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Functional polymorphisms of the *CCL2* and *MBL* genes cumulatively increase susceptibility to severe acute respiratory syndrome coronavirus infection

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Summary *Objectives:* To assess associations between the functional polymorphisms G-2518A at the chemokine (C–C motif) ligand 2 gene (*CCL2*) and mannose binding lectin (*MBL*) codon 54 variant (A/B) and susceptibility to SARS.

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Severe acute respiratory syndrome;
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Methods: We genotyped the *CCL2* G-2518A and *MBL* codon 54 variant (A/B) in 4 case–control populations of Chinese descent, totally consisting of 932 patients with SARS and 982 control subjects.
Results: Both the high-CCL2-producing GG genotype and the low-MBL-producing B allele were consistently associated with increased risks of SARS-CoV infection in all 4 case–control populations (joint $P = 1.6 \times 10^{-4}$ and 4.9×10^{-8}, for CCL2 and MBL respectively), with no interaction between polymorphisms could be detected. Furthermore, all the 4 case–control studies demonstrated a cumulative effect on risk of SARS-CoV infection for the combination of polymorphisms (joint $P = 1.3 \times 10^{-10}$). However, tests using the area under the curve (AUC) indicated that at this stage, the polymorphisms were unlikely to be appropriate for risk prediction testing because of low AUC values (all <66%). Additionally, no association was observed between the polymorphisms and severity of SARS.
Conclusions: The *CCL2* G-2518A and *MBL* codon 54 variant have a significantly cumulative effect on increased risk of SARS-CoV infection.

Introduction

Severe acute respiratory syndrome (SARS) is a newly emerged infectious disease of humans caused by a novel coronavirus (CoV), the SARS-CoV.[1] Pathogenesis of SARS is complex and host genetic background is considered to be one of the factors in determining susceptibility to, and outcome of SARS.[2] Previous reports have shown that polymorphisms of several putatively important genes affect an individual's susceptibility to SARS-CoV infection or disease severity of SARS.[3–10] In particular, our previous two independent association studies have implicated that a functional polymorphism at codon 54 in exon 1 (rs1800450, G230A, denoted as A/B variant) of mannose binding lectin (*MBL*), which encodes a protein belonging to the family of collectin and plays a critical role in the innate immune response, conferred a significantly increased susceptibility to SARS-CoV infection.[3,9] However, on the basis of the fact that the susceptibility to infectious disease is determined at different functional levels of innate and adaptive immunity,[11] we hypothesize that an unknown number of other unidentified genes are likely to mediate the susceptibility to SARS, including SARS-CoV infection and disease severity.

Chemokines play important role in cells trafficking during immune responses. Among the chemokine family, the chemokine (C–C motif) ligand 2 (CCL2), also designated as monocyte chemoattractant protein-1 (MCP-1), is known as a potent chemoattractant for monocytes and macrophages, and is considered to be involved in several diseases characterized by intense macrophage infiltration.[12] CCL2 influences both innate immunity, through effects on monocytes and macrophages, and adaptive immunity, through control of T helper cell polarization.[13] In the case of SARS, our and other studies have shown that CCL2 was one of the earliest and most prominent chemokines upregulated in either lung epithelial cells or monocyte derived dendritic cells infected with SARS-CoV.[14,15] The upregulation of CCL2 mediate the migration of monocytes and macrophages, which were the infiltrating cells indeed observed in the lung tissues of patients with SARS.[16] Notably, the overexpression of CCL2 was consistently detected in plasma of patients with SARS in several independent studies.[17–19] Furthermore, after treatment with corticosteroid, which is an effective cytokine modulator and has a beneficial effect on SARS patients, the level of plasma CCL2 was reduced significantly from 5 to 8 days.[17] Additionally, it has also been reported that the higher level of serum CCL2 in patients is correlated with more advanced disease severity of SARS.[17] In the lungs of BALB/c mice, the PDZ-binding motif of recombinant SARS-CoV envelope protein is a determinant of viral pathogenesis and induces the deleterious exacerbated immune response including increased expression of *CCL2*.[20] On the basis of the above relevance of the CCL2 in the pathogenesis of SARS, we hypothesize that the *CCL2* may be the excellent biologic candidate susceptibility gene for SARS. It is expected that the genetic variation within *CCL2* could contribute to inter-individual differences in susceptibility to, and outcome of SARS.

Recently, a functional single nucleotide polymorphism (SNP) (rs1024611, G-2518A) in the distal regulatory region of the *CCL2* at position −2518 relative to the transcription start site has been well characterized.[21] Compared with the *CCL2* -2518A allele, the −2518G allele conferred a greater *CCL2* transcriptional activity mediated by differential protein-DNA interactions, an increased CCL2 protein production *in vitro* and *in vivo*, and an enhanced leukocyte trafficking to tissues.[21–23] Furthermore, prevalence of the high-CCL2-producing −2518G allele has been shown to be associated with increased susceptibility or severity of infectious diseases, including HIV-1 infection and AIDS dementia,[21,24] HCV infection,[22] HBV clearance,[25] HCMV reactivation,[26] and pulmonary tuberculosis.[27] The role of this functional polymorphism in SARS, however, has never been specifically evaluated.

In this study, we therefore investigated whether the functional polymorphism G-2518A in the *CCL2* gene have any bearing on the SARS. To this end, we genotyped the *CCL2* G-2518A polymorphism in 4 independent case–control populations of Chinese descent, totally including 932 patients with SARS and 982 control subjects. We also reassessed the *MBL* codon 54 variant (A/B) in susceptibility to SARS-CoV infection in the present study. Furthermore, we investigated whether a combination of these two functional polymorphisms of *CCL2* and *MBL* would have a cumulative effect on risk of SARS-CoV infection.