MAJOR ARTICLE

Nasopharyngeal Shedding of Severe Acute Respiratory Syndrome–Associated Coronavirus Is Associated with Genetic Polymorphisms

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Background. A high initial or peak severe acute respiratory syndrome (SARS)–associated coronavirus (SARS-CoV) load in nasopharyngeal specimens was shown to be associated with a high mortality rate. Because all infected individuals were devoid of preexisting protective immunity against SARS-CoV, the biological basis for the variable virus burdens in different patients remains elusive.

Methods. The nationwide SARS database in Taiwan was analyzed, and genotyping of 281 single-nucleotide polymorphisms (SNPs) of 65 genes was performed for 94 patients with SARS, to identify SNPs for which distribution between patients with or without detectable nasopharyngeal shedding of SARS-CoV was biased.

Results. Titers of SARS-CoV shed in nasopharyngeal specimens varied widely, ranging from nondetectable to 10^8 SARS-CoV RNA copies/mL, and they were correlated positively with a high mortality rate ($P < .0001$, by trend test) and with early death (i.e., death occurring within 2 weeks of the onset of illness) ($P = .0015$, by trend test). Virus shedding was found to be higher among male patients ($P = .0014$, by multivariate logistic regression) and among older patients ($P = .015$, by multivariate logistic regression). Detectable nasopharyngeal shedding of SARS-CoV was associated with polymorphic alleles of interleukins 18 ($P = .014$) and 1A ($P = .031$) and a member of NFκB complex (reticuloendotheliosis viral oncogene homolog B [*RelB*]) ($P = .034$), all of which are proinflammatory in nature, as well as the procoagulation molecule fibrinogen-like protein 2 ($P = .008$).

Conclusion. The SARS-CoV load is a determinant of clinical outcomes of SARS, and it is associated with polymorphisms of genes involved in innate immunity, which might be regulated in an age- and sex-dependent manner. The findings of the present study provided leads to genes involved in the host response to SARS-CoV infection; if substantiated with functional studies, these findings may be applicable to other newly emerged respiratory viruses (e.g., the influenza pandemic strain).

Severe acute respiratory syndrome (SARS) is a recently identified infectious disease caused by a novel coronavirus, SARS-CoV, that causes significant mortality, especially among elderly patients and patients with comorbid factors [1–4]. The clinical course of SARS consists of initial onset of fever, followed by the development of respiratory symptoms that may progress to acute respiratory distress syndrome in some patients [2, 5] but may be quite self-limiting in others. Serial chest radiographs of patients with SARS have disclosed distinct patterns of disease progression [6] and have suggested prolonged recovery in correlation with the clinical severity of the disease. These findings, in conjunction with those of clinical studies of cytokines during the acute phase of SARS [7–9], have suggested that the development of acute respiratory distress syndrome in patients with SARS is due to an immunopathological response related to strong activation of T helper type 1 cell–mediated immunity and a hyperinnate inflammatory response, rather than to uncontrolled virus growth [2].

However, a high initial or peak nasopharyngeal virus load is associated with a high mortality rate [10, 11], and a high serum virus load correlates with an increased risk for admission to an intensive care unit [12], highlighting the importance of virus burden to the clinical

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outcome. High SARS-CoV titers have also been recovered from saliva samples and mouth wash specimens, raising the possibility that the virus also replicates in the upper airway [13]. Autopsy findings for patients with SARS who died within 2 weeks after the onset of illness showed that the presence of SARS-CoV was widespread in a number of tissues and organs [14, 15], and that, in some surviving patients, the virus remained for prolonged periods (up to 80 days) [16, 17]. Detection of virus in airway aspirates, serum samples, or fecal specimens beyond the 10th day of illness suggested continuing, unabated viral replication and indicated a poor prognosis [18]. In a small study of a series of postmortem lung tissues, detection of a high virus load in lung tissue was associated with a survival time of <21 days [19].

