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ACE1 polymorphism and progression of SARS

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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.

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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

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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 metallopeptidase 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 (AmpliQ Gold DNA polymerase, Applied Biosystems).

Nucleotide sequences of primers were as follows: ACE1: 5'-CATC CTTTCTCCCATTTC-3', ACE2: 5'-TGGGATTACAGCGTGA TACAG-3', and ACE3: 5'-ATTTCAGAGCTGGAATAAAATT-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 (<i>n</i> = 44)	Contacts (<i>n</i> = 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 (<i>n</i> = 44)			
	Non-hypoxemic group		Hypoxemic group	
	(<i>n</i> = 22)	(<i>n</i> = 22)	Without mechanical ventilation (<i>n</i> = 17)	With mechanical ventilation (<i>n</i> = 5)
Age (year), mean	37.7	41.0	36.8	55.4
Male/female (<i>n</i>)	6/16	7/15	5/12	2/3
Direct/indirect contact (<i>n</i>)	19/3	18/4	16/1	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 (<i>n</i> = 22)	Hypoxemic group (<i>n</i> = 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	Controls		References			
	(n = 44)	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.

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