

書籍

著者氏名	論文タイトル名	書籍全体の編集者	書籍名	出版社名	出版地	ページ	出版年
徳永勝士	HLAハプロタイプと連鎖不平衡. ヒトゲノム多様性の概要. 集団遺伝学との関連.	大谷文雄、木村彰方、小林賢、鈴木洋司、徳永勝士	「移植・輸血検査学」	講談社サイエンティフィク	東京	56-63, 159-163, 185-191	2004
徳永勝士	HLAハプロタイプ解析に基づく日本人の起源と形成.	柳佳之、笹月健彦、油谷浩幸	Molecular Medicine 臨時増刊号 (Vol. 41) 「ヒトゲノム」	中山書店	東京	197-202	2004
西田奈央、 徳永勝士	プライマーエクステンションを用いたSNP解析	辻本豪三、田中利男	実験医学別冊「ゲノム研究実験ハンドブック」	羊土社	東京	139-144	2004



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

Emi Hamano ^a, Minako Hijikata ^a, Satoru Itoyama ^a, Tran Quy ^b, Nguyen Chi Phi ^b,
Hoang Thuy Long ^c, Le Dang Ha ^d, Vo Van Ban ^e, Ikumi Matsushita ^a,
Hideki Yanai ^f, Fumiko Kirikae ^g, Teruo Kirikae ^g, Tadatoshi Kuratsuji ^h,
Takehiko Sasazuki ⁱ, Naoto Keicho ^{a,*}

^a Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, Japan

^b Bach Mai Hospital, Viet Nam

^c National Institute of Hygiene and Epidemiology, Viet Nam

^d Institute for Clinical Research in Tropical Medicine, Viet Nam

^e Hanoi-French Hospital, Viet Nam

^f The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan

^g Department of Infectious Diseases, Research Institute, International Medical Center of Japan, Japan

^h Research Institute, International Medical Center of Japan, Japan

ⁱ International Medical Center of Japan, Japan

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.

© 2005 Elsevier Inc. All rights reserved.

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.

* Corresponding author. Fax: +81 3 3207 1038.

E-mail address: nkeicho-tky@umin.ac.jp (N. Keicho).

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 *Mbo*II (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 *Acl*I (New England Biolabs). The PCR products of 306 bp were digested with *Acl*I 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 *Sgr*AI (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-1* 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-1* 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-1* 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-1* 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-1</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-1</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-1* 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 (n = 60)	Uninfected (n = 87)	p value	Controls without contact (n = 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 (n = 44)		p value
	Non-hypoxemic group (n = 22)	Hypoxemic group (n = 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.

