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Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population

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Received 13 February 2005

Abstract

We hypothesized that host antiviral genes induced by type I interferons might affect the natural course of severe acute respiratory syndrome (SARS). We analyzed single nucleotide polymorphisms (SNPs) of 2',5'-oligoadenylate synthetase 1 (OAS-1), myxovirus resistance-A (MxA), and double-stranded RNA-dependent protein kinase in 44 Vietnamese SARS patients with 103 controls. The G-allele of non-synonymous A/G SNP in exon 3 of OAS-1 gene showed association with SARS ($p = 0.0090$). The G-allele in exon 3 of OAS-1 and the one in exon 6 were in strong linkage disequilibrium and both of them were associated with SARS infection. The GG genotype and G-allele of G/T SNP at position -88 in the MxA gene promoter were found more frequently in hypoxemic group than in non-hypoxemic group of SARS ($p = 0.0195$). Our findings suggest that polymorphisms of two IFN-inducible genes OAS-1 and MxA might affect susceptibility to the disease and progression of SARS at each level.

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Keywords: Severe acute respiratory syndrome; SARS associated coronavirus; Association study; Polymorphism; Oligoadenylate synthetase 1; Myxovirus resistance-A; Interferon; Vietnam

Severe acute respiratory syndrome (SARS) is a new infectious disease that emerged towards the end of 2002, spreading from China to countries in Asia, Europe, and North America. During the outbreak, a total of 8098 cases of SARS were diagnosed and the mortality rate was 9.6% [1]. Risk factors for exacerbation of the

clinical progress in SARS have been reported as being patients in excess of 60 years of age, or having diabetes mellitus or other comorbid medical conditions [2,3]. However, little is known about host genetic factors associated with the development or progression of SARS, excepting human leukocyte antigens [4,5] and insertion/deletion polymorphism in the angiotensin converting enzyme 1 gene whose association with the disease [6] our research group had identified.

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It has been shown that SARS is caused by a newly identified SARS coronavirus (SARS-CoV) [7–10]. Among innate immunity against viral infection, type I interferons (IFN- α/β) induced by virus infection generally play an important role in the first line of defense, inducing intracellular antiviral proteins, such as 2',5'-oligoadenylate synthetase 1 (OAS-1), myxovirus resistance-A (MxA), and double-stranded RNA-dependent protein kinase (PKR) [11]. Although the induction of endogenous type I IFNs in the SARS-CoV infection in vivo has not yet been clarified, recent studies have shown that administration of exogenous type I IFNs could inhibit SARS-CoV replication both in vivo [12] and in vitro [13–19]. Investigations into the role of the IFN system against SARS-CoV infection are important, not only to understand the mechanisms of viral pathogenesis but also to adopt effective therapeutic strategies against SARS.

Host genetic factors that influence antiviral effects of IFNs have been well studied in the field of viral hepatitis. Type I IFNs have been widely used as antiviral agents, mainly to treat hepatitis C virus (HCV) infection. Host genetic factors that affect the outcome of IFN treatment in chronic hepatitis C have been investigated, and a single nucleotide polymorphism (SNP) in the promoter region of IFN-inducible *MxA* gene was associated with the response to IFN treatment in the Japanese [20,21] and Caucasian populations [22]. The SNP in *MxA* gene and SNPs in *OAS-1* gene and in *PKR* gene were also shown to be associated with self-limiting infection of HCV by Knapp et al. [22]. Their report indicated that the SNPs in IFN-inducible genes were not only associated with the result of IFN treatment but also with the natural course of HCV infection.

It has been highly suspected that host genetic factors affect the course of various viral infections, including cases of SARS-CoV infection. In the present study, we have tried to determine whether the polymorphisms in IFN-inducible genes are associated with SARS-CoV infection, development, and progression of SARS. This was carried out by investigating 44 Vietnamese SARS cases, with 103 controls of individuals with a history of contact with SARS patients and 50 controls of individuals with no such contact history.

