doi: 10.1111/j.1744-313X.2007.00741.x

Lack of association between HLA-A, -B and -DRB1 alleles and the development of SARS: a cohort of 95 SARS-recovered individuals in a population of Guangdong, southern China

P. Xiong,* X. Zeng,† M. S. Song,‡ S. W. Jia,§ M. H. Zhong,* L. L. Xiao,¶ W. Lan,§ C. Cai,† X. W. Wu,* F. L. Gong* & W. Wang‡****

Summary

Severe acute respiratory syndrome (SARS), caused by infection with a novel coronavirus (SARS-CoV), was the first major novel infectious disease at the beginning of the 21st century, with China especially affected. SARS was characterized by high infectivity, morbidity and mortality, and the confined pattern of the disease spreading among the countries of South-East and East Asia suggested the existence of susceptible factor(s) in these populations. Studies in the populations of Hong Kong and Taiwan showed an association of human leucocyte antigen (HLA) polymorphisms with the development and/or severity of SARS, respectively. The aim of the present study was to define the genotypic patterns of HLA-A, -B and -DRB1 loci in SARS patients and a co-resident population of Guangdong province, southern China, where the first SARS case was reported. The samples comprised 95 cases of recovered SARS patients and 403 unrelated healthy controls. HLA -A, -B and -DRB1 alleles were genotyped using polymerase chain reaction with sequence-specific primers. The severity of the disease was assessed according to the history of lung infiltration, usage of assisted ventilation and occurrence of lymphocytopenia. Although the allelic frequencies of A23, A34, B60, DRB1*12 in the SARS group were slightly higher, and A33, -B58 and -B61 were lower than in the controls, no statistical significance was found when the *Pc* value was considered. Similarly, no association of HLA alleles with the severity of the disease was detected. Thus, variations in the major histocompatibility complex are unlikely to have contributed significantly to either the susceptibility or the severity of SARS in the population of Guangdong.

* Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, † Guangdong Provincial TCM Hospital, Guangzhou, China, ‡ College of Life Sciences, Graduate School of the Chinese Academy of Sciences, Beijing, China, § Peking University Shenzhen Hospital, Shenzhen, China, ¶ Tissue-typing Center of Sun Yat-sen University, Guangzhou, China, and ** School of Public Health and Family Medicine, Capital Medical University, Beijing, China.

P. Xiong, X. Zeng and M. S. Song contributed equally to this work.

Received 5 August 2007; revised 5 August 2007; accepted 27 November 2007

Correspondence: Wei Wang, Department of Biology, College of Life Sciences, Graduate School of the Chinese Academy of Sciences, No. 19A, Yuquan Road, Beijing 100049, China. Tel: +86 10 8825 6373; Fax: +86 10 8825 6373; E-mail: wei6014@yahoo.com

Introduction

Severe acute respiratory syndrome (SARS) originated in southern China in November 2002, reached Hong Kong in February 2003, and thereafter spread rapidly to 29 countries/regions on five continents. By the end of the epidemic, the global cumulative patient total was 8098 with 774 deaths. Seven Asian countries/regions were among the top 10 populations affected, China and Hong Kong accounting for 87% of all cases and 84% of deaths from SARS (Lam *et al*., 2003). SARS was mostly confined to the populations of South-East and East Asia (Mainland Chinese, Hong Kong residents, Vietnamese, Singaporeans and Taiwanese). The disease is caused by infection with a novel single-stranded RNA coronavirus (Peiris *et al*., 2003) and starts with an influenza-like illness characterized by non-specific, systemic symptoms, followed by the rapid development of an unspecific bronchopneumonia associated with lower respiratory tract or gastrointestinal symptoms. Most patients recovered after 1 or 2 weeks, but some developed an acute respiratory distress syndrome (Zhong *et al*., 2003). The confined geographical and ethnic spread pattern of the disease suggested the possibility of genetic variations that had influenced susceptibility to infection with SARS-CoV and the development of the disease itself.