References

- [1] World Health Organization (2003), Consensus document on the epidemiology of severe acute respiratory syndrome (SARS), WHO/CDS/CSR/GAR/2003.11, Geneva.
- [2] C.M. Booth, L.M. Matukas, G.A. Tomlinson, A.R. Rachlis, D.B. Rose, H.A. Dwosh, S.L. Walmsley, T. Mazzulli, M. Avendano, P. Derkach, I.E. Eptimios, I. Kitai, B.D. Mederski, S.B. Shadowitz, W.L. Gold, L.A. Hawryluck, E. Rea, J.S. Chenkin, D.W. Cescon, S.M. Poutanen, A.S. Detsky, Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area, *Jama* 289 (2003) 2801–2809.
- [3] J.W. Chan, C.K. Ng, Y.H. Chan, T.Y. Mok, S. Lee, S.Y. Chu, W.L. Law, M.P. Lee, P.C. Li, Short term outcome and risk factors for adverse clinical outcomes in adults with severe acute respiratory syndrome (SARS), *Thorax* 58 (2003) 686–689.
- [4] M. Lin, H.K. Tseng, J.A. Trejaut, H.L. Lee, J.H. Loo, C.C. Chu, P.J. Chen, Y.W. Su, K.H. Lim, Z.U. Tsai, R.Y. Lin, R.S. Lin, C.H. Huang, Association of HLA class I with severe acute respiratory syndrome coronavirus infection, *BMC Med. Genet.* 4 (2003) 9.
- [5] M.H. Ng, K.M. Lau, L. Li, S.H. Cheng, W.Y. Chan, P.K. Hui, B. Zee, C.B. Leung, J.J. Sung, Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome, *J. Infect. Dis.* 190 (2004) 515–518.
- [6] S. Itoyama, N. Keicho, T. Quy, N.C. Phi, H.T. Long, D. Ha le, V.V. Ban, J. Ohashi, M. Hijikata, I. Matsushita, A. Kawana, H. Yanai, T. Kirikae, T. Kuratsuji, T. Sasazuki, ACE1 polymorphism and progression of SARS, *Biochem. Biophys. Res. Commun.* 323 (2004) 1124–1129.
- [7] J.S. Peiris, S.T. Lai, L.L. Poon, Y. Guan, L.Y. Yam, W. Lim, J. Nicholls, W.K. Yee, W.W. Yan, M.T. Cheung, V.C. Cheng, K.H. Chan, D.N. Tsang, R.W. Yung, T.K. Ng, K.Y. Yuen, Coronavirus as a possible cause of severe acute respiratory syndrome, *Lancet* 361 (2003) 1319–1325.
- [8] T.G. Ksiazek, D. Erdman, C.S. Goldsmith, S.R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J.A. Comer, W. Lim, P.E. Rollin, S.F. Dowell, A.E. Ling, C.D. Humphrey, W.J. Shieh, J. Guarner, C.D. Paddock, P. Rota, B. Fields, J. DeRisi, J.Y. Yang, N. Cox, J.M. Hughes, J.W. LeDuc, W.J. Bellini, L.J. Anderson, A novel coronavirus associated with severe acute respiratory syndrome, *N. Engl. J. Med.* 348 (2003) 1953–1966.
- [9] C. Drosten, S. Gunther, W. Preiser, S. van der Werf, H.R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R.A. Fouchier, A. Berger, A.M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J.C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H.D. Klenk, A.D. Osterhaus, H. Schmitz, H.W. Doerr, Identification of a novel coronavirus in patients with severe acute respiratory syndrome, *N. Engl. J. Med.* 348 (2003) 1967–1976.
- [10] T. Kuiken, R.A. Fouchier, M. Schutten, G.F. Rimmelzwaan, G. van Amerongen, D. van Riel, J.D. Laman, T. de Jong, G. van Doornum, W. Lim, A.E. Ling, P.K. Chan, J.S. Tam, M.C. Zambon, R. Gopal, C. Drosten, S. van der Werf, N. Escriou, J.C. Manuguerra, K. Stohr, J.S. Peiris, A.D. Osterhaus, Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome, *Lancet* 362 (2003) 263–270.
- [11] C.E. Samuel, Antiviral actions of interferons, *Clin. Microbiol. Rev.* 14 (2001) 778–809.
- [12] B.L. Haagmans, T. Kuiken, B.E. Martina, R.A. Fouchier, G.F. Rimmelzwaan, G. van Amerongen, D. van Riel, T. de Jong, S. Itamura, K.H. Chan, M. Tashiro, A.D. Osterhaus, Pegylated interferon-alpha protects type I pneumocytes against SARS coronavirus infection in macaques, *Nat. Med.* 10 (2004) 290–293.
- [13] J. Cinatl, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, H.W. Doerr, Treatment of SARS with human interferons, *Lancet* 362 (2003) 293–294.
- [14] L.E. Hensley, L.E. Fritz, P.B. Jahrling, C.L. Karp, J.W. Huggins, T.W. Geisbert, Interferon-beta 1a and SARS coronavirus replication, *Emerg. Infect. Dis.* 10 (2004) 317–319.
- [15] M. Spiegel, A. Pichlmair, E. Muhlberger, O. Haller, F. Weber, The antiviral effect of interferon-beta against SARS-coronavirus is not mediated by MxA protein, *J. Clin. Virol.* 30 (2004) 211–213.
- [16] U. Stroher, A. DiCaro, Y. Li, J.E. Strong, F. Aoki, F. Plummer, S.M. Jones, H. Feldmann, Severe acute respiratory syndrome-