Materials and methods

Subjects. This study was reviewed and approved by ethics committees in the Ministry of Health in Vietnam as well as the International Medical Center in Japan. Written informed consent had been obtained from all subjects and detailed characteristics of the subjects had been described beforehand [6]. In short, the study population comprised 44 SARS patients in Vietnam, 103 staff members of the same hospital as control subjects, who had come into contact with SARS patients but had not developed SARS, and 50 individuals reflecting the general Vietnamese population, having had no contact

history with SARS patients. Out of 44 SARS patients, 22 required oxygen therapy because of hypoxemia, with the other 22 cases, not being hypoxemic, not receiving any such oxygen therapy. There was a significant correlation between the degree of lung involvement in chest radiographs and the requirement of supplementary oxygen. Because of this finding, the progression of SARS in the lung could be reasonably determined from the status of supplementary oxygen ascertained in our previous study [6]. Peripheral blood samples were obtained in all subjects and the genomic DNAs were subsequently extracted [6]. Anti-SARS-CoV antibodies in the blood samples were tested by SARS ELISA (Genelabs Diagnostics, Singapore).

Genotyping of allelic variants of the *OAS-1*, *MxA*, and *PKR* genes. The SNPs analyzed in this study were all genotyped utilizing PCR and restriction fragment length polymorphism (RFLP) methods.

It was once held that *OAS-1* gene consisted of 8 exons [23]. However, according to the current database of RefSeq gene NM_016816, it comprises six exons. As a result, the A/G SNP (rs#2660) in exon 8 of *OAS-1* gene associated with outcome of HCV infection in the previous report by Knapp et al. [22] should have been located in exon 6, which falls on the 3'-untranslated region of long transcript E18 (NM_016816). To detect the SNP, genomic DNA was amplified by AmpliTaq Gold DNA polymerase (Applied Biosystems) with primers 25AS-e6F (5'-GAG GAC TGG ACC TGC ACC ATC CTC-3') and 25AS-e6R (5'-AGA AAG TCA AGG CTG GAA TTT CAT-3'), and the PCR products of 309 bp were digested with *MboII* (New England Biolabs) at 37 °C for 1 h. The 309 bp product was not cut in the presence of G-allele, but was cut into fragments in the presence of A-allele. Subsequently, the fragment was separated into 188 and 121 bp units on 2% agarose gels with ethidium bromide.

We found a non-synonymous SNP in exon 3 of the *OAS-1* gene registered in the JSNP database (No. IMS-JST093062, i.e., rs#3741981). The A/G SNP in exon 3 was genotyped by PCR with primers 25AS-e3F (5'-ATC AGG AAT GGA CCT CAA GAC TTC-3') and 25AS-e3R (5'-CGG ATG AGG CTC TTG AGC TTG GT-3'), and RFLP with *AccI* (New England Biolabs). The PCR products of 306 bp were digested with *AccI* and electrophoresed on 3% agarose gels to analyze undigested 306 bp band and digested parts of 159 and 147 bp bands.

The G/T SNP at position –88 in the promoter region of *MxA* gene was analyzed by PCR–RFLP methods as described previously [20]. The G/T SNP at position –88 was associated with the result of IFN treatment in chronic hepatitis C [20–22] and with the result of HCV infection [22].

The T/C SNP at position –168 in the promoter region of *PKR* gene, associated with result of HCV infection [22], was genotyped as follows. PCR was carried out with primers PKR-pF (5'-GTG GAA CCC TTG ATT CGA GAA CCT AGT-3') and PKR-pR (5'-GCG GCT TCG GGA GAG CTG GTT CTC AGT-3') using TaKaRa Ex Taq with GC buffer I (TaKaRa). The cycling condition is 45 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 1 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and digested with *SgrAI* (New England Biolabs). Digested DNA was electrophoresed on a 5% agarose gel. The presence of T-allele was demonstrated by 169 and 155 bp fragments, and the presence of C-allele was indicated by 169, 136, and 19 bp fragments.

Statistical analysis. Possible differences deriving from the distribution of age and gender between two groups were evaluated with the unpaired *t* test and χ^2 test, respectively. Disease associations were assessed by the χ^2 test. *p* values less than 0.05 were considered significant in all the tests, and data analysis was carried out using JMP version 5 (SAS Institute). Genotype distribution of tested polymorphisms in the control population was in Hardy–Weinberg equilibrium. We calculated Lewontin's $|D'|$ and r^2 to assess the extent of pairwise linkage disequilibrium between polymorphisms [24]. These indices were calculated with the use of haplotype frequencies estimated by the PHASE algorithm (PHASE, version 2.1.1) based on Bayesian methods.

