and controls were shown in Table 2. When the analysis was performed comparing the incidence of *MBL2* polymorphisms between DF versus DHF and DSS, we found that there was no significant difference in the MBL genotype between the two groups and frequencies of MBL genotypes were shown in Table 3.

DISCUSSION

MBL interacts with wide range of pathogens, including many different bacteria, viruses, fungi and protozoa. MBL deficiency increases the susceptibility of an individual to infectious diseases including human immunodeficiency virus, influenza A, *Cryptosporidium parvum*, *Neisseria meningitidis*, severe acute respiratory syndrome coronavirus and is also associated with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (Worthley *et al,* 2005; Ip *et al,* 2005; Tsutsumi *et al,* 2001). MBL may be a candidate gene for dengue infection because the envelope of dengue virus has complete high-mannose glycans which MBL recognizes repetitive oligosaccharide moieties present on a wide array of pathogens.

In the present study, both heterozygous and homozygous for *YB* is associated with low serum levels of MBL, which is similarly found in Chinese and Caucasian populations. Although there was no associ-

Table 3

Frequencies of MBL genotypes in DF patients versus DHF and DSS patients.

Variable	DF (%) (n = 30)	DHF and DSS (%) (n = 80)	p-value
MBL genotypes			0.186
YA/YA	12 (40.0)	25 (31.2)	
YA/XA	8 (26.7)	28 (35.0)	
XA/XA	2 (6.7)	6 (7.5)	
YA/YB	6 (20.0)	18 (22.5)	
XA/YB	-	3 (3.7)	
YB/YB	2 (6.7)	-	

ation between *MBL2* gene polymorphisms and susceptibility to dengue infection, but the frequency of *YB* was higher in dengue patients than in controls, suggesting the likelihood of an association.

In recent years, several publications reporting correlations between *MBL2* polymorphisms and disease susceptibility or disease protection have been published. Alagarasu *et al* (2012) found that deficiency of MBL may be associated with primary DHF. Based on the cutoff value of 500 ng/ml, 50% of primary DHF cases had MBL deficiency as compared to 10% of primary DF cases (*p*=0.038, odds ratio (OR) 9; 95% confidence limits 0.84-120). This difference was not observed in secondary infections.

For disease protection, Acioli-Santos *et al* (2008) reported that dengue patients with lower MBL serum levels were less likely to develop thrombocytopenia than the MBL wide-type group, suggesting a protective role of *MBL2* allele *O* toward dengue-related thrombocytopenia, whereas the presence of the *MBL2* AA genotype was associated with augmented risk of thrombocytopenia. Loke *et al* (2002) have investigated MBL genotype in a Vietnam-ese cohort to identify possible correlation between the codon 54 *MBL2* polymorphism and DHF. There were no significant differences in MBL genotypes or allele frequencies between DHF patients and controls. However, the relatively low frequency of the variant allele in this population limits the statistical power of this analysis.

The findings of various studies concerning association between *MBL2* polymorphism and susceptibility to dengue infection were controversial. Further studies with increased sample size are warranted since the underpowered sample size may be unable to detect such association. In addition, demonstration of MBL binding to dengue virus and neutralization of infection is needed.

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