- related coronavirus is inhibited by interferon- α , *J. Infect. Dis.* 189 (2004) 1164–1167.
- [17] E.L. Tan, E.E. Ooi, C.Y. Lin, H.C. Tan, A.E. Ling, B. Lim, L.W. Stanton, Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs, *Emerg. Infect. Dis.* 10 (2004) 581–586.
- [18] B. Zheng, M.L. He, K.L. Wong, C.T. Lum, L.L. Poon, Y. Peng, Y. Guan, M.C. Lin, H.F. Kung, Potent inhibition of SARS-associated coronavirus (SCOV) infection and replication by type I interferons (IFN- α /beta) but not by type II interferon (IFN- γ), *J. Interferon Cytokine Res.* 24 (2004) 388–390.
- [19] B. Sainz Jr., E.C. Mossel, C.J. Peters, R.F. Garry, Interferon-beta and interferon-gamma synergistically inhibit the replication of severe acute respiratory syndrome-associated coronavirus (SARS-CoV), *Virology* 329 (2004) 11–17.
- [20] M. Hijikata, Y. Ohta, S. Mishiro, Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon, *Intervirology* 43 (2000) 124–127.
- [21] F. Suzuki, Y. Arase, Y. Suzuki, A. Tsubota, N. Akuta, T. Hosaka, T. Someya, M. Kobayashi, S. Saitoh, K. Ikeda, M. Matsuda, K. Takagi, J. Satoh, H. Kumada, Single nucleotide polymorphism of the MxA gene promoter influences the response to interferon monotherapy in patients with hepatitis C viral infection, *J. Viral Hepat.* 11 (2004) 271–276.
- [22] S. Knapp, L.J. Yee, A.J. Frodsham, B.J. Hennig, S. Hellier, L. Zhang, M. Wright, M. Chiramonte, M. Graves, H.C. Thomas, A.V. Hill, M.R. Thursz, Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR, *Genes Immun.* 4 (2003) 411–419.
- [23] P. Benech, Y. Mory, M. Revel, J. Chebath, Structure of two forms of the interferon-induced (2'-5') oligo A synthetase of human cells based on cDNAs and gene sequences, *Embo J.* 4 (1985) 2249–2256.
- [24] R.C. Lewontin, On measures of gametic disequilibrium, *Genetics* 120 (1988) 849–852.
- [25] D. Rebouillat, A.G. Hovanessian, The human 2',5'-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties, *J. Interferon Cytokine Res.* 19 (1999) 295–308.
- [26] A. Ghosh, S.N. Sarkar, T.M. Rowe, G.C. Sen, A specific isozyme of 2'-5' oligoadenylate synthetase is a dual function proapoptotic protein of the Bcl-2 family, *J. Biol. Chem.* 276 (2001) 25447–25455.
- [27] S.K. Ghosh, J. Kusari, S.K. Bandyopadhyay, H. Samanta, R. Kumar, G.C. Sen, Cloning, sequencing, and expression of two murine 2'-5'-oligoadenylate synthetases. Structure-function relationships, *J. Biol. Chem.* 266 (1991) 15293–15299.
- [28] J.D. Mosca, P.M. Pitha, Transcriptional and posttranscriptional regulation of exogenous human beta interferon gene in simian cells defective in interferon synthesis, *Mol. Cell. Biol.* 6 (1986) 2279–2283.
- [29] M.O. Diaz, S. Ziemien, M.M. Le Beau, P. Pitha, S.D. Smith, R.R. Chilcote, J.D. Rowley, Homozygous deletion of the alpha and beta 1-interferon genes in human leukemia and derived cell lines, *Proc. Natl. Acad. Sci. USA* 85 (1988) 5259–5263.
- [30] J. Cinatl Jr., G. Hoever, B. Morgenstern, W. Preiser, J.U. Vogel, W.K. Hofmann, G. Bauer, M. Michaelis, H.F. Rabenau, H.W. Doerr, Infection of cultured intestinal epithelial cells with severe acute respiratory syndrome coronavirus, *Cell. Mol. Life Sci.* 61 (2004) 2100–2112.
- [31] M. Hijikata, S. Mishiro, C. Miyamoto, Y. Furuichi, M. Hashimoto, Y. Ohta, Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (-123 and -88) in vivo and in vitro, *Intervirology* 44 (2001) 379–382.
- [32] N. Fernandez-Arcas, A. Blanco, M.J. Gaitan, M. Nyqvist, A. Alonso, A. Reyes-Engel, Differential transcriptional expression of the polymorphic myxovirus resistance protein A in response to interferon- α treatment, *Pharmacogenetics* 14 (2004) 189–193.



ACE1 polymorphism and progression of SARS

Satoru Itoyama^a, Naoto Keicho^{a,*}, Tran Quy^b, Nguyen Chi Phi^b, Hoang Thuy Long^c,
Le Dang Ha^d, Vo Van Ban^e, Jun Ohashi^f, Minako Hijikata^a, Ikumi Matsushita^a,
Akihiko Kawana^g, Hideki Yanai^h, Teruo Kirikaeⁱ, Tadatoshiki Kuratsuji^j, Takehiko Sasazuki^k

^a Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, Japan

^b Bach Mai Hospital, Viet Nam

^c National Institute of Hygiene and Epidemiology, Viet Nam

^d Institute for Clinical Research in Tropical Medicine, Viet Nam

^e Hanoi-French Hospital, Viet Nam

^f Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Japan

^g Division of Pulmonology, International Medical Center of Japan, Japan

^h The Research Institute of Tuberculosis Japan Anti-Tuberculosis Association, Japan

ⁱ Department of Infectious Diseases and Tropical Medicine, Research Institute, International Medical Center of Japan, Japan

^j Research Institute, International Medical Center of Japan, Japan

^k International Medical Center of Japan, Japan

Received 23 July 2004

Abstract

We have hypothesized that genetic predisposition influences the progression of SARS. Angiotensin converting enzyme (ACE1) insertion/deletion (I/D) polymorphism was previously reported to show association with the adult respiratory distress syndrome, which is also thought to play a key role in damaging the lung tissues in SARS cases. This time, the polymorphism was genotyped in 44 Vietnamese SARS cases, with 103 healthy controls who had had a contact with the SARS patients and 50 controls without any contact history. SARS cases were divided into either non-hypoxemic or hypoxemic groups. Despite the small sample size, the frequency of the D allele was significantly higher in the hypoxemic group than in the non-hypoxemic group ($p = 0.013$), whereas there was no significant difference between the SARS cases and controls, irrespective of a contact history. ACE1 might be one of the candidate genes that influence the progression of pneumonia in SARS.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Angiotensin converting enzyme; Severe acute respiratory syndrome; Association study; Hypoxemia; Polymorphism

The severe acute respiratory syndrome (SARS) spread worldwide as an emergent pneumonia [1]. Typical clinical features of SARS are high fever, myalgia, and other symptoms caused by systemic inflammation and the subsequent atypical pneumonia [2–4]. Approximately 40% of SARS cases developed hypoxemia [5] and further advanced cases were presented as acute respiratory distress syndrome (ARDS). Pathological analysis

of the lung at autopsy in cases of SARS revealed diffuse alveolar damage, characterized by desquamation of pneumocytes, inflammatory infiltrates, edema, and hyaline-membrane formation [6–8]. Such pathological feature of the lung in SARS cases was consistent with that in ARDS.