Results

Demographic information is shown in Table 1. The mean age was not different between SARS cases and contacts ($p = 0.1781$). Although females appeared more frequently in SARS cases than contacts, the male/female ratio was not statistically different between the two groups ($p = 0.0869$). Sixteen individuals out of 103 contacts were revealed to have anti-SARS-CoV antibodies. When we classified these individuals into an infected group together with SARS patients, age and gender showed no significant difference between the infected and non-infected groups ($p = 0.2139$; 0.2065). SARS cases were classified by the requirement for oxygen therapy. Age and gender did not differ between these subgroups either ($p = 0.4198$; 0.7411).

We analyzed SNPs of IFN-inducible genes showing association with HCV infection as described above [20–22], and compared their genotypes and allele frequencies between 44 SARS cases and 103 controls with contact history to SARS patients (Table 2).

We observed a higher frequency of the G-allele positive genotypes (GA and GG) of non-synonymous SNP in exon 3 of *OAS-I* gene in SARS patients (odds ratio 2.68; 95% CI; 1.17–6.15; $p = 0.0178$). Allele frequency of the G-allele in exon 3 was significantly higher in SARS patients ($p = 0.0090$). Allele frequency of the G-allele in exon 6 of *OAS-I* was also found more frequently in SARS patients than in the controls that showed marginal significance ($p = 0.0542$).

The genotype and allele frequencies of *OAS-I* polymorphisms were compared among 60 SARS-CoV infected individuals and 87 uninfected individuals (Table 3). It was shown that frequencies of the G-alleles in exon 3 and exon 6 were significantly higher in infected individuals than in uninfected ($p = 0.0156$ and $p = 0.0176$, respectively). These two polymorphisms in the *OAS-I* gene were in strong linkage disequilibrium ($|D'| = 0.931$, $r^2 = 0.530$). Genotype and allele frequencies in 50 controls with no contact history with SARS patients are also shown in Table 3. In controls of subjects having no contact history, allele frequencies of G-allele in exons 3 and 6 were lower than those of the infected group, but higher than those of the uninfected group.

The SNPs in *MxA* gene and *PKR* gene were not associated with the development of SARS (Table 2). However, on comparison of the *MxA* genotype between hypoxemic SARS patients requiring oxygen therapy

Table 2

Genotype and allele frequencies in SARS cases and controls with contact history

| | SARS cases ($n = 44$) | Controls ($n = 103$) | p value |
|---------------------|-------------------------|------------------------|-----------|
| <i>OAS-I</i> exon 6 | | | |
| Genotype | | | |
| AA | 18 (40.9%) | 60 (58.3%) | 0.0537 |
| AG | 21 (47.7%) | 36 (35.0%) | |
| GG | 5 (11.4%) | 7 (6.7%) | |
| Allele | | | |
| A | 0.65 | 0.76 | 0.0542 |
| G | 0.35 | 0.24 | |
| <i>OAS-I</i> exon 3 | | | |
| Genotype | | | |
| AA | 9 (20.5%) | 42 (40.8%) | 0.0178 |
| AG | 24 (54.5%) | 48 (46.6%) | |
| GG | 11 (25.0%) | 13 (12.6%) | |
| Allele | | | |
| A | 0.48 | 0.64 | 0.0090 |
| G | 0.52 | 0.36 | |
| <i>MxA</i> –88 | | | |
| Genotype | | | |
| GG | 23 (52.3%) | 43 (41.7%) | 0.2400 |
| GT | 16 (36.4%) | 52 (50.5%) | |
| TT | 5 (11.3%) | 8 (7.8%) | |
| Allele | | | |
| G | 0.70 | 0.67 | 0.5597 |
| T | 0.30 | 0.33 | |
| <i>PKR</i> –168 | | | |
| Genotype | | | |
| CC | 18 (41.9%) | 49 (47.6%) | 0.5278 |
| CT | 22 (51.2%) | 46 (44.7%) | |
| TT | 3 (7.0%) | 8 (7.8%) | |
| Allele | | | |
| C | 0.67 | 0.70 | 0.6780 |
| T | 0.33 | 0.30 | |

and non-hypoxemic SARS patients who did not, GG genotype was found more frequently in patients of the former category (odds ratio 3.75; 95% CI 1.08–10.7; $p = 0.0346$). It was also shown that the G-allele was more frequent in the former group ($p = 0.0195$) (Table 4). The other SNPs did not show any significant p values between these two groups (data not shown).

Discussion

Our study showed that the polymorphisms in the IFN-inducible *OAS-I* gene might affect susceptibility to SARS-CoV infection or the development of SARS.

