準備中

2. 学会発表

　準備中

H. 知的所有権の出願・登録状況

1. 特許取得

　な し

2. 実用新案登録

　な し

3. その他

　な し

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院内集団発生事例で分離された *Clostridium difficile* の型別および簡便検出試験法の開発

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研究要旨：*Clostridium difficile* は、院内感染性下痢症の主要な原因病原体である。本研究では、1)4病院の入院症例より分離された *C. difficile* 菌株をタイピングにより解析し、2) Loop-mediated isothermal amplification (LAMP)法による toxin B 遺伝子検出法を確立した。1) 4病院の入院症例より分離された 69 株を PCR ribotyping によりタイピングしたところ、11 タイプに分けられ、69 株中 50 株が同一のタイプ type smz であった。このタイプは検討した4病院すべてにおいて優勢であった。同 69 株において *slpA* sequence typing (*slpA* ST)を行ったところ、15 タイプが認められた。PCR ribotyping は比較的施行が簡便であるため、それぞれの病院に迅速に報告が可能であった。*slpA* ST は、PCR ribotyping よりさらにサブタイピングが可能であり、さらに、バンドパターンの比較解析によるタイピングと異なり、研究室間で菌株の交換を行うことなくタイピング結果の比較が可能である点が利点と考えられた。2) 本研究で確立したプライマーセットによる LAMP 法により *C. difficile* の toxin B 遺伝子を特異的に検出することが可能であった。74 糞便検体から抽出した DNA における LAMP 法による toxin B 遺伝子検出は、検体によっては *C. difficile* の分離培養よりも感度がよかった。本法は PCR よりも増幅反応が迅速で、サーマルサークラーや電気泳動装置が不要である点、反応チューブを開ける必要がないので検査室環境の汚染の心配がない点が利点と考えられた。

研究協力者：加藤はる（感染研　細菌第二部）
　　　　　　吉村由美子（　同上　）

A. 研究目的

1) タイピングによる院内集団発生の解析

　*Clostridium difficile* は抗菌薬関連下痢症の主要な原因菌である。一方、本菌は院内感染の原因菌として重要であり、近年、入院症例の高齢化、医療内容の高度化等に伴い、本菌による院内集団発生事例が増加している。本研究では第一に、院内集団発生事例における調査を目的に、院内感染性下痢症が疑われた症例から分離された *C. difficile* 菌株においてタイピングによる解析を行った。

2) Loop-mediated isothermal amplification (LAMP)法による toxin B 遺伝子(*tcdB*)検出法の確立

*C. difficile* による院内感染の調査および予防・対策には、まず個々の症例の診断が適切になされなければならない。*C. difficile* 関連下痢症／腸炎の細菌学的診断法として、感度および特異性が高く、加えて施行が迅速かつ簡便な方法が必要である。本研究では第二に、臨床分離株の toxin B 産生能の解析および糞便検体中の *tcdB* 検出法として、LAMP による *tcdB* 検出法を確立した。

B. 研究材料と方法

1) タイピング解析は、4 病院の入院症例の糞便検体から培養分離した 69 菌株において行った。解析は、16S rRNA 遺伝子と 23 rRNA 遺伝子の間の intergenic spacer region を PCR により増幅し増幅されるバンドパターンを比較する方法 (PCR ribotyping)、および surface layer protein をコードする遺伝子(*slpA*)のシークエンスを比較することによる方法(*slpA* sequence typing, *slpA*

ST)により行った。

2)LAMP 法による *tcdB* 検出は、菌株から抽出した DNA および臨床的に抗菌薬関連下痢症／腸炎と診断された 74 症例から採取された糞便検体から直接抽出した DNA において行った。*C. difficile* の分離培養は芽胞選択ののち、選択培地を用いて行った。分離された菌株の毒素産生能は PCR による toxin A 遺伝子および *tcdB* の検出により行った。