The early reports showed that higher age, diabetes mellitus, and heart disease are risk factors for the prognosis of SARS [9,10], whereas there has been so far little discussion on contribution of genetic factors for the development or progression of SARS [11,12]. To date, there are only a few reports showing genetic involvement

* Corresponding author. Fax: +81 3 3207 1038.

E-mail address: nkeicho-tky@umin.ac.jp (N. Keicho).

in ARDS as well [13,14]. In the present study, we focused on the angiotensin converting enzyme (ACE) deletion (D) allele that was once reported to be associated with poor outcome in ARDS [13].

ACE is a metalloproteinase that converts angiotensin I (AT-I) to angiotensin II (AT-II), acting as a vasoconstrictor, and it also degrades bradykinin, acting as a vasodilator. ACE is a prototype of angiotensin converting enzyme, which is distinct from its human homologue ACE2 also known as a SARS receptor [15]. It is well known that ACE is rich in microvascular endothelium and is likely involved in angiopathy of the lung. The human ACE gene on chromosome 17q23 consists of 26 exons, and has insertion (I) or deletion (D) of a 287-bp Alu repeat sequence in intron 16 [16]. Although the function of this polymorphism remains unclear, the genotype or allele frequency of this polymorphism has been often reported to be associated with pathological status including the development of myocardial infarction and deterioration of the renal function in diabetes mellitus in humans [17]. In the present study, we explored the possibility of this polymorphism, based on the assumption that it might be associated with progression of SARS in Vietnamese cases.

Although Vietnam experienced the outbreak of SARS in February 2003, they successfully controlled SARS and Vietnam was removed from the list of the affected areas just nine weeks later. Consequently, in Vietnam, there were 62 probable cases of SARS; 87% was nosocomial infection in a single hospital and the community-acquired infection was only 13% [1].

Materials and methods

Study population. This study was reviewed and approved by Ethics Committees in Ministry of Health of Vietnam as well as International Medical Center of Japan. Out of 62 cases fulfilling the World Health Organization case definition of probable SARS [18], five cases died, three were not Vietnamese, such that they were excluded from this study. In the remaining 54, written informed consent was obtained from 44 individuals, who were enrolled in this study as cases. Furthermore, 103 staff members who came in contact with SARS patients in the hospital A without development of SARS, and 50 individuals reflecting general Vietnamese population who had no contact history participated in this study. Peripheral blood sample of all the subjects was taken, blood cells were separated from plasma by centrifuge method, and genomic DNA was subsequently extracted by a method described elsewhere [19].

Genotyping of ACE I/D polymorphism. ACE I/D genotype was determined by three-primer polymerase chain reaction amplification method that was described previously [20]. Briefly, primers ACE1 and ACE3 were chosen outside the insertion sequence and another primer ACE2 was placed on the insertion sequence. Reactions were performed with 25 pmol of each primer ACE1 and ACE3, and with 7.5 pmol of primer ACE2 in a final volume of 25 μ l, containing 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.001% (W/V) of gelatin, 0.2 mM of each dNTP, and 1 U *Taq* polymerase (Ampli[®]Taq Gold DNA polymerase, Applied Biosystems).

Nucleotide sequences of primers were as follows: ACE1: 5'-CATCCTTTCTCCCATTTCTC-3', ACE2: 5'-TGGGATTACAGGCGTGA TACAG-3', and ACE3: 5'-ATTTTCAGAGCTGGAATAAAATT-3'.

After the initial denaturation at 94 °C for 10 min, the reaction mixture was subjected to 30 cycles of 94 °C for 1 min and 55 °C for 1 min. This method yields amplification products of 84 bp for the D allele and 65 bp for the I allele, and products were electrophoresed and visualized on 4% agarose gels with ethidium bromide.

Clinical profiles and backgrounds of the subjects. Clinical profiles and backgrounds of all subjects were extracted from medical records and questionnaires taken by trained interviewers. Information about age, sex, degree of contacts with SARS patients, and requirement of supplementary oxygen was obtained. Chest radiographs of SARS cases on the acute phase were also available. Entire lung field on the chest X-ray films was divided into six zones, which were right upper, middle, and lower zones and left upper, middle, and lower zones. The number of affected zones on the film where the lung is most severely inflamed during the clinical course was counted in each case. Two different chest physicians judged the number of involved zones in the entire lung field independently.