Given the assumption that all patients had neither preexisting protective immunity against SARS-CoV nor access to antiviral drugs, which can effectively alter the level of virus shedding, the biological basis for the differences in the virus burden in different patients is intriguing and has not yet been elucidated. In this report, the virus load of patients with confirmed SARS-CoV infection at the time of admission to the hospital was analyzed in relation to sex, age, and the genetic polymorphism of genes involved in proinflammation and innate immunity responses.

MATERIAL AND METHODS

The database. The nationwide SARS database in Taiwan includes all laboratory-confirmed cases of SARS reported from March through June 2003. The details of data compilation have been described elsewhere [20, 21]. Our analysis focused on the initial nasopharyngeal SARS-CoV load at the time of admission to the hospital and included 154 patients whose virus loads were quantified using a commercially available RT-PCR assay (Artus Real Art HPA-Coronavirus; Artus), as well as 111 patients who had successive negative results of testing of nasopharyngeal samples and for whom diagnoses of SARS were based on a positive finding of seroconversion of SARS-neutralizing antibody.

The genetic study. The genetic study was designed to compare the genetic background of patients who had SARS with or without detectable nasopharyngeal SARS-CoV. All surviving patients with SARS were followed up, and 108 genetically unrelated patients with laboratory-confirmed SARS consented to participate in this study. A reference group of 94 Taiwanese subjects was selected, on the basis of an age- and sex-stratified random sampling scheme, from a biobank compiled as part of a nationwide population study that enrolled 3312 Han Chinese descendants residing in Taiwan [22]. The study protocol, including an informed consent statement that conforms to the fifth edition of the Helsinki Declaration, was approved by the Medical Research Ethics Committee of the Institute of Biomedical Sciences (Taipei, Taiwan) (application #AS-IBMS-MREC-92-11).

Genes and single-nucleotide polymorphism (SNP) selection. We focused on genes whose products are either known to or have been predicted to interact with SARS-CoV in antiviral responses, inflammation, or immunostimulation (table 1) [23]. SNPs of these genes were identified in 5 databases: the SNP Consortium, the National Center for Biotechnology Information, GeneSNPs Public Internet Resource, GeneCards, and the Japan SNP Database. A total of 281 SNPs in 65 genes (table 1) were studied, with 127 (45.2%) of these 281 SNPs showing no variants (<1%) according to the results of a test run of 94 DNAs selected from the reference Han population (power, >80%) [25].

SNP genotyping. Genotyping was performed using either (1) the Sequenom MassArray MALDI-TOF system (Sequenom) at the Taiwan National Genotyping Center core facility, by use of primers for SNP typing that were designed using SpectroDesigner software (Sequenom), or (2) the ABI 7000 system (Applied Biosystems), if the Sequenom system could not design the primers. For quality control, genotyping of up to 20% of the samples was repeated; any discrepancy was verified by sequence analysis, and the error rate was <1%.

Statistical analysis. Data analysis was performed, unless stated otherwise, by use of SAS, version 8, or SAS/Genetics (SAS Institute). Hardy-Weinberg equilibrium was tested for each SNP result of the Han reference population, and the genotyping assay was repeated or another method was used for testing if the results violated Hardy-Weinberg equilibrium. Haplotypes for all SNPs of each gene were predicted using the Phase 2.0.2 program [26]. For each SNP, univariate analysis was performed by comparing the frequencies of alleles and genotypes between patients with or without detectable SARS-CoV in nasopharyngeal specimens. Tests for models of dominant/recessive or codominant traits were performed to identify the susceptible genotypes of the SNP. The empirical P value was calculated using the permutation test of 10,000 rounds for each SNP for which an association with the virus load was demonstrated. Tests of significance that were performed included the χ^2 test, the Cochran-Armitage trend test, Fisher's exact test, and the exact Mantel-Haenszel test. The continuous variables (i.e., age and virus load) were compared using the Wilcoxon rank sum test, to analyze factors that could have potentially affected the virus load, including demographic characteristics, the source of infection, and the duration of illness. Associations between nasopharyngeal SARS-CoV shedding and multiple variables were analyzed simultaneously in a cumulative logistic model. Interactions between genes were tested in a cumulative logistic model for virus detection.