（倫理面への配慮）

糞便検体採取にあたっては、患者様より文書にてインフォームドコンセントを取った。

C．研究結果

1) タイピングを行った 69 株は、PCR ribotyping により 11 タイプに分かれ、さらに *slpA* ST により 15 のサブタイプに分類された。各病院の 27 株中 24 株、17 株中 12 株、15 株中 7 株、および 10 株中 7 株が PCR ribotype smz であり、検討した 4 病院すべてにおいて優勢であった。PCR ribotype smz とサブタイプされた菌株は *slpA* ST により 1 アミノ酸の差で 3 タイプに分けられ、2 病院では smz-1 と smz-2 の両方が認められ、1 病院では smz-1 のみ認められた。Type smz-4 は残る 1 病院にのみ認められた。

2) 検討したすべての toxin A 陽性 toxin B 陽性および toxin A 陰性 toxin B 陽性株において LAMP 法により *tcdB* が検出された。Toxin A 陰性 toxin B 陰性株においては陰性であった。検討した糞便検体では、74 検体中 39 検体において toxin A 陽性 toxin B 陽性あるいは toxin A 陰性 toxin B 陽性 *C. difficile* が分離培養され、そのうち 38 検体において糞便検体から抽出した DNA において LAMP により *tcdB* が検出された。分離培養陰性で LAMP 陽性であった検体が 10 検体認められた。本研究の詳細については、添付の論文を参照してください。

D．考　察

1) 検討した 4 病院において、PCR ribotype smz が優勢であり、本タイプ(PCR ribotype smz)は、少なくとも日本の病院において院内感染を起こしやすい性質を持っていると考えられた。PCR ribotyping は施行が簡便であり、タイピング結果を病院側に比較的迅速に報告することが可能であった。*slpA* ST は、さらにサブタイプに分けることが可能であり、またバンドパターンの比較によるタイピングと異なり、菌株や抗血清の交換をしなくても異なる研究室間でのタイピング結果の比較解析が可能であることが利点の一つと考えられた。

2) LAMP 法は、*C. difficile* の分離菌株の toxin B 産生能の同定に有用であり、糞便検体から直接抽出した DNA における *tcdB* 検出では、検体によっては培養法よりも感度が良好であった。PCR 法よりも増幅反応が迅速であること、サーマルサイクラーや電気泳動等の特殊な装置が不要であること、さらに PCR と異なり反応チューブを開ける必要がないので、増幅産物による環境の汚染の心配がないことが利点と考えられる。したがってこの試験法は、DNA 増幅装置や電気泳動装置を持たない一般の細菌検査室でも十分に実用的な試験法であり、その普及により、*C. difficile* の検出率の向上とその対策の強化に貢献する事が期待される。

E．結　論

　国内の医療施設では、院内感染で広がり入院患者に抗菌薬関連下痢症を引き起こしやすい特定の遺伝子型の *C. difficile*(PCR ribotype smz)が、複数の医療施設に広く分布している事が、再確認された。

　また、*C. difficile* の検出には嫌気培養などを必要とするが、臨床材料から簡便に *C. difficile* のトキシン B の遺伝子を簡便に検出可能な、LAMP 法用プライマーの設計に成功し、特許申請を行った。

F．健康危険情報

　*C. difficile* による抗菌薬関連下痢症を院内感

染症として引き起こしやすい特定の遺伝子型の
株（PCR ribotype smz）が複数の国内の複数の
施設に優勢的に分布している事が確認された
ため、今後、各医療施設において、それらに対
する監視と伝播防止策の強化が必要と考えら
れる。

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I. 知的所有権の取得状況

*C. difficile* の *tcdB* 遺伝子検出用の特異的
LAMP primer について、特許出願を完了（出願
番号 2005-162776）

## *Clostridium difficile* A 毒素非産生 B 毒素産生株の疫学調査

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研究要旨　　つくば・土浦地区の4基幹病院で，*Clostridium difficile* A 毒素非産生 B 毒素産生（A-/B+）株を収集し，分離頻度および分子疫学的解析を行った．当該病院で 2005 年 1 月から 6 月までに *C. difficile* 毒素検査用に提出された 332 検体を調べたところ，158 検体から *C. difficile* を検出し，そのうちの 10 株が A-/B+株だった．それらの株を解析したところ，PCR リボタイピングでは，全株同一パターンを示したが，パルスフィールド電気泳動での解析では，特定の病院から検出した9株と他病院から検出した残りの 1 株とは異なるバンドパターンを示した．以上より，今のところ *C. difficile*(A-/B+) 株は当該地域では流行していないこと，分子疫学解析には PCR リボタイピングよりパルスフィールド電気泳動法による解析の方が優れていることがわかった．