Statistical analysis. Disease associations were assessed by the χ^2 test. Inter-observer variability for the number of involved zones in the lung was assessed with the Spearman rank correlation coefficient. Logistic regression analysis was performed to identify predictors of hypoxemia or progression of SARS. The *p* values less than 0.05 were considered significant in all the tests and data analysis was carried out using the SAS system for Windows version 8.2 (SAS Institute, Cary, NC).

Results

Demographics

Demographic information of 44 SARS cases and 103 healthy contacts in this study is shown in Table 1. Of the 44 cases, 13 were male and 31 were female. Their mean age was 39.3 years old. Two cases had hypertension but they had neither diabetes mellitus nor heart disease. Of the 103 healthy contacts, 46 were male and 57 were female. Their mean age was 36.5 years old and this was comparable with that of SARS patients. One case had hypertension.

Individuals who faced SARS patients directly were defined as "direct contacts." Health care workers working in the hospital A during the outbreak but did not come in direct contact with SARS patient were described as "indirect contacts."

Validation of parameters for progression of SARS

Based on the requirement of supplementary oxygen when pneumonia deteriorated, cases were separated into two groups. Oxygen was supplied to maintain a partial pressure of arterial oxygen of higher than 60 mmHg, or arterial oxygen saturation of more than 90% at room air on the basis of Provisional guidelines issued by Ministry of Health in Vietnam. Half of the cases did not require supplementary oxygen and were defined as "non-hypoxemic" group and the other half required supplementary oxygen and constituted "hypoxemic"

Table 1
Characteristics of SARS cases and healthy contacts

Characteristics	SARS cases (n = 44)	Contacts (n = 103)
Age (years), mean [range]	39.3 [17–76]	36.5 [15–68]
Male/female	13/31	46/57
<i>Underlying diseases</i>		
Diabetes mellitus	0	0
Hypertension	2	1
Heart disease	0	0
<i>Lung disease</i>		
COPD	3	2
Bronchial asthma	0	2
Tuberculosis	1	1
Pulmonary embolism	0	1
<i>Contact status</i>		
Direct	37	63
Indirect	7	40
<i>Occupation</i>		
Nurse	16	24
Doctor	5	13
Co-medical staff	8	22
Non-medical staff	1	44
Visitor	2	—
Inpatient	2	—
Family or maid of patient	5	—
Others	5	—

group (Table 2). Five cases of the hypoxemic group further received mechanical ventilation because they could not keep spontaneous breathing.

Serial chest X-rays were taken in all cases during the acute stage. We compared the number of involved lung zones with requirement of supplementary oxygen (Table 3). Assessment of the number of involved lung zone correlated well between two medical observers (Spearman rank correlation coefficient, $r = 0.879$, $p < 0.0001$). The number of involved lung zones was significantly associated with requirement of supplementary oxygen (χ^2 value = 31.5; $df = 3$; $p < 0.0001$). In 13 out of 22 cases with hypoxia, more than three zones of the lung were involved on chest X-rays, whereas in 14 out of 22 cases without hypoxia, only one zone was involved.

Genotypic and allele frequencies of ACE I/D polymorphism

Genotypic distribution and allele frequency of the ACE I/D polymorphism in SARS cases and controls

Table 2
Characteristics of subgroups of SARS cases

Characteristics	SARS cases (n = 44)		
	Non-hypoxemic group (n = 22)	Hypoxemic group	
		Without mechanical ventilation (n = 17)	With mechanical ventilation (n = 5)
Age (year), mean	37.7	41.0	36.8
Male/female (n)	6/16	7/15	5/12
Direct/indirect contact (n)	19/3	18/4	16/1
			55.4
			2/3
			2/3

Table 3
The number of involved lung zones on chest radiographs in the subgroups of SARS cases

Number of involved zones	Non-hypoxemic group (n = 22)	Hypoxemic group (n = 22)
One	14	1
Two	6	6
Three	2	2
More than three	0	13

with or without contact history to SARS patients were compared (Table 4). Genotypic and allele frequencies of ACE I/D polymorphism were not different among those groups. Roughly, one-third of the alleles were D in the Vietnamese population, which were comparable with the previous data from Asians including Japanese and Chinese, but quite different from those in British population, where a half of them were D allele.

As shown in Table 5, frequency of the D allele of the hypoxemic group was significantly higher than that of the non-hypoxemic group (9 of 44 alleles versus 20 of 44 alleles; χ^2 value = 6.22, $df = 1$, $p = 0.013$).

Logistic regression analysis on progression of SARS

In a univariate logistic regression analysis, the number of ACE D allele in SARS cases was a significant risk factor for hypoxemia of SARS cases (odds ratio 3.04; 95% CI 1.15–8.02; $p = 0.025$) (Table 6). Even when age, gender, and contact status were added to the ACE allele to carry out multivariate logistic regression analysis, contribution of the D allele as a risk factor for hypoxemia was still robust (Table 6). Age did not contribute to this parameter. However, the mean age of 5 hypoxemic cases that received mechanical ventilation was 55.6 and obviously higher than 36.6 of hypoxemic cases that did not require mechanical ventilation (Table 2). Comparison of parameters between survivors and non-survivors was not possible, because no DNA samples were available from non-survivors.