Table 1. Genes studied.

Gene symbol[a]	No. of SNPs[b]
ACE2	2/16
ACP5	2/2
ADAR	2/4
AIP	0/3
ANPEP[c]	2/4
B2M	2/3
CAT	2/2
CCL5	1/3
CD209	8/9
CIITA	3/5
CXCL9	1/1
CXCL10	5/5
CYP17A1	1/1
EIF2AK3[c]	4/6
EIF2S1	5/6
EIF4G1	6/8
ESR1	1/2
FGL2[d]	3/4
FN1	2/5
G6PD	1/6
GNB3[c]	6/8
GPX1	2/7
GSS	0/1
HMOX1	2/2
IFNAR1	4/10
IFNAR2	1/1
IFNG	1/1
IFNGR1	2/2
IFNGR2	0/2
IL1A[d]	1/1
IL1B	2/3
IL1RN	1/1
IL4	1/1
IL6	0/1
IL10	5/11
IL10RB	1/3
IL12A	2/5
IL15	1/2
IL18[d]	2/6
IRF1[c]	3/4
IRF3	1/3
IRF7	0/1
MBL2	1/2
MX1	4/9
NFRKB	0/4
OAS1	2/7
PRDX2	0/1
PRKRA	2/2
PTGS2	2/13
RelB[d]	1/2
RFX5	0/6
RNASEL[c]	3/5
SERPINB3[c]	2/2
SH2DIA	1/1
SLAMF1	7/7
SOCS1	0/3
SOCS3	1/2
SOD1	0/1
TBF	1/3
TFRC	0/1
TGFB1	1/2
TLR3	4/11
TLR4	3/19
TRAF6	1/5
WSX1	0/2

(continued)

Table 1. ***(Continued.)***

NOTE. Detailed information on the SNPs is provided in [23]. SNP, single-nucleotide polymorphism.

[a] HUGO Gene Nomenclature Committee–approved gene symbol [24].

[b] Data are no. of polymorphic loci/total no. of loci studied per gene.

[c] Genes containing SNPs that showed a >10% difference in allele distribution between patients with SARS with or without detectable levels of virus but for which statistical significance could not be determined because of a lack of power (figure 3).

[d] Genes containing SNPs with a statistically significant association ($P < .05$) with virus load.

RESULTS

Nasopharyngeal SARS-CoV load. The virus load in the respiratory tract at the time of admission to the hospital or diagnosis of SARS ranged widely (from undetectable to 10^8 SARS-CoV RNA copies/mL) (figure 1). Although a longitudinal study of patients with SARS indicated that the nasopharyngeal virus load increased between the fifth and the 10th days of illness and decreased by the 15th day of illness [2], the virus loads at the time of admission of all patients with SARS in our data set did not reflect this trend of a rise and fall occurring during the first 2 weeks of the clinical course of SARS. Rather, on any given day within the first week of the clinical course of SARS, the nasopharyngeal virus load in different patients ranged widely. Overall, 111 (41.9%) of 265 patients with SARS had an undetectable level of nasopharyngeal virus shedding, and this lack of detection of virus did not correlate with the time of specimen collection, because successive samples were obtained from these patients, and the test results for these specimens remained negative.

Factors influencing the level of virus shedding. Among patients with SARS, undetectable levels of nasopharyngeal