## A. 研究目的

　*Clostridium difficile* は，抗菌薬関連下痢症（AD）の主要起因菌である．*C. difficile* が産生する毒素には，主に A 毒素と B 毒素がある．従来両方の毒素を産生する株（A+/B+株）のみが AD を起こすと考えられていたが，最近 B 毒素のみを産生し A 毒素を産生しない株（A-/B+株）も AD を起こすことがわかってきた．しかしわが国における A-/B+株による AD の発生頻度は，よくわかっていない．そこで，つくば・土浦地区の基幹病院での *C. difficile* A-/B+株の分離率を調べ，あわせて分離株の分子疫学的解析を行った．

## B. 研究方法

　2005 年 1 月から 6 月にかけて，つくば・土浦地区の4基幹病院で，*C. difficile* 毒素検査用に提出された糞便を収集し，*C. difficile* を分離した．また，PCR 法で毒素産生遺伝子の確認を行い，毒素産生型を決定した．分離した A-/B+株の分子疫学的解析には，PCR リボタイピングおよびパルスフィールド電気泳動（PFGE）を用いた．

## C. 研究結果

　収集した 332 検体のうち，159 検体から *C. difficile* を分離した．そのうち 137 株（86%）が A+/B+株，10 株（6%）が A-/B+株，12 株（8%）が毒素非産生株だった．各病院の A-/B+株の分離数は，0 件（依頼検体あたり 0%）から 9 件（11%）とばらつきがあった．同一病院で分離した A-/B+株 9 株では，PCR リボタイピングおよび PFGE 解析ともに同一起源由来を示した．しかし，残りの 1 株は，PCR リボタイピングでは他の株と同じパターンを示したものの，PFGE 解析では異なるパターンを示した．

D. 考察

　　今回の調査で分離した *C. difficile* の多くは A+/B+株であり，A-/B+株の大きな流行は認めなかった．しかし，既に A-/B+株の院内流行事例が国内外で報告されていることから，今後 A-/B+株による院内感染にも，A+/B+株によるものと同様の注意を払う必要がある．また，A-/B+株の分子疫学的解析では，PCR リボタイピングより PFGE 法の方が感度よく株を区別できた．これは，A+/B+株でよく起こる DNA の非特異的分解が今回解析した株では起こらなかったためと考えた．現在わが国では，*C. difficile* の A 毒素を検出する検査薬のみが保険適用になっており，臨床現場で A-/B+株を検出することは極めて困難である．今後，国外では既に汎用されている B 毒素検出キットを早期に国内でも使用できるようにし，各臨床現場での A-/B+株に対する意識を高める必要があると考える．

E. 結論

　　今回調査を行った病院では，A-/B+株の大規模な流行は認めなかったが，施設内での院内伝播を示唆する事例を認めた．また，A-/B+株の分子疫学的解析には，PCR リボタイピングより PFGE 法の方が優れていた．

F. 健康危険情報
　　なし

G. 研究発表
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H. 知的財産権の出願・登録状況
　　なし

# III. 研究成果の刊行に関する一覧表

## III. 研究成果の刊行に関する一覧表

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書籍名 | 出版社名 | 出版地 | 出版年 | ページ |
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# IV. 研究成果の刊行物・別刷

# 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

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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. © 2005 Wiley-Liss, Inc.

KEY WORDS: angiotensin-converting enzyme 2 (ACE2); severe acute respiratory syndrome (SARS); SARS associated coronavirus (SARS Co-V); 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 an etiological agent of SARS [Drosten et al., 2003; Ksiazek et al., 2003; Kuiken 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 [Jeffers 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-Δ32 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-exon 1s (5'-CAA AGG CTG ATA AGA 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'-CCT AGA CTA AAA CCT CCT CA-3'), and ACE2-5'Outer2 and ACE2-5'Inner2 (5'-GAA GTA AGA AAG CCT CCA CA-3' and 5'-CTC CTG ATC CTC TGT AGC CA-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 Institute) with the sense primer New-exon (5'-TTC TTA CTT CCA CGT GAC CT-3') or Extended-exon 1 (5'-GCT CAG CAG ATT GTT TAC TG-3') and the antisense primer ACE2-5'Outer1.