Discussion

In Vietnam, 87% of SARS cases were medical staff members, in-patients with another disease or visitors to the hospital in question [1]. Although a standard

Table 4
Genotypic and allele frequencies of ACE I/D polymorphisms in SARS cases and controls

	SARS cases (n = 44)	Controls		References			
		With contact (n = 103)	Without contact (n = 50)	Japanese (n = 341) [21]	Chinese (n = 102) [22]	Korean (n = 135) [23]	British (n = 1903) [13]
<i>Genotype frequency</i>							
DD	6 (13.6%)	12 (11.7%)	3 (6.0%)	13.1%	16.7%	24.4%	26.1%
DI	17 (38.6%)	44 (42.7%)	25 (50.0%)	40.5%	47.0%	43.0%	49.8%
II	21 (47.8%)	47 (45.6%)	22 (44.0%)	46.3%	36.3%	32.6%	24.1%
<i>Allele frequency</i>							
D	0.33	0.33	0.31	0.33	0.4	0.46	0.51
I	0.67	0.67	0.69	0.67	0.6	0.54	0.49

Table 5
Genotypic and allele frequencies of ACE I/D polymorphism in the subgroups of SARS cases

	SARS cases (n = 44)	
	Non-hypoxemic group (n = 22)	Hypoxemic group (n = 22)
<i>Genotype frequency</i>		
DD	1 (4.5%)	5 (22.7%)
DI	7 (31.8%)	10 (45.5%)
II	14 (63.6%)	7 (31.8%)
<i>Allele frequency</i>		
D	0.2	0.45
I	0.8	0.55

Table 6
Logistic regression analysis on hypoxemia of SARS

Variable	Odds ratio (95% CI)	p value
<i>Univariate analysis</i>		
ACE (No. of D allele)	3.04 (1.15–8.02)	0.025
<i>Multivariate analysis</i>		
ACE (No. of D allele)	3.04 (1.12–8.25)	0.029
Age (>60 years)	2.12 (0.19–24.1)	0.546
Female	1.07 (0.25–4.52)	0.928
Direct contact	0.53 (0.09–3.12)	0.484

precaution was taken, this nosocomial infection was neither modified by isolation of patients nor protected by measures such as wearing N95 masks against the new pathogen, because the cause of this atypical pneumonia was unknown in the early phase of the outbreak [24]. All the infection spread originally from one case from Hong Kong within 2 months. It implies that natural course of nosocomial infection of SARS was observed in Vietnam. This was an unfortunate event but was a major advantage for us to assess genetic risk factors, minimizing other confounding factors. Moreover, the study population was limited to be Vietnamese ethnicity for genetic analysis. Questionnaires taken by trained interviewers and chest radiographs of all cases in this study, and clinical records of most of the cases were available to evaluate clinical status in detail. As a result, 44 out of 54 accessible cases of ethnic of Vietnamese were enrolled and this achieved high coverage

of this study. Although the number of cases is not enough to reach a definite conclusion in a case-control design, we were able to extract an attractive candidate gene in this study.

Herein, 50% of the cases required supplementary oxygen. This was comparable with a report by others [5]. Oxygen was given on the basis of local guidelines, when hypoxemia was detected at room air. There was a close correlation between the number of involved zones of chest radiography and status of supplementary oxygen; therefore, we considered that progression of SARS in the lung is reasonably evaluated by the requirement of supplementary oxygen.

In the SARS cases, the frequency of D allele in hypoxemic group was higher than that of non-hypoxemic group with statistical significance, despite the small sample size. This indicates that ACE gene could be one of the candidates which influence the progression of SARS, although another gene that is located close to the gene may be responsible for the disease. The frequency of D allele of SARS cases was not different from that of the healthy contacts, and the D allele did not appear to influence development of SARS itself. There was also no difference in the frequency of D allele between healthy contacts and non-contacts. In other words, contact history did not affect the genotype distribution.

Most severe cases of SARS show diffuse alveolar damage. This pathological status is characterized by increased permeability of alveolar–capillary barrier, which consists of two separate barriers, microvascular endothelium and alveolar epithelium [25]. When these barriers are damaged, the permeability of alveolar–capillary barrier increases and protein-rich edema fluids flow into alveolar spaces from blood vessel [25]. A previous report indicated that AT-I increased vascular permeability in Rat [26]. Another report showed that AT-II induces apoptosis of human alveolar epithelial cells [27]. The degree of epithelial injury seems to influence the outcome of ARDS [25]. The serum ACE levels of individuals who have genotype DD are almost twice as high as that of genotype II [16]. It is conceivable that D allele might influence the activity of renin–angiotensin system via ele-

vation of serum or local ACE level, and then this may lead to damage of endothelium or epithelium of the lung.