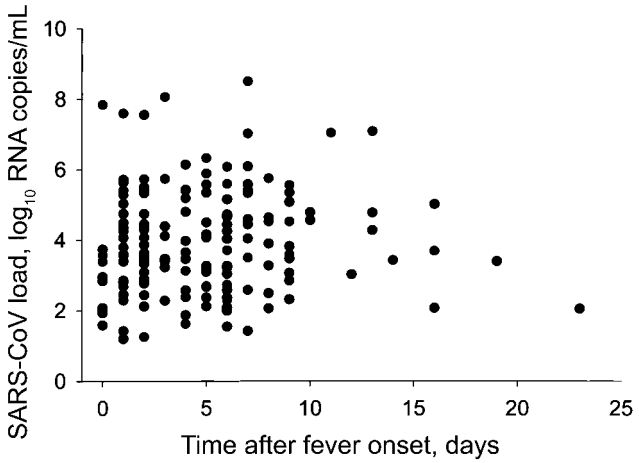

Figure 1. Scatter plot of titers of severe acute respiratory syndrome–associated coronavirus (SARS-CoV) in nasopharyngeal specimens from 154 patients vs. the day of specimen collection.

SARS-CoV shedding were found more often among female patients (50.0%) than among male patients (27.4%), and they were also found more often among younger patients (53.1% of patients <30 years of age) than among older patients (37.7% of patients ⩾30 years of age) (table 2 and figure 2). When only the virus titer of patients with detectable virus shedding was the focus of investigation, female patients consistently shed lower titers of virus than did male patients ($P = .045$, by the multivariate logistic regression model). Furthermore, in a multivariate cumulative logistic regression model in which (1) the date of sample collection was controlled, and (2) any underlying diseases and the source of infection (identifiable or not) were considered simultaneously, female sex ($P = .001$) and younger age ($P = .005$) were independently associated with a lower virus load, whereas having an underlying disease or a known source of infection did not influence virus shedding. Because the lack of virus detection may be the result of lower levels of virus shedding occurring at the time of sample collection during the early (<5 days of illness) or later (>15 days of illness) clinical phase of disease [2], the multivariate model confirmed that nasopharyngeal specimens in which levels of virus shedding were undetectable were collected at the same time relative to disease onset as were specimens in which virus levels were detectable; lack of detection of virus did not correlate with early sample collection.

Clinical significance of virus shedding. We further analyzed the mortality rate relative to the levels of virus shedding, to validate the biological significance of detection of virus in nasopharyngeal specimens (table 2). The mortality rate was 46.2% among patients with $>10^5$ SARS-CoV RNA copies/mL detected in nasopharyngeal specimens, whereas the mortality rate was only 9.9% among patients with undetectable levels of virus in nasopharyngeal specimens. Furthermore, the duration of survival for the patients whose cases of SARS were fatal was significantly shortened (to <2 weeks), with increasing levels of virus shedding occurring during the acute phase of the disease (table 3). Of the patients with fatal cases of SARS who had $>10^5$ SARS-CoV RNA copies/mL detected in nasopharyngeal specimens, 77.8% died within the first 2 weeks of SARS illness, whereas, of the patients with fatal cases of SARS who did not have virus detected in nasopharyngeal specimens, only 18% died within the first 2 weeks of illness ($P = .001$, by Cochran-Armitage trend test).

Polymorphic genes associated with the detection of SARS-CoV in nasopharyngeal specimens. With mortality data validating the biological significance of undetectable nasopharyngeal virus shedding, we then compared the genotype distribution of SNPs between the 2 groups of patients with detectable or undetectable virus shedding (table 4 and figure 3). Of the 281 SNPs tested, 4 SNPs of 4 genes showed a significant association with virus load. IL-18 *(IL18)* of −607T (rs1946518) in the promoter, IL-1A *(IL1A)* of −889T (rs1800587) upstream from the ATG, *RelB* (reticuloendotheliosis viral oncogene homolog B) in intron 5 at +23962T (rs2288918) from the ATG, and the fibrinogen-like protein 2 *(FGL2)* of +158A (rs2075761) (resulting in an amino acid substitution from glycine to glutamic acid) were overrepresented in patients with detectable levels of SARS-CoV. For the genotype distribution of each SNP, empirical *P* values were calculated using permutation tests of 10,000 rounds and confirmed the statistical significance of $P < .05$ (table 4).