### Genomic DNA Samples for the Association Study

An association study between SARS patients and controls was reviewed and approved by local ethics committees. Of 62 cases fulfilling the World Health Organization case definition of probable SARS in Vietnam [WHO, 2003], 5 fatal cases and 3 non-Vietnamese cases were excluded from this study. In the remaining 54 cases, 44 individuals agreed to participate in this study as cases. One hundred and three Vietnamese staff members, who did not develop SARS but may have come in contact with SARS patients in the hospital where nosocomial infection of SARS had arisen, were enrolled as contacts. Furthermore, 50 medical staff members who had been working in a separate building and those considered having no history of contact with SARS patients joined in this study as non-contacts, according to information obtained by questionnaire. Peripheral blood samples of all the subjects were collected and genomic DNA was extracted from the blood cells by a method described elsewhere [Wang et al., 1994].

### Testing for Antibody Response to the SARS-CoV

To detect the antibody to the SARS-CoV in serum, all the 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 I). 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).

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 TTG C | 676 bp |
| | ACE2-pro-1-anti | AAT AGT GGA GGC ATA GAT AAA | |
| 5' flanking region | ACE2-pro-2-sense | TTT GTG AGC TGC TTT ATT TT | 618 bp |
| | ACE2-pro-2-anti | TGC CAG AGT GTA TGT ATG AG | |
| New alternate exon | ACE2-new-sense | TTA TTG CAA TGT CAC CTG A | 470 bp |
| | ACE2-new-anti | TTA TGA CTA CTC TCC ACT CCA | |
| 5' flanking region | ACE2-pro-3-sense | TTT GAA TAG GTA AGT GAA GG | 669 bp |
| | ACE2-pro-3-anti | TAG AAC TAG GGA TCA TGA AGA | |
| 5' flanking region | ACE2-pro-4-sense | TGA ATT CCA TAA AGA CAA GG | 653 bp |
| | ACE2-pro-4-anti | AAA CTT GTC CAA AAA TGT CTT | |
| Exon 1 | ACE2-ex1-sense | ATC TTT AAC AGC TTT CTA GGA | 644 bp |
| | ACE2-ex1-anti | AAC ATC CAA TCT CAC AAC TC | |
| Exon 2 | ACE2-ex2-sense | AAC TCA TCT ATG TCA CAG CAC | 636 bp |
| | ACE2-ex2-anti | AAA TTA TAT GGA CAC CTT ACC | |
| Exon 3 | ACE2-ex3-sense | ACT TCT TTG GGT TTT GGT AG | 627 bp |
| | ACE2-ex3-anti | ACA TCA GGT CAT AAA GTG GTT | |
| Exon 4 | ACE2-ex4-sense | TCA TTT CAG TGG TTT ATT TTC | 521 bp |
| | ACE2-ex4-anti | CTT TTC TTT TTC CCC AGT A | |
| Exon 5 | ACE2-ex5-sense | CTT GTA TGG TTC TTG TGC TT | 535 bp |
| | ACE2-ex5-anti | GGG CTG TCC TAT TAT TCT CTA | |
| Exon 6 | ACE2-ex6-sense | ACC TGT GTT CTC CCA AGT A | 568 bp |
| | ACE2-ex6-anti | CTT TAT CAT TTG AAT TGC AG | |
| Exon 7 | ACE2-ex7-sense | TCA CCA AGT TAA GTA CAC GAA | 562 bp |
| | ACE2-ex7-anti | TAC ACC TGC AAT TCA AGT TAT | |
| Exon 8 | ACE2-ex8-1-sense | TTG CAG TGA GAA CAT TTG AAA | 560 bp |
| | ACE2-ex8-1-anti | CCT CTG TTG TCT CCC ATT T | |
| Exon 8 | ACE2-ex8-2-sense | GCT GTG CAG TAG ATC TCA AA | 643 bp |
| | ACE2-ex8-2-anti | CAG ATT GTC CAC AGG TTC A | |
| Exon 9 | ACE2-ex9-sense | CTA TGA GCA AGA GAA CAG G | 577 bp |
| | ACE2-ex9-anti | TCA CCA GTA GTA ATT TCC AGT | |
| Exon 10 | ACE2-ex10-sense | AGG GAG GAA ACT GAA ACT AAT | 587 bp |
| | ACE2-ex10-anti | GGT ATC CAA ATG GAG ACT AAA | |
| Exon 11 | ACE2-ex11-sense | GTG CAC ACC TAT AAA CCA AG | 615 bp |
| | ACE2-ex11-anti | TGA GCA TGT TTA GGG TAG AC | |
| Exon 12 | ACE2-ex12-sense | GTG AAA GGG CTA TTA ATC TGT | 612 bp |
| | ACE2-ex12-anti | GAG AGG GCT GTA GTT ATG A | |
| Exon 13 | ACE2-ex13-sense | CAG GAA CCT AGA CCA TAC AA | 636 bp |
| | ACE2-ex13-anti | GTT GCT TTC ACT ATG TCT CA | |
| Exon 14 | ACE2-ex14-sense | GTA CAA ATT AGG TCA TGG C | 550 bp |
| | ACE2-ex14-anti | GAC GAG AGT CAA TTG AAA G | |
| Exon 15 | ACE2-ex15-sense | ATT ATT GGG TTT CAT CTC G | 637 bp |
| | ACE2-ex15-anti | TAT AGG TCA ATG AAG GCA G | |
| Exon 16 | ACE2-ex16-sense | CAG AAC AAA TAG TGC CAA A | 610 bp |
| | ACE2-ex16-anti | CAT AGT GGT AAC TTG CTT GAT | |
| Exon 17 | ACE2-ex17-sense | GCT CTG TCA CCT AGG CTA G | 633 bp |
| | ACE2-ex17-anti | CTA GGA AGA TGA ACT GCT GAT | |
| Exon 18 | ACE2-ex18-1-sense | TTA AGA TGA ATC CTA GCA GTG | 655 bp |
| | ACE2-ex18-1-anti | CAT TTA GAT TAT CCC TGA ACA | |
| Exon 18 | ACE2-ex18-2-sense | TCT GGA TTT GAC TTC TGT TC | 623 bp |
| | ACE2-ex18-2-anti | AAC ACT GTG AGC AAA TAC AAA | |
| Exon 18 | ACE2-ex18-3-sense | GAA CAG GTA GAG GAC ATT G | 531 bp |
| | ACE2-ex18-3-anti | GGG TAG TGA CTG TGA GAA ATA | |