In this study, neither higher age nor underlying disease was found to be risk factors for hypoxemia in SARS. One of the reasons might be that SARS cases in Vietnam were mostly nosocomial infection among relatively young medical staff members who did not have underlying diseases. This situation may be different from ones in other countries. However, as described above, the number of cases that had specific underlying diseases, which may influence the prognosis of SARS, was too few to conclude the role of underlying disease as a risk factor in our cases.

Although no case of SARS had cardiovascular disorders or diabetes mellitus in Vietnam, it is interesting that heart disease and diabetes mellitus have been reported to be risk factors in the prognosis of SARS cases in other countries where incidence rates of these life-style related diseases are rather high [9,10]. The ACE insertion/deletion polymorphism has also been reported to be a risk factor of the diseases mentioned above [17] and this might be associated with systemic angiopathy and influence progression of SARS in the lung.

The reason why we used hypoxia as a parameter of disease progression and did not use other parameters such as requirement of mechanical ventilation or outcome of death was that there were only 5 cases which required mechanical ventilation in our samples tested and no samples were obtained from the fatal cases. Autopsies were not performed in Vietnamese SARS patients for religious reasons. It would promote further studies to clarify genetic predisposition of infection, development or progression of SARS in the near future in other countries where a larger number of cases were reported.

In conclusion, we showed that ACE insertion/deletion polymorphism was statistically associated with hypoxemic status in SARS cases of Vietnam. Genetic predisposition may be one of the risk factors for the progression of SARS.

Acknowledgments

The authors thank Dr. Nguyen Le Hang, Ms. Pham Thi Phuong Thuy, and Ms. Nguyen Thi Thu Ha for their help in management and coordination of this study in Vietnam and Drs. Shuzo Kanagawa and Yukihiko Ishizaka in Japan. The authors also thank Ms. Kazuko Tanabe for her critical reading of the manuscript. This work was supported by Special Coordination Funds for Promoting Science and Technology and by a grant for International Health Cooperation Research (14C-9) from the Ministry of Health, Labour and Welfare in 2003.

References

- [1] World Health Organization, Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). Available from: <<http://www.who.int/csr/sars/en/WHOconsensus.pdf>> (accessed 27 May 2004).
- [2] J.S. Peiris, K.Y. Yuen, A.D. Osterhaus, K. Stohr, The severe acute respiratory syndrome, *N. Engl. J. Med.* 349 (2003) 2431–2441.
- [3] T.H. Vu, J.F. Cabau, N.T. Nguyen, M. Lenoir, SARS Northern Vietnam, *N. Engl. J. Med.* 348 (2003) 2035.
- [4] H.T. Vu, K.C. Leitmeyer, D.H. Le, M.J. Miller, Q.H. Nguyen, T.M. Uyeki, M.G. Reynolds, J. Aagesen, K.G. Nicholson, Q.H. Vu, H.A. Bach, A.J. Plan, Clinical description of a completed outbreak of SARS in Vietnam, February–May 2003, *Emerg. Infect. Dis.* 10 (2004) 334–338.
- [5] J.S. Peiris, C.M. Chu, V.C. Cheng, K.S. Chan, I.F. Hung, L.L. Poon, K.I. Law, B.S. Tang, T.Y. Hon, C.S. Chan, K.H. Chan, J.S. Ng, B.J. Zheng, W.L. Ng, R.W. Lai, Y. Guan, K.Y. Yuen, HKU/UCH SARS study group, clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study, *Lancet* 361 (2003) 1767–1772.
- [6] J.M. Nicholls, L.L. Poon, K.C. Lee, W.F. Ng, S.T. Lai, C.Y. Leung, C.M. Chu, P.K. Hui, K.L. Mak, W. Lim, K.W. Yan, K.H. Chan, N.C. Tsang, Y. Guan, K.Y. Yuen, J.S. Peiris, Lung pathology of fatal severe acute respiratory syndrome, *Lancet* 361 (2003) 1773–1778.
- [7] T.J. Franks, P.Y. Chong, P. Chui, J.R. Galvin, R.M. Lourens, A.H. Reid, E. Selbs, C.P. McEvoy, C.D. Hayden, J. Fukuoka, J.K. Taubenberger, W.D. Travis, Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore, *Hum. Pathol.* 34 (2003) 743–748.
- [8] G.M. Tse, K.F. To, P.K. Chan, A.W. Lo, K.C. Ng, A. Wu, N. Lee, H.C. Wong, S.M. Mak, K.F. Chan, D.S. Hui, J.J. Sung, H.K. Ng, Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS), *J. Clin. Pathol.* 57 (2004) 260–265.
- [9] C.M. Booth, L.M. Matukas, G.A. Tomlinson, A.R. Rachlis, D.B. Rose, H.A. Dwosh, S.L. Walmsley, T. Mazzulli, M. Avendano, P. Derkach, I.E. Epthimos, I. Kitai, B.D. Mederski, S.B. Shadowitz, W.L. Gold, L.A. Hawryluck, E. Rea, J.S. Chenkin, D.W. Cescon, S.M. Poutanen, A.S. Detsky, Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area, *JAMA* 289 (2003) 2801–2809.
- [10] J.W. Chan, C.K. Ng, Y.H. Chan, T.Y. Mok, S. Lee, S.Y. Chu, W.L. Law, M.P. Lee, P.C. Li, Short term outcome and risk factors for adverse clinical outcomes in adults with severe acute respiratory syndrome (SARS), *Thorax* 58 (2003) 686–689.
- [11] M. Lin, H.K. Tseng, J.A. Trejaut, H.L. Lee, J.H. Loo, C.C. Chu, P.J. Chen, Y.W. Su, K.H. Lim, Z.U. Tsai, R.Y. Lin, R.S. Lin, C.H. Huang, Association of HLA class I with severe acute respiratory syndrome coronavirus infection, *BMC Med. Genet.* 4 (2003) 9.
- [12] M.H. Ng, K.M. Lau, L. Li, S.H. Cheng, W.Y. Chan, P.K. Hui, B. Zee, C.B. Leung, J.J. Sung, Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome, *J. Infect. Dis.* 190 (2004) 515–518.
- [13] R.P. Marshall, S. Webb, G.J. Bellingan, H.E. Montgomery, B. Chaudhari, R.J. McAnulty, S.E. Humphries, M.R. Hill, G.J. Laurent, Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome, *Am. J. Respir. Crit. Care Med.* 166 (2002) 646–650.
- [14] Z. Lin, C. Pearson, V. Chinchilli, S.M. Pietschmann, J. Luo, U. Pison, J. Floros, Polymorphisms of human SP-A, SP-B, and SP-D

