Identification of an Alternative 5′-Untranslated Exon and New Polymorphisms of Angiotensin-Converting Enzyme 2 Gene: Lack of Association With SARS in the Vietnamese Population

Satoru Itoyama,1 Naoto Keicho,1* Minako Hijikata,1 Tran Quy,2 Nguyen Chi Phi,3 Hoang Thuy Long,3 Le Dang Ha,7 Vo Van Ban,5 Ikumi Matsushita,1 Hideki Yanai,6 Fumiko Kirikae,7 Teruo Kirikae,7 Tadatoshi Kuratsuji,8 and Takehiko Sasazuki9

1Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, Tokyo, Japan
2Bach Mai Hospital, Hanoi, Vietnam
3National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
4Institute for Clinical Research in Tropical Medicine, Vietnam
5Hanoi-French Hospital, Vietnam
6The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan
7Department of Infectious Diseases, Research Institute, International Medical Center of Japan, Japan
8Research Institute, International Medical Center of Japan, Japan
9International Medical Center of Japan, Japan

We analyzed genetic variations of angiotensin-converting enzyme 2 (ACE2), considering that it might influence patients’ susceptibility to severe acute respiratory syndrome-associated coronavirus (SARS-CoV) or development of SARS as a functional receptor. By cloning of the full-length cDNA of the ACE2 gene in the lung, where replication occurs on SARS-CoV, it was shown that there are different splicing sites. All exons including the new alternative exon, exon-intron boundaries, and the corresponding 5′-flanking region of the gene were investigated and 19 single nucleotide polymorphisms (SNPs) were found. Out of these, 13 SNPs including one non-synonymous substitution and three 3′-UTR polymorphisms were newly identified. A case control study involving 44 SARS cases, 16 anti-SARS-CoV antibody-positive contacts, 87 antibody-negative contacts, and 50 non-contacts in Vietnam, failed to obtain any evidence that the ACE2 gene polymorphisms are involved in the disease process in the population. Nevertheless, identification of new 5′-untranslated exon and new SNPs is considered helpful in investigating regulation of ACE2 gene expression in the future.

KEY WORDS: angiotensin-converting enzyme 2 (ACE2); severe acute respiratory syndrome (SARS); SARS-associated coronavirus (SARS-CoV); virus receptor; polymorphism; association study

INTRODUCTION

Severe acute respiratory syndrome (SARS) is an emerging infectious disease characterized by systemic inflammation followed by atypical pneumonia [Peiris et al., 2003b]. Shortly after the initial worldwide outbreak in 2003, SARS-associated coronavirus (SARS-CoV) was discovered as a putative agent of SARS [Drosten et al., 2003; Ksiazek et al., 2003; Kikum et al., 2003; Peiris et al., 2003a], and then angiotensin-converting enzyme 2 (ACE2) was identified as a functional receptor of this newly arrived virus [Li et al., 2003]. More recently, CD209L was reported as being another alternative receptor for the virus, but it appears to be a less efficient entry site than ACE2 [Jefferies et al., 2004].

Virus receptors generally play a key role in the entry of the pathogen into the host cells and may influence development or progression of viral diseases. For example, it is well known that genetic polymorphism of chemokine receptor 5 (CCR5), a co-receptor for human immunodeficiency virus-1 (HIV-1), influences the natural history of HIV-1 infection. The mutant allele CCR5-A32 does not produce a functional protein and has been shown to protect host cells against HIV-1 infection, and progression into acquired immunodeficiency syndrome is delayed after seroconversion takes place [Dean et al., 1996; Liu et al., 1996; Samson et al., 1996]. By analogy with the above, we considered that genetic polymorphisms of ACE2 could influence SARS-CoV infection or clinical manifestations of SARS.

ACE2 is a homologue of ACE1 and exhibits 40% identity of amino acid sequence to its N- and C-terminal domains [Tipnis et al., 2000]. Similar to ACE1, ACE2 is a metalloprotease that constitutes a renin-angiotensin system. Human full-length ACE2 cDNAs have been cloned already from lymphoma (GenBank accession No. AF241254) [Tipnis et al., 2000], cardiac left ventricle (AF291820) [Donoghue et al., 2000], and testis (AY623811) [Douglas et al., 2004]. Based on published data, it has been said that the ACE2 gene (ACE2) contains 18 exons, and spans approximately 40 kb of genomic DNA on the human X-chromosome. Although ACE2 mRNA expressions were demonstrated in the lung by the method of quantitative reverse transcription-PCR (RT/PCR) [Harmer et al., 2002] and its protein expression was obviously shown by immunohistochemistry [Hamming et al., 2004], full-length ACE2 cDNA has not been cloned from the lung so far. This is considered to be
very likely as being an important replication site of SARS-CoV [Haagmans et al., 2004].