## Subgrouping of Subjects Based on the Status of Anti-SARS-CoV Antibody

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
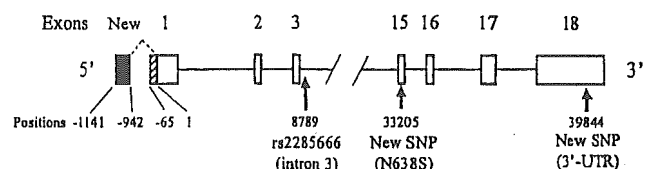


Fig. 1. A schematic diagram of the *ACE2* gene structure and the positions of SNPs. The known exons are depicted as open boxes. A solid box and a striped box indicate the new exon and the new extended region of the exon 1, respectively. The arrows represent locations of the SNPs analyzed in a case-control study. The broken line depicts an alternative-splicing site.

TABLE II. Demographic Findings of Subjects and Subgroups

| Groups | SARS cases (n = 44) | Contacts (n = 103) | Anti-SARS-CoV antibody | | Non-contacts (n = 50) |
|---|---|---|---|---|---|
| | | | Positive (n = 16) | Negative (n = 87) | |
| Age (years), mean [range] | 39.3 [17–76] | 36.5 [15–68] | 36.0 [25–50] | 36.6 [15–68] | —[a] |
| Male/female | 13/31 | 46/57 | 7/9 | 39/48 | 17/33 |

[a]Data not available.