- genes: association of SP-B Thr131Ile with ARDS, *Clin. Genet.* 58 (2000) 181–191.
- [15] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, *Nature* 426 (2003) 450–454.
- [16] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, F. Soubrier, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, *J. Clin. Invest.* 86 (1990) 1343–1346.
- [17] B. Baudin, New aspects on angiotensin-converting enzyme: from gene to disease, *Clin. Chem. Lab. Med.* 40 (2002) 256–265.
- [18] World Health Organization, Global surveillance for severe acute respiratory syndrome (SARS), *Wkly. Epidemiol. Rec.* 78 (2003) 100–119.
- [19] L. Wang, K. Hirayasu, M. Ishizawa, Y. Kobayashi, Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS, *Nucleic Acids Res.* 22 (1994) 1774–1775.
- [20] A.E. Evans, O. Poirier, F. Kee, L. Lecerf, E. McCrum, T. Falconer, J. Crane, D.F. O'Rourke, F. Cambien, Polymorphisms of the angiotensin-converting-enzyme gene in subjects who die from coronary heart disease, *Q. J. Med.* 87 (1994) 211–214.
- [21] K. Furuya, E. Yamaguchi, A. Itoh, N. Hizawa, N. Ohnuma, J. Kojima, N. Kodama, Y. Kawakami, Deletion polymorphism in the angiotensin I converting enzyme (ACE) gene as a genetic risk factor for sarcoidosis, *Thorax* 51 (1996) 777–780.
- [22] W. Huang, C. Xie, H. Zhou, T. Yang, M. Sun, Association of the angiotensin-converting enzyme gene polymorphism with chronic heart failure in Chinese Han patients, *Eur. J. Heart Fail.* 6 (2004) 23–27.
- [23] S.J. Hong, H.I. Yang, M.C. Yoo, C.S. In, S.V. Yim, S.Y. Jin, B.K. Choe, J.H. Chung, Angiotensin converting enzyme gene polymorphism in Korean patients with primary knee osteoarthritis, *Exp. Mol. Med.* 35 (2003) 189–195.
- [24] D.H. Le, S.A. Bloom, Q.H. Nguyen, S.A. Maloney, Q.M. Le, K.C. Leitmeyer, H.A. Bach, M.G. Reynolds, J.M. Montgomery, J.A. Comer, P.W. Horby, A.J. Plant, Lack of SARS transmission among public hospital workers, Vietnam, *Emerg. Infect. Dis.* 10 (2004) 265–268.
- [25] L.B. Ware, M.A. Matthay, The acute respiratory distress syndrome, *N. Engl. J. Med.* 342 (2000) 1334–1349.
- [26] Z.H. Xu, K. Shimakura, T. Yamamoto, L.M. Wang, S. Mine-shita, Pulmonary edema induced by angiotensin I in rats, *Jpn. J. Pharmacol.* 76 (1998) 51–56.
- [27] R. Wang, C. Ramos, I. Joshi, A. Zagariya, A. Pardo, M. Selman, B.D. Uhal, Human lung myofibroblast-derived inducers of alveolar epithelial apoptosis identified as angiotensin peptides, *Am. J. Physiol.* 277 (1999) L1158–L1164.