Table 1

Characteristics of SARS cases and healthy contacts

| Characteristics | Contacts | | | SARS cases | | |
|--------------------------|---------------|-----------------------------------|-----------------------------------|--------------|----------------------------------|------------------------------|
| | ($n = 103$) | Anti-SARS-CoV Ab (–) ($n = 87$) | Anti-SARS-CoV Ab (+) ($n = 16$) | ($n = 44$) | Non-hypoxemic group ($n = 22$) | Hypoxemic group ($n = 22$) |
| Age (year), mean [range] | 36.5 [15–69] | 36.6 [15–69] | 36.6 [25–50] | 39.3 [17–76] | 37.7 [17–61] | 41.0 [23–76] |
| Male/female (n) | 46/57 | 39/48 | 7/9 | 13/31 | 6/16 | 7/15 |

Table 3
Genotype and allele frequencies of *OAS-1* polymorphisms in SARS infected, uninfected, and controls without contact history

| <i>OAS-1</i> | SARS infected (<i>n</i> = 60) | Uninfected (<i>n</i> = 87) | <i>p</i> value | Controls without contact (<i>n</i> = 50) |
|---------------|-----------------------------------|--------------------------------|----------------|--|
| Exon 6 | | | | |
| Genotype | | | | |
| AA | 25 (41.7%) | 53 (60.9%) | 0.0215 | 27 (54.0%) |
| AG | 28 (46.7%) | 29 (33.3%) | | 17 (34.0%) |
| GG | 7 (11.7%) | 5 (5.7%) | | 6 (12.0%) |
| Allele | | | | |
| A | 0.65 | 0.76 | 0.0176 | 0.71 |
| G | 0.35 | 0.24 | | 0.29 |
| Exon 3 | | | | |
| Genotype | | | | |
| AA | 14 (23.3%) | 37 (42.5%) | 0.0163 | 17 (34.0%) |
| AG | 33 (55.0%) | 39 (44.8%) | | 26 (52.0%) |
| GG | 13 (21.7%) | 11 (12.6%) | | 7 (14%) |
| Allele | | | | |
| A | 0.51 | 0.65 | 0.0156 | 0.60 |
| G | 0.49 | 0.35 | | 0.40 |

Table 4
Genotype and allele frequencies of *MxA* –88 G/T polymorphism in the subgroups of SARS cases

| | SARS cases (<i>n</i> = 44) | | <i>p</i> value |
|----------|---|-------------------------------------|----------------|
| | Non-hypoxemic group (<i>n</i> = 22) | Hypoxemic group (<i>n</i> = 22) | |
| Genotype | | | |
| GG | 8 (36.4%) | 15 (68.2%) | 0.0346 |
| GT | 10 (45.4%) | 6 (27.3%) | |
| TT | 4 (18.2%) | 1 (4.5%) | |
| Allele | | | |
| G | 0.59 | 0.82 | 0.0195 |
| T | 0.41 | 0.18 | |

In the presence of double-stranded RNA (dsRNA), *OAS-1* catalyzes the 2',5'-oligomers of adenosine in order to permit the binding and activation of a latent ribonuclease, RNase L, which cleaves cellular and viral RNAs [11,25]. *OAS-1* gene has two major transcripts that are generated by alternative splicing at the last two exons [23]. E16 (NM_002534) is a short transcript with 5 exons and is translated to p40 isoform. E18 (NM_016816) is a long transcript with 6 exons and is translated to p46 isoform. Another transcript 9-2 is generated using a different splice acceptor site that comes from E18 at exon 6 and is translated to 9-2 protein [26]. The 9-2 protein has a unique property due to the Bcl-2 homology domain 3 present in its unique carboxyl-terminal region. This is also distinctive in causing cellular apoptosis by binding to the anti-apoptotic proteins of the Bcl-2 family [26]. Therefore, *OAS-1* has dual functions representing the synthesis of 2',5'-oligomers of adenosine and the promotion of cellular apoptosis.