Analysis performed by further stratification of these 4 genes did not suggest any interactions between these genes that contributed to the level of virus shedding. Multivariate analysis confirmed that all loci were independently associated with virus load (table 4). Furthermore, the homozygous individuals possessing 2 alleles of −607T *IL18* had increased risk of virus shedding, compared with the heterozygous individuals possessing only 1 allele when genotypes of the other 3 alleles were controlled. A total of 13 SNPs in the 4 genes were typed, and polymorphisms were found at 2 of 6 *IL18* loci (−607 and −137), at 1 of 2 *RelB* loci, at 1 *IL1A* locus, and at 3 of 4 *FGL2* loci (−1285, −656, and +158). The haplotypes were analyzed for *IL18* and *FLG2;* only a single locus of *IL18* (−607) and *FGL2* (+158) showed an association with virus load. We compared both the allelic and genotypic distributions of these 4 loci between the 94 patients with SARS and the 94 healthy Taiwanese in the reference group, and we found no statistical difference (table 4).

DISCUSSION

The clinical significance of the virus burden is intuitive, but it has been difficult to directly demonstrate. In the present report, we showed the dramatic interindividual difference in virus bur-

Table 2. Distribution of 265 patients with severe acute respiratory syndrome (SARS), according to nasopharyngeal (NP) SARS-associated coronavirus (SARS-CoV) load at the time of admission, by demographic and clinical characteristics.

Demographic or clinical characteristic	Patients with undetectable NP SARS-CoV load (N = 111)		Patients with NP SARS-CoV load, RNA copies/mL						P[b]
			$<10^3$ (N = 39)		10^3–10^5 (N = 76)		$>10^5$ (N = 39)		
	Distribution, %[a]	No.	Distribution, %[a]	No.	Distribution, %[a]	No.	Distribution, %[a]	No.	
Sex									.0015
Male (n = 95)	27.4	26	14.7	14	33.7	32	24.2	23	
Female (n = 170)	50.0	85	14.7	25	25.9	44	9.4	16	
Age,[c] years									.0055
<30 (n = 81)	53.1	43	14.8	12	22.2	18	9.9	8	
30–49 (n = 107)	45.8	49	14.0	15	26.2	28	14.0	15	
50–64 (n = 42)	31.0	13	11.9	5	38.1	16	19.0	8	
⩾65 (n = 34)	17.7	6	20.6	7	38.2	13	23.5	8	
Time of swab sampling[d]									.603
⩽3 days (n = 86)	43.0	37	12.8	11	29.1	25	15.1	13	
>3 days (n = 179)	41.3	74	15.7	28	28.5	51	14.5	26	
Source of infection									.346
Known (n = 47)	31.9	15	19.2	9	31.9	15	17.0	8	
Unknown (n = 218)	44.0	96	13.8	30	28.0	61	14.2	31	
Underlying disease									.589
Present (n = 219)	46.1	101	13.3	29	26.9	59	13.7	30	
Absent (n = 46)	21.7	10	21.7	10	37.0	17	19.6	9	
Death,[e] by patient age									
⩽40 years	4.6	3/65	15.8	3/19	20.0	6/30	26.7	4/15	.0001[f]
>40 years	17.8	8/45	45.0	9/20	53.3	24/45	58.3	14/24	.0004[f]

[a] Percentages were calculated by dividing the no. of patients with an undetectable or detectable NP SARS-CoV load by the no. of patients with a demographic or clinical characteristic.
[b] Multivariate logistic regression model controlled by the date of specimen collection.
[c] Data were missing for 1 patient.
[d] After onset of fever.
[e] Data in the "No." columns are no. of patients who died/no. of patients with the characteristic. P = .0002, multivariate logistic regression model controlled by sex and age.
[f] P value determined using the Cochran-Armitage trend test.

den and the direct correlation of such virus burden with clinical outcomes. Our analysis further indicated that a high SARS-CoV load is associated with early death, in addition to a high mortality rate, as previously reported elsewhere [10–12]. Therefore, our results suggest that (1) the level of nasopharyngeal virus shedding during the acute phase of SARS (<2 weeks of illness) can be monitored as an index for therapeutic and clinical progress, and (2) identification of genes associated with virus load can potentially provide leads to pathways involved in virus clearance during the early phase of infection. To the best of our knowledge, this is the first study to probe into the factors influencing the SARS-CoV load.

