High Prevalence of the CD14-159CC Genotype in Patients Infected with Severe Acute Respiratory Syndrome-Associated Coronavirus

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To investigate whether genetic factors of innate immunity might influence susceptibility and/or progression in individuals infected with SARS, we evaluated the CD14 gene polymorphism in 198 Hong Kong blood donors and 152 Hong Kong severe acute respiratory syndrome (SARS) patients who were previously genotyped for FcγRIIA polymorphisms. The prevalence of the CD14-159CC polymorphism was significantly higher in the patients with severe SARS than in those with mild SARS or controls (31% versus 15% [mild SARS] or 20% [controls]; mild SARS: P = 0.029; odds ratio, 2.74; 95% confidence interval, 1.15 to 6.57; controls, P = 0.04; odds ratio, 2.41; 95% confidence interval, 1.05 to 5.54), and both CD14-159CC and FcγRIIA-RR131 are risk genotypes for severe SARS-CoV infection.

The study of the relevance of polymorphisms of immunity-related genes in infectious diseases has been an important area of investigation, especially with regard to how these polymorphisms influence both susceptibility to the infection and the course of disease development. Previously, we investigated FcγRIIA and MBL polymorphisms in a group of 180 people from Hong Kong who were infected with severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and revealed that in addition to age and comorbidity, FcγRIIA polymorphisms in individuals may influence outcome after infection with the SARS-CoV, the causative agent of SARS, as a significant association between FcγRIIA-R/R131 genotype and severe SARS was found (10). It is important to investigate whether other host genetic factors could influence susceptibility to SARS-CoV infection and its subsequent clinical course. The innate immune system plays a role in limiting an infectious challenge in the early stages after exposure, during the lag time required to initiate long-lasting adaptive immunity. The impairment of Toll-like receptors (TLRs) due to polymorphisms of TLR genes can alter immune response to a wide variety of microbial ligands, including viruses, and polymorphisms in TLR2 and TLR4 have been linked to infectious diseases in human. In particular, TLR-Arg677Trp was reported to be present in Korean patients with lepromatous leprosy exclusively (6), and this polymorphism was also found to be associated with tuberculosis in a Tunisian population (3). TLR2 and TLR4 SNPs also exhibit ethnic variation, with low and high frequencies in some Asian populations compared with Caucasians and Africans (7). To examine the presence of polymorphisms in TLR2, TLR4, and their coreceptor CD14 in both healthy controls and SARS patients in Hong Kong, we further genotyped 152 patients who were diagnosed as having SARS both clinically and serologically in the Prince of Wales Hospital in Hong Kong and a group of 198 blood donors from Hong Kong (10) for polymorphisms in TLR2, TLR4, and CD14. The study was approved by the Institutional Human Ethics committees of the Prince of Wales Hospital and the Hong Kong Red Cross Blood Transfusion Service.

TLR2-Arg677Trp/Arg753Gln (2180C/T and 2408G/A), TLR4-Asp299Gly/Thr399Ile (12874A/G and 13174C/T), and CD14-159C/T were genotyped in individuals using PCR and restriction fragment length polymorphism analysis as described previously (2, 8, 9) with the following slight modifications: the PCR was performed by adding 1 μl DNA to a 13-μl solution containing 67 mM Tris base (pH 8.8), 16.6 mM ammonium sulfate, 2.5 mM magnesium chloride, 0.1% Tween-20, 200 μM of each deoxynucleoside triphosphate, 10 pmol of each oligonucleotide of a pair of appropriate primers, and 1 U Platinum Taq polymerase (Invitrogen); after PCR, 5 μl of the PCR product was used for overnight digestion at 37°C with the appropriate restriction enzymes (New England Biolabs): for TLR2 (Arg753Gln and Arg677Trp) polymorphisms, 2.5 U of AciI was used, for TLR4 (Asp299Gly and Thr399Ile), 2 U NcoI or 2 U HinfI was used, and for CD14-159C/T, 5 U of AvaII was used. The digests were run on 2.5% high-resolution agarose gels. Data were analyzed for differences in distribution of CD14-159CC,-CT, and -TT genotypes among groups using the chi-squared test for linear trend or independence (two-by-three contingency tables and χ² analysis). Frequencies of combined genotypes (CD14-159 and FcγRIIA) were compared

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among groups using Fisher’s exact test (two-by-two contingency tables, two sided). Differences were considered significant when \( P < 0.05 \). TLR4-Asp299Gly and TLR4-Thr399Ile polymorphisms, which are common in Caucasians though polymorphisms of TLR4-299/399 and TLR2-677/753 are rare or nonexistent in Hong Kong populations, the question of what exact role TLRs play in the clinical and pathologic features of SARS remains.

### REFERENCES


### TABLE 1. Comparison CD14 polymorphisms and “risk” genotypes (CD14-159CC, FcγRIIA-R/R131) between Hong Kong patients with SARS and control group of Hong Kong blood donors

<table>
<thead>
<tr>
<th>Factor</th>
<th>SARS patients (n = 152)</th>
<th>Blood donors (n = 198)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of CD14-159 polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19 (15)</td>
<td>8 (31)</td>
</tr>
<tr>
<td>CT</td>
<td>67 (53)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>TT</td>
<td>40 (32)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Presence of risk genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 (24)</td>
<td>12 (46)</td>
<td>53 (27)</td>
</tr>
<tr>
<td>Absence of risk genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 (76)</td>
<td>14 (54)</td>
<td>145 (73)</td>
</tr>
</tbody>
</table>

* Mild SARS refers to patients who recovered without the need for intensive care or ventilation.  
* The difference in linear trend distribution of CD14-159 genotypes (CC, CT, and TT) between the ICU and mild SARS groups was significant (two-by-three contingency table; \( P = 0.027 \)).  
* In comparison with the mild SARS group and controls, the incidence of risk genotypes in the severe SARS (ICU) group is significantly higher (two-by-two contingency table; for mild SARS, two-sided \( P < 0.05 \); odds ratio, 2.74 [1.15 to 6.57]; for controls, \( P = 0.04 \); odds ratio, 2.41 [1.05 to 5.54]) for controls.