In the present study, we attempted a full-length cloning of ACE2 cDNA from the human lung and found a new alternative, the 5'- untranslated exon. During this process, an extended region of the original exon 1 was identified in the testis' RNAs. Then, we explored genetic polymorphisms within 19 exons including new regions and the 5'-flanking region of ACE2 and tried to determine whether the polymorphisms of ACE2 are associated with SARS in Vietnamese.

MATERIALS AND METHODS

Cloning of ACE2 cDNA From the Lung

Cloning was performed by combination of RT/PCR and 5'- and 3'- rapid amplification of cDNA ends (RACE) procedures, using human lung total RNA (Stratagene, La Jolla, CA) and human testis total RNA (Stratagene) as a control. The total RNAs were reverse-transcribed using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) with oligo(dT)12-18, and then cDNA was amplified using Platinum Taq DNA Polymerase High Fidelity (Invitrogen) with primers ACE2-exon15'-CAA AGC CAG ATG AAC GAG AA-3' and ACE2-exon 18 as (5'-GAA CAG AAG TCA AAT CCA GA-3') to amplify the transcript of 2721 bp encompassing the original 18 exons of ACE2 gene on database.

The First Choice RLM-RACE Kit (Ambion, Austin, TX) was used for 5'- and 3'- RACE procedures following the manufacturer's recommendation. Gene-specific primer sets for 5'-RACE were ACE2-5'Outer1 and ACE2-5'Inner1 (5'-GTG GAT ACA TTT GGG CAA GT-3') and 5'-CTC CTG ATC TCT CTC TGT AGC AC-3'). Gene specific primer set for 3'-RACE was ACE2-3'Outer and ACE2-3'Inner (5'-CAA TGA TGC TTT CCG TCT GA-3' and 5'-ACA CTT GGA CCT CCT AAC CA-3'). Nucleotide sequences of PCR products were directly determined by the automated DNA sequencer (PRISM 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA).

To investigate expression of the exons on the 5'-side, RT/PCR procedures were performed on the total RNAs of human lung, testis, trachea (Stratagene), primary-cultured bronchial epithelial cells [Lechner and LaVeck, 1985], small intestine (Ambion), and on the human major organ cDNAs (Bio Chain Diagnostics Pte. Ltd., Singapore Science Park, Singapore) in blood samples were tested with SARS ELISA (Genelabs Diagnostics Pte. Ltd., Singapore Science Park, Singapore) in accordance with the manufacturer's recommendation [Guan et al., 2004].

Identification of Polymorphisms Within ACE2 Gene

Of the 44 SARS cases and 103 contacts recruited, a half of the samples were randomly selected for searching polymorphisms within the ACE2 gene. PCR primers were designed to amplify 19 exons including the new alternative exon, exon-intron boundaries and approximately 1.000 bp of the 5'-flanking region of the new exon, reaching 2.000 bp upstream of the 5'-end of the original exon 1 (Table 1). Genomic DNA of each sample was subjected to PCR amplification followed by direct sequencing.

Genotyping of Identified Polymorphisms

Non-synonymous nucleotide substitutions and other variations with a minor allele frequency higher than 0.05 were subjected to genotyping in all SARS cases, contacts and non-contacts. Consequently, one novel non-synonymous substitution, two possible non-synonymous polymorphisms in the database (dbSNP identification nos. rs4646116 and rs11798104), and variations of 3'-UTR in exon 18 (position 39844) and of intron 3 (rs2285666, position 8789) were genotyped by the combination of direct sequencing method and single-strand conformation polymorphism (SSCP) analysis or PCR-based restriction fragment length polymorphism (RFLP) analysis.

Statistical Analysis

Disease associations were assessed by the chi-square test. The 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, Inc., Cary, NC).