Knapp et al. [22] described how the GG genotype in exon 6 of *OAS-1* gene was more frequent in persistent

HCV infection than in self-limiting infection. In our study, the G-allele was more frequently observed in SARS-CoV infected individuals than in the uninfected group. In both these studies, the G-allele was susceptible to virus infection. The A/G polymorphism in exon 6 is located downstream of the stop codon for E18 transcript meaning therefore that it is included in the 3'-untranslated region. However, it is located upstream of the stop codon for 9-2, and the A/G SNP results in amino acid substitution Arg397Gly of 9-2 protein, which is located near the Bcl-2 homology domain (amino acid positions 372–393). It will be an interesting aspect if this phenomenon occurs with any functional importance. We also analyzed the A/G polymorphism in exon 3 of *OAS-1* gene and found that there was strong linkage disequilibrium between the two SNPs. The A/G polymorphism in exon 3 causes amino acid substitution Ser162Gly in three isoforms, which is located near the dsRNA binding domain (amino acid positions 104–158) of *OAS-1* [27]. We are unable at this point to determine which SNP is directly related to susceptibility to SARS or SARS-CoV infection. One can also consider that the other unidentified polymorphism of strong linkage disequilibrium with these SNPs may serve as the basis for any functional difference. Judging from the results obtained in this study, polymorphisms in *OAS-1* gene are likely to be involved in SARS-CoV infection or the development of SARS, at least in part, bearing in mind the fact that *OAS-1* might have antiviral potential against SARS-CoV.

SARS-CoV is usually cultured in Vero E6 cell line [13–17,19], which cannot produce IFNs because it lacks *IFN* genes [28,29]. Recently, Cinatl et al. [30] infected permissive Caco-2 cells with SARS-CoV and analyzed the effects of SARS-CoV on cellular gene expression by high-density oligonucleotide arrays. They found that SARS-CoV infection of Caco-2 cells up-regulated IFN-inducible *OAS-2*, *OASL*, and *MxA* but not *PKR* genes. *OAS-2* and *OASL* are members of the human *OAS* gene family [25]. The role of *OAS-1* as an inhibitor of SARS-CoV replication should be clarified to examine the hypothesis that Caco-2 cells permitted considerable infection with SARS-CoV because they did not induce *OAS-1*.

As regards the G/T polymorphism at position –88 in promoter region of *MxA* gene, GG genotype and G-allele were found to be more frequent in patients with an enhanced clinical progression, requiring oxygen therapy, although the number of cases was rather small. GG genotype was found more frequently in non-responders of IFN treatment in hepatitis C, and a luciferase reporter assay revealed that the *MxA* promoter sequence of G haplotype had lower promoter activity than that of T haplotype [31]. Recently, Arcas et al. [32] reported that GG genotype expressed lower amount of *MxA* mRNA than GT or TT genotype in IFN-treated peripheral

blood mononuclear cells in vitro. Spiegel et al. [15] reported that SARS-CoV replication was not affected in Vero E6 cells that were stably expressing MxA. They concluded that antiviral effect of IFN against SARS-CoV was not mediated by MxA. In our study, –88 SNP in *MxA* promoter was not related to disease susceptibility. Taking these observations together, MxA may not have a strong inhibitory effect on replication of SARS-CoV, but lower MxA expression may play a role in the worsening of SARS clinical progression.

If SARS re-emerges, IFN could be a promising candidate to treat SARS patients [12–19]. In the present study, the SNPs in *OAS-1* were associated with SARS-CoV infection or development of SARS, and the SNP in *MxA* was associated with the progression of SARS. It could be interesting to consider that they may also be related to the response of SARS patients to IFNs, and that SARS patients with AA genotype of the A/G SNP in exon 3 of *OAS-1* may respond to IFN treatment more effectively than those with AG or GG genotypes. During the course of our study, age was not a risk factor contributing to any worsening of SARS, probably because the majority of the patients consisted of relatively young medical staff members [6].

In conclusion, we showed that the polymorphisms in *OAS-1* gene were associated with SARS-CoV infection or development of SARS and that the polymorphism in *MxA* gene was also associated with hypoxemic status in SARS cases in Vietnam. These findings may lead to an understanding of IFN-induced antiviral response to SARS infection.

Acknowledgments

The authors thank Dr. Nguyen Le Hang, Ms. Pham Thi Phuong Thuy, and Ms. Nguyen Thi Thu Ha for their help in the management and coordination of this study in Vietnam. The authors also thank Kazuko Tanabe D.V.M. and Mr. John Crosskey for their critical reading of the manuscript and Dr. Goh Tanaka for his help in statistical analysis. This work was supported by a grant for International Health Cooperation Research (14C-9) and for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare in 2004.

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