The exploratory nature of this genetic study necessitated the screening of a large number of SNPs; applying the conventional Bonferroni correction for multiple tests (n = 281) would not be most appropriate. However, the internal validity of results based on a small sample size in an epidemic setting was further substantiated by the results of permutation tests. On the basis of a preset level of statistical significance, 4 SNPs were singled out for further analysis and discussion. An additional 10 SNPs of 6 genes, which showed differences of >10% in the allele frequency between patients with or without detectable SARS-CoV but for which the differences did not reach the level considered to be significant (figure 3), were also highlighted and should be investigated if the opportunity arises. These candidate genes were selected on the basis of the biological plausibility of innate immune responses to viral infections in a broad sense, rather than specific to SARS-CoV; thus, similar considerations may be pertinent and applicable to a potential influenza pandemic in which no individuals have preexisting immunity and in which the effects of the innate immunity of the hosts would be most conspicuous.

Three of the 4 SNPs *(IL18, IL1A,* and *RelB)* belong to proinflammatory genes. Both *IL18* and *IL1* signal through pathways

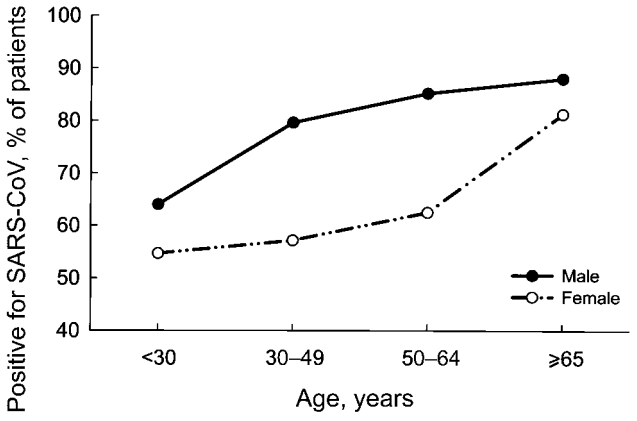

Figure 2. Rate of detection of shedding of severe acute respiratory syndrome–associated coronavirus (SARS-CoV) in nasopharyngeal specimens from 265 patients, as determined by RT-PCR, according to sex and age.

involving the transcription factor *NFκB* [27–29], to which several proinflammation pathways converge and *RelB* is a subunit. *RelB* belongs to the *Rel* proteins that are evolutionarily conserved mediators of immune responses and are especially important to cytokine expression and the differentiation and survival of cells in the immune system [30]; *RelB* mutant mice display a severe and fatal multiorgan inflammation. The induction of *NFκB,* to which *RelB* is a subunit, is reduced in T lymphocytes from old mice [31] and is a potential mechanism to explain the senescence of immunity. Whether the genetic variant of *RelB,* which is associated with higher virus load, represents a suppressed or an overly reactive function of *RelB* (i.e., inflammation signaling) remains elusive.

IL18, which is a member of the *IL1* family and also is a pleiotropic proinflammatory cytokine in activating natural killer cells and enhancing the T helper type 1 cell immune response, is constitutively expressed in the lung [32]. *IL18* knockout mice were shown to clear influenza virus more effectively [33], and the polymorphic alleles of *IL1A* (−889) have been reported to predict control of plasma viremia in patients with HIV-1 infection who are undergoing antiretroviral therapy [34], suggesting that both *IL18* and *IL1A* participate in the clearance of viral infection in a broad sense. Estrogen has been shown to reduce *IL18* mRNA levels in the mouse uterus [35], suggesting that regulation of *IL18* expression could be sex dependent. The outcomes of infection or the level of response to vaccination has been noted to demonstrate sex differences (see Beagley et al. [36] and Morales-Montor et al. [37] for reviews); the innate and adaptive immunity may be regulated through sex hormones [38]. A higher mortality rate among male patients with SARS was suggested in an analysis of the Hong Kong and Taiwan databases [39]; it would be more informative if the analysis had included information on nasopharyngeal virus burden, which is currently a commonly used method of diagnosing SARS and other viral infections.