RESULTS

Full-Length ACE2 cDNAs From the Lung and Expression of the Transcripts

By the use of the RT/PCR encompassing all known exons of ACE2 and 3'-RACE method, we could amplify ACE2 cDNA as PCR fragments completely corresponding to the published sequence of ACE2 cDNA (AF241254). The 5'-RACE procedure on the total RNA of the lung demonstrated the presence of a new alternative exon (registered as AB193259), which consisted of a segment between position –1141 and –942 and was connected to the 5'-end of the original exon 1. The 5'-end of transcripts was extended to position –1141 repeatedly by both sets of gene-specific primers. In addition, novel 65 nucleotides on the 5'-side (registered as AB193260), extending the 5'-end of the original exon 1 upstream, were amplified from the total RNA of testis. A schematic diagram of the exon-intron structure is shown in Figure 1.

RT-PCR revealed that the expression of the new alternative exon could be seen not only in the lung but also in the testis, trachea, bronchial epithelial cells, small intestine, and various major organs (data not shown). The new extended region was expressed not only in the testis but also in other organs including bronchial epithelial cells and the small intestine (data not shown).
Basic characteristics and sub-grouping of subjects are shown in Table II. The 44 SARS cases, 103 contacts, and 50 non-contacts were analyzed in the present study. Based on anti-SARS-CoV antibody titer in serum, the contacts were further divided into two subgroups, antibody-positive contacts, and antibody-negative contacts (data not shown).

**Identification of Polymorphisms Within ACE2 Gene**

All exons including the new exon, exon-intron boundaries and the corresponding 5'-flanking region of ACE2 were tested

### TABLE I. Primers Used to Identify Polymorphisms Within the ACE2 Gene

| Region                | Primer name      | Primer sequence (5'-3') | Product size |
|-----------------------|------------------|-------------------------|--------------|
| 5' flanking region    | ACE2-pro-1-sense | TAA TTC AGT CAG TGC TGG C | 676 bp       |
| 5' flanking region    | ACE2-pro-1-anti  | AAT AGT GGA GGC ATA GAT AAA | 618 bp       |
| New alternate exon    | ACE2-new-sense   | TTA TTT CCA TGG CTC TTC ACT CCA | 470 bp       |
| 5' flanking region    | ACE2-new-anti    | TTA TGG CTA CTC TCC ACT CCA | 669 bp       |
| 5' flanking region    | ACE2-pro-4-sense | TAG AAC TAG GGA TCA TGA AGA | 653 bp       |
| Exon 1                | ACE2-ex1-sense   | ATC TTT TAA AGC TTG TTA CCA | 644 bp       |
| Exon 2                | ACE2-ex2-sense   | AAC ATC CAA TCT CAC AAT TC | 636 bp       |
| Exon 3                | ACE2-ex3-sense   | ACA TCA GGT CAT AAA GTG GAT | 627 bp       |
| Exon 4                | ACE2-ex4-sense   | TTC TCT TTG TTC CCC AGT A | 521 bp       |
| Exon 5                | ACE2-ex5-sense   | CTT GTA TGG TTC TTG TGC TT | 535 bp       |
| Exon 6                | ACE2-ex6-sense   | ACC TGT GTT CTC CCA AGT A | 568 bp       |
| Exon 7                | ACE2-ex7-sense   | TCA CCA AGT TAA GTA CAC GAA | 562 bp       |
| Exon 8                | ACE2-ex8-sense   | TTT GTG AGC TGC TTT ATT TT | 618 bp       |
| Exon 9                | ACE2-ex9-sense   | TTC TCT TTG TTC CCC AGT A | 521 bp       |
| Exon 10               | ACE2-ex10-sense  | CCT CTG TCC TAT TCT CTA | 568 bp       |
| Exon 11               | ACE2-ex11-sense  | GCT GTG CAG TAG ATC TCA AA | 643 bp       |
| Exon 12               | ACE2-ex12-sense  | CAG ATT GTC CAC AGG TTA A | 577 bp       |
| Exon 13               | ACE2-ex13-sense  | TCA TGA GTA GTA ATT TCC AGT T | 636 bp       |
| Exon 14               | ACE2-ex14-sense  | GGA GAG GAA ACT CAC AGT A | 587 bp       |
| Exon 15               | ACE2-ex15-sense  | GAT ATC CAA ATG GAC ACT AAA | 615 bp       |
| Exon 16               | ACE2-ex16-sense  | GTG CAC ACC TAT AAA CCA AG | 615 bp       |
| Exon 17               | ACE2-ex17-sense  | TGA CCA TGT TTA GGG TAG AC | 612 bp       |
| Exon 18               | ACE2-ex18-sense  | GAG AGG GCT GTC GAT GAT A | 637 bp       |
| Exon 19               | ACE2-ex19-sense  | CAG AAC AAA TAG TGC TGG CAA A | 610 bp       |
| Exon 20               | ACE2-ex20-sense  | CAT AGT GGT AAC TTG CTT GAT | 633 bp       |
| Exon 21               | ACE2-ex21-sense  | GCT CTG TCA CCT AGG TCA A | 633 bp       |
| Exon 22               | ACE2-ex22-sense  | CTA GGA AGA TGA ACT GCT GAT | 655 bp       |
| Exon 23               | ACE2-ex23-sense  | TTA AGA TGA ATC AGA TGG GAA | 623 bp       |
| Exon 24               | ACE2-ex24-sense  | AAC ACT GTC AGC AAA TAC AAA | 531 bp       |