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) were identified. All SNPs but one in intron 3 (rs2285666, position 8789) 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-Δ32 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 III. SNPs Within the *ACE2* Gene

| Region | Position[a] | 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[b] | 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[c]* |
| 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 | 28946 | 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 | 33121 | NEW | G/C | — | 1 | 0 |
| Exon 15 | 33205 | NEW | A/G | N/S | 0 | 1 |
| Intron 16 | 36655 | NEW | G/A | — | 0 | 1 |
| Intron17 | 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[c]* |
| | | | | | No. of samples tested = 20 | No. of samples tested = 57 |

[a]Position numbers indicate distance from 5′ end of the original exon 1.
[b]Newly identified SNPs are shown as NEW.
[c]Minor allele frequencies of the SNPs shown in bold and italic were higher than 0.05.

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

| | | | | SARS cases | Contacts Antibody (+) | Contacts Antibody (−) | Non-contacts |
|---|---|---|---|---|---|---|---|
| Intron 3 (rs2285666) | | | | | | | |
| | Male | Genotype/allele[a] 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 (0.98) | 33 (1.00) |
| | | | A/G | 0 (0.00) | 1 (0.11) | 1 (0.02) | 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) | G | 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) | 0 (0.00) | 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) |

[a]Genotype distribution is the same as allele distribution in male.

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 exon was definitely shown in the lung and small intestine. Also, we found the extended region of the original exon 1, 65 bp on the 5′ side. Neither the new alternative exon nor the new extended region of 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.

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

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## Abstract

We hypothesized that host antiviral genes induced by type I interferons might affect the natural course of severe acute respiratory syndrome (SARS). We analyzed single nucleotide polymorphisms (SNPs) of 2',5'-oligoadenylate synthetase 1 (OAS-1), myxovirus resistance-A (MxA), and double-stranded RNA-dependent protein kinase in 44 Vietnamese SARS patients with 103 controls. The G-allele of non-synonymous A/G SNP in exon 3 of OAS-1 gene showed association with SARS ($p = 0.0090$). The G-allele in exon 3 of OAS-1 and the one in exon 6 were in strong linkage disequilibrium and both of them were associated with SARS infection. The GG genotype and G-allele of G/T SNP at position −88 in the MxA gene promoter were found more frequently in hypoxemic group than in non-hypoxemic group of SARS ($p = 0.0195$). Our findings suggest that polymorphisms of two IFN-inducible genes OAS-1 and MxA might affect susceptibility to the disease and progression of SARS at each level.
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Severe acute respiratory syndrome (SARS) is a new infectious disease that emerged towards the end of 2002, spreading from China to countries in Asia, Europe, and North America. During the outbreak, a total of 8098 cases of SARS were diagnosed and the mortality rate was 9.6% [1]. Risk factors for exacerbation of the clinical progress in SARS have been reported as being patients in excess of 60 years of age, or having diabetes mellitus or other comorbid medical conditions [2,3]. However, little is known about host genetic factors associated with the development or progression of SARS, excepting human leukocyte antigens [4,5] and insertion/deletion polymorphism in the angiotensin converting enzyme 1 gene whose association with the disease [6] our research group had identified.

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

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

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

## Materials and methods

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

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

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

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

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

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

The T/C SNP at position −168 in the promoter region of PKR gene, associated with result of HCV infection [22], was genotyped as follows. PCR was carried out with primers PKR-pF (5′-GTG GAA CCC TTG ATT CGA GAA CCT AGT-3′) and PKR-pR (5′-GCG GCT TCG GGA GAG CTG GTT CTC AGT-3′) using TaKaRa Ex Taq with GC buffer I (TaKaRa). The cycling condition is 45 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 1 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and digested with SgrAI (New England Biolabs). Digested DNA was electrophoresed on a 5% agarose gel. The presence of T-allele was demonstrated by 169 and 155 bp fragments, and the presence of C-allele was indicated by 169, 136, and 19 bp fragments.

*Statistical analysis.* Possible differences deriving from the distribution of age and gender between two groups were evaluated with the unpaired $t$ test and $\chi^2$ test, respectively. Disease associations were assessed by the $\chi^2$ 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 |
|---|---|---|---|
| *OAS-1* 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 |  |
| *OAS-1* 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 |  |
| *MxA* −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 |  |
| *PKR* −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 |