FGL2, an IFN-γ–inducible protein expressed by lymphocytes, macrophages, and endothelium, is a prothrombinase that has been reported to contribute to fibrin deposition during viral hepatitis, and its expression is associated with a number of pathological conditions [40–42]. The inclusion of *FGL2* in the present study was based on reports that *FGL2* expression could be induced by the nucleocapsid protein of virulent mouse hepatitis virus, also a coronavirus [43]. The *FGL2* SNP at +158 also shows an association with the clinical severity of SARS (W.-Y. Tsai, C.S.J.F., W.-J.C., V.O., C.-C.K., Y.-M.A.C., Y.-T. Lu, C.-L. Liu, C.-L. Chao, H.-L.C., H.-W.K., B.-L. Chiang, F.L., I.-J. Su, and M.-S.H., unpublished data), and *FGL2* protein is highly expressed in the respiratory tract, according to the findings of immunohistochemical analysis (M.-L. Huang, L.-Y. Chao, and M.-S.H., unpublished data). Human *FGL2* mRNA was shown to be preferentially expressed in memory T lymphocytes ($CD3^+/CD45R0^+$) in one study [44]. Patients with SARS who were of advanced age had a higher mortality rate [1–4] and higher virus loads than did younger patients in this study; how these observations are related to the preferential expression of *FGL2* in memory T lymphocytes remains to be investigated.

Most patients with the highest level of virus shedding (>10^6 SARS-CoV RNA copies/mL) were not included in our genetic analysis because of their early death. Therefore, it was not possible to conduct a multivariate analysis to simultaneously examine the effects of age, sex, and genetic polymorphism on death. The biological basis of the increased virus load in older patients remains elusive, be it the senescence of immunity (see Stout and Suttles [45] for a review), which undermines the efficacy of both innate and adaptive immunity, or the enhanced virus infection, for which age is a confounder [21].

Because the Han descendants, who constitute >98% of the population of Taiwan, are genetically homogenous, according to a recent study of the SNPs of the major histocompatibility complexes [46], population stratification that confounds our

Table 3. Nasopharyngeal severe acute respiratory syndrome (SARS)–associated coronavirus (SARS-CoV) load and length of survival for 71 patients with fatal cases of SARS.

	No. (%) of patients, by nasopharyngeal SARS-CoV load			
Length of survival[a]	Undetectable (n = 11)	≤10^5 RNA copies/mL (n = 42)	>10^5 RNA copies/mL (n = 18)	P[b]
≤14 days	2 (18.2)	20 (47.6)	14 (77.8)	.0015
>14 days	9 (81.8)	22 (52.4)	4 (22.2)	

[a] After the onset of SARS symptoms.
[b] Determined by Cochran-Armitage trend test.

Table 4. Genetic polymorphisms and nasopharyngeal virus loads of 94 patients with severe acute respiratory syndrome (SARS).

Gene (position from start ATG), genotype	Nasopharyngeal SARS-CoV load			Multivariate logistic[a]		All patients with SARS (n = 94)	Reference group[c] (n = 94)	
	Detectable (n = 49)	Not Detectable (n = 45)	P[b]	OR (95% CI)	P			P
IL1A (−889)			.024/.028		.008			1.0
CC	37	42		1		79	79	
TC	12	3		10.2 (1.82–56.8)		15	15	
IL18 (−607)			.010/.032		.014			.78
GG	9	18		1		27	25	
GT	27	22		4.47 (1.25–17.4)		49	46	
TT	13	5		10.6 (2.03–55.0)		18	23	
FGL2 (+158)			.070/.041		.031			.61
GG	24	32		1		56	61	
GA	23	11		4.0 (1.39–11.48)		34	30	
AA	2	2		4.57 (0.27–75.2)		4	3	
RelB (+23962)			.043/.033		.034			.16
CC	6	11		1		17	29	
CT	26	26		1.95 (.50–7.52)		52	46	
TT	17	8		7.20 (1.47–35.3)		25	19	

NOTE. Data are no. of patients, unless indicated otherwise. SARS-CoV, SARS-associated coronavirus.