**Fig. 1.** A schematic diagram of the ACE2 gene structure and the positions of SNPs. The known exons are depicted as open boxes. A solid box and a striped box indicate the new exon and the extended new region of the exon 1, respectively. The arrows represent locations of the SNPs analyzed in a case-control study. The broken line depicts an alternative-splicing site.
to identify variations of ACE2 among SARS cases and contacts. As shown in Table III, 19 single nucleotide polymorphisms (SNPs) were identified. Six of them have already registered on dbSNP database, and 13 SNPs including one non-synonymous substitution, from asparagine to serine at 638 (N638S) in the exon 15 (position 33205) and another in exon 18 (position 39844) were found to be considerably rare among both SARS cases and contacts tested. In subsequent analysis, we therefore chose polymorphisms, and analyzed possible non-synonymous substitution, excluding rare non-coding variants among SARS patients and contacts.

**Genotype and Allele Frequency of Three SNPs**

Two SNPs in intron 3 and exon 18 with minor allele frequencies higher than 0.05 and a newly identified non-synonymous SNP, N638S in exon 15 were analyzed in all samples (Table IV). Relative positions of these SNPs are shown in Figure 1. Genotyping results by direct sequencing method were confirmed by RFLP or SSCP methods. Because ACE2 is located to the X chromosome in humans, samples from both males and females were analyzed, respectively. Two possible non-synonymous SNPs that are shown in the dbSNP database (rs4646116 and rs11798104) were not found in our samples this time. When the antibody-negative contacts group was compared with antibody-positive group including SARS cases in either males or females, no difference was observed between the two groups both in regards to genotype and allele frequencies. Comparison between antibody-positive contacts and SARS cases, and comparison between contacts and non-contacts did not show any significant differences in genotype and allele frequencies of the tested polymorphisms.

**DISCUSSION**

During the worldwide outbreak of SARS in 2003, a subset (about 20%–30%) of SARS patients required mechanical ventilation, having developed pneumonia. The fatality rate was 11%, although the majority of patients recovered without unfavorable outcome [Peiris et al., 2003b]. As a natural consequence, asymptomatic individuals produce antibodies against SARS-CoV in their sera [Ip et al., 2004; Woo et al., 2004]. In one of the studies, it was shown that 2.3% of contacts who did not develop clinical SARS had serum antibody titer over the threshold [Ip et al., 2004], and this implies the presence of asymptomatic individuals.

We hypothesized that the functional polymorphism of ACE2, which is considered as being a virus receptor of SARS-CoV, might influence the clinical history of SARS-CoV infection at least in part. This is because, a variation of the co-receptor to HIV, CCR5-D32 where allele frequency is approximately 10% in the European population [Martinson et al., 1997], has been well known to resist HIV infection and alter its clinical course [Dean et al., 1996; Liu et al., 1996; Samson et al., 1996].