[a] Multivariate logistic model including age and sex.

[b] P values for univariate analysis were determined by exact Mantel-Haenszel/permutation test.

[c] Healthy Han Chinese reference population.

results is not of major concern. Furthermore, this work constitutes a population-based study in that all surviving patients with SARS were solicited, and no selection bias was involved in the classification of participating patients with SARS into case and control groups that were based on results of tests to determine virus burden.

In conclusion, the SARS-CoV load during the initial phase of infection, which is crucial to the clinical outcome of patients

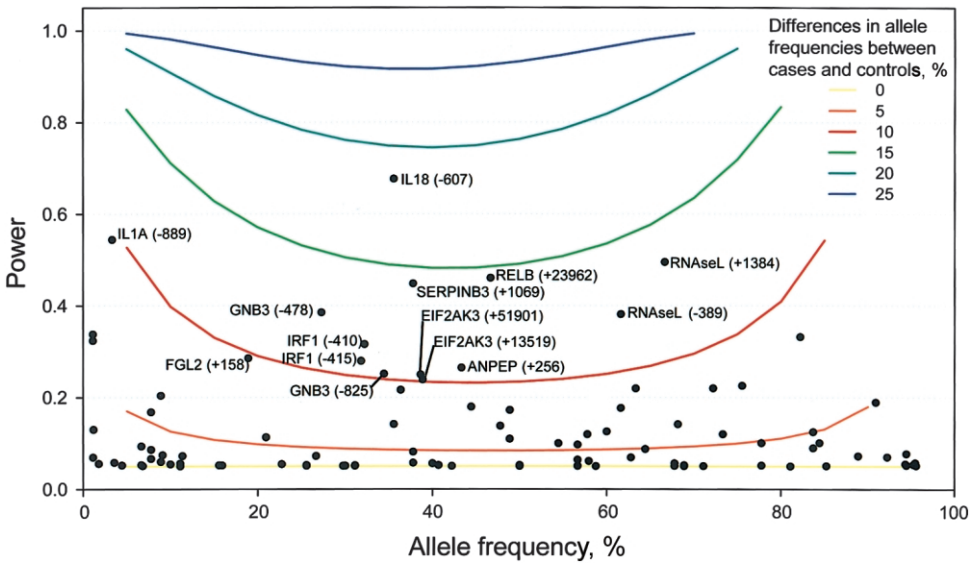

Figure 3. Power plotted against the allele frequency of each single-nucleotide polymorphism. Power is calculated based on the allele frequencies and the sample size of patients with severe acute respiratory syndrome (SARS) with (no. of case patients, 49) or without (no. of control patients, 45) detectable virus shedding. The curved lines denote the zones of differences between the allele frequencies of the case patients and control patients. Each black dot (·) denotes one single-nucleotide polymorphism plotted according to the allele frequency of the control patients with SARS without detectable SARS-associated coronavirus.

with SARS, is shown, for what we believe is the first time, to be associated with polymorphisms of genes involved in innate immunity. These genes, although not claimed to be a comprehensive list, have exemplified potential pathways of innate immunity in response to SARS-CoV or, more broadly, to other viral infections. It is imperative to proceed with functional studies to test whether these genes are indeed functionally active in signaling pathways to the direct suppression of virus replication or to the clearance of virally infected cells. Understanding how the interplay between the virus load during the early stage (<2 weeks of illness) and the later stage (>2 weeks of illness) of host response might contribute to the clinical outcome of SARS should be helpful in the clinical management of patients with SARS.

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