**TABLE II. Demographic Findings of Subjects and Subgroups**

| Groups | SARS cases (n = 44) | Contacts (n = 103) | Anti-SARS-CoV antibody | Non-contacts (n = 50) |
|--------|-------------------|-------------------|-----------------------|----------------------|
| Age (years), mean [range] | 39.3 [17–76] | 36.5 [15–68] | 36.0 [25–50] | 36.6 [15–68] |
| Male/female | 13/31 | 46/57 | 7/9 | 39/48 | 17/33 |
| aData not available. |

**TABLE III. SNPs Within the ACE2 Gene**

| Region | Positiona | dbSNP rs# cluster ID | Change of nucleotide (major/minor allele) | Change of amino acid (major/minor allele) | No. of individuals who had the minor allele |
|--------|-----------|---------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| SARS cases | Contacts |
| 5' flanking region | –751 | NEW | C/T | — | 1 | 1 |
| 5' flanking region | –671 | NEW | G/A | — | 1 | 1 |
| 5' flanking region | –634 | NEW | C/G | — | 1 | 0 |
| Intron 3 | 8789 | rs2285666 | A/G | — | 15 | 32 |
| Intron 6 | 13286 | rs4646140 | G/A | — | 0 | 1 |
| Intron 9 | 25082 | NEW | G/A | — | 0 | 1 |
| Intron 10 | 25424 | NEW | G/A | — | 0 | 1 |
| Intron 10 | 27418 | rs4646165 | G/A | — | 0 | 1 |
| Intron 12 | 23946 | rs2301693 | C/T | — | 0 | 2 |
| Intron 12 | 29018 | rs2301692 | A/G | — | 0 | 2 |
| Intron 14 | 30816 | NEW | A/G | — | 1 | 1 |
| Intron 14 | 30867 | rs4646174 | C/G | — | 0 | 2 |
| Intron 14 | 31212 | NEW | G/C | — | 1 | 0 |
| Exon 15 | 33205 | NEW | A/G | N/S | 0 | 1 |
| Exon 16 | 36655 | NEW | G/A | — | 0 | 1 |
| Exon 17 | 38926 | NEW | C/T | — | 0 | 1 |
| Exon 18 (3'-UTR) | 39663 | NEW | C/G | — | 0 | 1 |
| Exon 18 (3'-UTR) | 39705 | NEW | A/G | — | 0 | 1 |
| Exon 18 (3'-UTR) | 39844 | NEW | G/A | — | 3 | 4 |

aPosition numbers indicate distance from 5' end of the original exon 1.
bNewly identified SNPs are shown as NEW.
cMinor allele frequencies of the SNPs shown in bold and italic were higher than 0.05.
Using the PCR-based cloning procedure, we identified for the first time an alternative exon upstream of the original exon 1 of ACE2 that is expressed in various organs, including the lung and trachea, primary-cultured bronchial epithelial cells, and the small intestine. These are considered to be important replication sites of SARS-CoV [Haagmans et al., 2004]. Both 5′- and 3′-ends of the intron between the new alternative exon and the original exon 1 followed the GT/AG rule of Breathnach and Chambon [1981]. Although the organ specificity of the transcripts was not confirmed in this study due to the limitation of non-quantitative PCR amplification, implication of the new alternative exon was definitely shown in the lung and small intestine. Also, neither the new alternative exon nor the new extended region of the original exon 1 gave rise to a new coding region and they were considered as 5′-untranslated region.

It was recently reported that genetic variations of ACE2 did not affect SARS susceptibility or outcome in Hong Kong [Chiu et al., 2004]. In that study, five intronic SNPs (rs2106809, rs2285666, rs4646142, rs714205, and rs2074192) were chosen and analyzed in a case-control manner, based on the previously known exon-intron structure and SNPs already registered in the database. By contrast, we attempted to analyze not only previously known SNPs but also variations newly identified among actual SARS patients and contacts. Based on the information from the exon-intron structure of ACE2 cloned by ourselves, we searched for nucleotide sequences in all the exons including the new alternative exon and the corresponding 5′-flanking region, which are thought to contain promoters of the new exon and the original exon 1. We found one novel non-synonymous substitution N638S and 18 non-coding SNPs including two relatively common SNPs with minor allele frequency higher than 5%. We selected these SNPs and analyzed them furthermore in a case-control manner, because, while they are rare occurrence, non-synonymous substitution may directly modulate the function of the protein, and because relatively common SNPs can often be used as markers to ascertain a causative variation. Of 19 SNPs found in this study, 13 were new polymorphisms, 3 of which were located in 3′-UTR. Two possible non-synonymous SNPs in dbSNP database were not found in the population tested. Judging from the results so far obtained in this case-control study, there was no statistical evidence that ACE2 polymorphisms affect SARS infection or alter its clinical course. However, type II error was not negligible because of a relatively small size of samples tested.

Taking also into consideration, the results from a previous study of ACE2 polymorphisms by others [Chiu et al., 2004], it is unlikely that the genetic defect of ACE2 is involved in the disease resistance that has been shown in CCR5-Δ32 in HIV-1 infection cases. Nevertheless, this newly identified alternative 5′-untranslated exon expressed in the lung, and also newly recognized polymorphisms in this study might be of great help concerning investigations into the regulation of ACE2 gene expression and the possible significance of the variations in further more in-depth studies.

**ACKNOWLEDGMENTS**

The authors thank Dr. Nguyen Le Hang, Pham Thi Phuong Thuy, and Nguyen Thi Thu Ha for their help in the management and coordination of this study in Vietnam and Dr. Shuzo

---

**TABLE IV. Genotype and Allele Distribution of Three Single Nucleotide Polymorphisms (SNPs)**

|                      | Contacts | SARS cases | Antibody (+) | Antibody (−) | Non-contacts |
|----------------------|----------|------------|--------------|--------------|--------------|
| **Intron 3 (rs2285666)** |          |            |              |              |              |
| Male Genotype/allele no. (frequency) |          |              |              |              |              |
| A 5 (0.38) | 4 (0.57) | 21 (0.54) | 5 (0.31) |
| G 8 (0.62) | 3 (0.43) | 18 (0.46) | 11 (0.69) |
| Total no. | 13 | 7 | 39 | 16 |
| Female Genotype no. (frequency) |          |              |              |              |              |
| A/A 12 (0.39) | 4 (0.44) | 15 (0.31) | 11 (0.33) |
| A/G 16 (0.51) | 3 (0.33) | 24 (0.50) | 17 (0.52) |
| G/G 3 (0.10) | 2 (0.22) | 9 (0.19) | 5 (0.15) |
| Total no. | 31 | 9 | 48 | 33 |
| Allele no. (frequency) |          |              |              |              |              |
| A 40 (0.65) | 11 (0.61) | 54 (0.56) | 39 (0.59) |
| G 22 (0.35) | 7 (0.39) | 42 (0.44) | 27 (0.41) |

| **Exon 15 (N638S)** |          |            |              |              |              |
| Male Genotype/allele no. (frequency) |          |              |              |              |              |
| A 13 (1.00) | 7 (1.00) | 39 (1.00) | 17 (1.00) |
| G 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Total no. | 13 | 7 | 39 | 17 |
| Female Genotype no. (frequency) |          |              |              |              |              |
| A/A 31 (1.00) | 8 (0.89) | 47 (0.88) | 33 (1.00) |
| A/G 0 (0.00) | 1 (0.11) | 2 (0.04) | 0 (0.00) |
| G/G 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Total no. | 31 | 9 | 48 | 33 |
| Allele no. (frequency) |          |              |              |              |              |
| A 62 (1.00) | 17 (0.94) | 95 (0.99) | 66 (1.00) |
| G 0 (0.00) | 1 (0.06) | 1 (0.01) | 0 (0.00) |

| **Exon 18 (3′-UTR)** |          |            |              |              |              |
| Male Genotype/allele no. (frequency) |          |              |              |              |              |
| A 12 (0.92) | 7 (1.00) | 37 (0.95) | 17 (1.00) |
| A 1 (0.08) | 0 (0.00) | 2 (0.05) | 0 (0.00) |
| Total no. | 13 | 7 | 39 | 17 |
| Female Genotype no. (frequency) |          |              |              |              |              |
| G/G 27 (0.87) | 8 (0.89) | 46 (0.96) | 29 (0.88) |
| A/G 4 (0.13) | 1 (0.11) | 2 (0.04) | 4 (0.12) |
| A/A 0 (0.00) | 0 (0.00) | 4 (0.08) | 0 (0.00) |
| Total no. | 31 | 9 | 48 | 33 |
| Allele no. (frequency) |          |              |              |              |              |
| G 58 (0.94) | 17 (0.94) | 94 (0.98) | 62 (0.94) |
| A 4 (0.06) | 1 (0.06) | 2 (0.02) | 4 (0.06) |

*Genotype distribution is the same as allele distribution in male.*
REFERENCES

Breatnach R, Chambon P. 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu Rev Biochem 50:349–383.

Chiu RW, Tang NL, Hui DS, Chim SS, Chan KC, Sung YM, Chan LY, Tong YK, Lee WS, Chan PK, Lo YM. 2004. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. Clin Chem 50:1683–1686.

Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Donfeld SF, Vlahov D, Kaslow R, Saah A, Rinaldo C, Detels R, O’Brien SJ. 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CRK5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science 273:1856–1862.

Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breatnach RE, Acton S. 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 87:E1–E9.

Douglas GC, O’Byran MP, Hedger MR, Lee DK, Vassart G, Smith MW. 2004. The novel angiotensin-converting enzyme (ACE) homolog, ACE2, is selectively expressed by adult Leydig cells of the testis. Endocrinology 145:4703–4711.

Drosten C, Gunther S, Preiser W, van der Werf S, Böttiger M, Becker S, Burguiere AM, Cinatl J, Eickmann M, Escriot N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klentz HD, Osterhaus AD. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1953–1966.

Guo M, Chen HY, Foy SY, Tan YJ, Goh PY, Wee SH. 2004. Recombinant protein-based enzyme-linked immunosorbent assay and immunochromatographic tests for detection of immunoglobulin G antibodies to severe acute respiratory syndrome (SARS) coronavirus in SARS patients. Clin Diag Lab Immunol 11:287–291.

Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Borms J, Driessen CA, van Amerongen G, van Riel D, de Jong T, Imaura S, Chan KH, Tashiro M, Osterhaus AD. 2004. Pegylated interferon-α protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat Med 10:290–293.

Hamming I, Timens W, Buithuis ML, Lely AT, Navis GJ, van Geer H. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203:631–637.

Harmer D, Gilbert M, Borman R, Clark KL. 2002. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 527:107–110.

Ip M, Chan PK, Lee N, Wu A, Ng TK, Chan L, Ng A, Kwan HM, Tsang L, Chu I, Cheung JL, Sung JJ, Tam JS. 2004. Seroprevalence of antibody to severe acute respiratory syndrome (SARS)-associated coronavirus among health care workers in SARS and non-SARS medical wards. Clin Infect Dis 38:e116–118.

Jeffers SA, Tsuelli BM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, Thomas WD Jr, Thackray LB, Young MD, Masen BJ, Ambrosino DM, Wentworth DE, Demartini JC, Holmes KV. 2004. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci USA 101:15748–15753.

Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery T, Seng S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SP, Ling AE, Humphrey CD, Shi JY, Guarnier P, Paddock CD, Rota PA, Fields BS, Delisi J, Yang YJ, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LF, SARS Working Group. 2003. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348:1953–1966.

Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G, Lim W, Ling AE, Chan PK, Tam JS, Zambon MC, Goyal R, Drosten C, van der Werf S, Escriot N, Manuguerra JC, Stoehr R, Peiris JS, Osterhaus AD. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 362:263–270.

Lechner JF, LaVeek MA. 1985. A serum-free method for culturing human bronchial epithelial cells at clonal density. J Tissue Cult Methods 9:43–48.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Parren P. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426:450–454.

Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR. 1996. Homozygous defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86:377–387.

Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. 1997. Global distribution of the CCR5 gene 32-basepair deletion. Nat Genet 16:100–103.

Peiris JS, Lai ST, Poon LL, Guan Y, Lam LY, Lim W, Nicholls J, Yue WK, Yan WW, Cheung MT, Cheng WC, Chan KH, Tsang DN, Yung RW, Ng TK, Yuen KY. 2003a. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 363:1319–1325.

Peiris JS, Yuen KY, Osterhaus AD, Stoehr K. 2003b. The severe acute respiratory syndrome. N Engl J Med 349:2431–2441.

Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Forceille C, Muyldermans S, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. 1996. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR5 gene. Nature 382:722–725.

Tospnas SV, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. 2000. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captoril-insensitive carboxypeptidase. J Biol Chem 275:33238–33243.

Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. 1994. Purification of genomic DNA from human whole blood by isopropanol-fractonation with concentrated NaI and SDS. Nucleic Acids Res 22:1774–1775.

WHO. 2003. Global surveillance for severe acute respiratory syndrome (SARS). Wkly Epidemiol Rec 78:100–119.

Woo PC, Lau SK, Tsio HW, Chan KH, Wong BH, Che HY, Tam VK, Tam SC, Cheng VC, Hung IF, Wong SS, Zheng BJ, Guan Y, Yuen KY. 2004. Relative rates of non-pneumatic SARS coronavirus infection and SARS coronavirus pneumonia. Lancet 363:841–845.