Recent reports have suggested that a single nucleotide polymorphism (SNP) in the 3′ UTR of the OAS-1 and MxA promoter region is associated with host susceptibility to SARS infection in the Han Chinese population (Hamano *et al*., 2005; He *et al*., 2006). Also, an association study that examined polymorphisms in the cytokine response genes [interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha and interleukin (IL)-10] showed that the 874 A/T IFN-gamma SNP was a risk factor for SARS infection, although no association was seen with either TNF-alpha or IL-10 polymorphisms (Chong *et al*., 2006). Chan *et al*. (2006) reported that individuals who were homozygous for CLEC4M (a binding receptor for SARS

Co-V) were less susceptible to SARS (Chan *et al.*, 2006). Conversely, association studies failed to demonstrate that ACE2 SNPs were implicated in SARS (Itoyama *et al.*, 2005), and that the ACE I/D polymorphism was neither directly related to increased susceptibility to SARS-coronavirus infection nor the clinical outcome of SARS patients (Chan *et al.*, 2005).

The human leucocyte antigen (HLA) system is widely used to investigate the aetiology of infectious diseases and autoimmune disorders. Studies in Hong Kong (Ng *et al.*, 2004) and Taiwan (Lin *et al.*, 2003) populations showed an association of HLA-B*0703–DRB1*0301 with the development of SARS, and HLA-B46 with the severity of SARS, respectively. However, the data on which these reports were based were limited in number, and no comparable data from Mainland China have been available. In this study, samples from 95 SARS patients and 403 healthy controls were typed for HLA-A, -B and -DRB1 alleles to determine if an association existed between HLA variations and SARS.

Subjects and methods

Subjects

The cohort of 95 SARS-recovered individuals comprised patients admitted to the Guangdong Provincial TCM Hospital in Guangzhou (82 cases) and the Peking University Shenzhen Hospital in Shenzhen (13 cases) between March and June 2003. All patients were diagnosed according to the World Health Organization (WHO) case definition of probable SARS case (WHO, 2003). All of the SARS subjects were ethnic Chinese and none were biological relatives (only the first infected patient in a family was included if several family members had been diagnosed). The age range of the patients was 19–72 years, and the male:female ratio was 0.76. All 95 SARS patients showed radiographic evidence of lung infiltrates and were seropositive for SARS-CoV antibody during the SARS episode. A sex- and age-matched healthy control group of 403 healthy volunteers resident in the same geographical area were also genotyped. The mean age of the controls was 45.6 years, and the male:female ratio was 0.73. Informed written consent was obtained from all tested subjects.

The hospital records of the 95 SARS cases indicated that 63 patients (66.3%) had lymphocytopenia ($< 1 \times 10^9$ L^{-1}), with two patients (2.11%) showing diffuse infiltration of both lungs. Auxiliary ventilation was applied by mask in 30 cases (31.6%), with intubation additionally used in two cases (2.1%). Lymphocytopenia, lung involvement and auxiliary ventilation were considered as indicative of disease severity in our study.

HLA allele typing

Peripheral blood samples were collected in EDTA vacutainers, with genomic DNA prepared by standard phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation for immediate storage at –20 °C. DNA quantity and quality were assessed by spectroscope (Pharmacia Biotech, Uppsala, Sweden) absorbance at 260 and 280 nm. The identification of HLA-A, -B and -DRB1 alleles in all subjects was performed by DNA typing, using polymerase chain reaction with sequence-specific primers (PCR-SSP) based on the protocol of Bunce *et al.* (1995), with slight modification to correspond with serological typing results. The conversion was based on the WHO nomenclature table of correspondence (Marsh *et al.*, 2002).

Briefly, PCR mixtures consisted of 67 mM Tris base pH 8.8, 16.6 mM ammonium sulphate, 2 mM magnesium chloride, 0.01% (v/v) Tween 20, 200 pmol of each dNTP, 1–4 pmol of each allele-specific primer, 0.1 pmol of DRB1 control primers or 2 pmol of APC control primers, between 0.1 and 0.01 pg DNA and 0. 1875 U Taq polymerase. The primer concentrations in the primer mixes were empirically titrated with positive and negative samples to ensure maximum efficiency. PCR amplications were carried out in an MJ Research PTC-100 (Waltham, MA, USA) machine. The cycling parameters for 13 µl reactions in rapid-cycling PCR machines were: 1 min at 96 °C followed by five cycles of 25 s at 96 °C, 45 s at 70 °C, 45 s at 72 °C, followed by 21 cycles of 25 s at 96 °C, 50 s at 65 °C, 45 s at 72 °C, followed by four cycles of 25 s at 96 °C, 60 s at 55 °C and 20 s at 72 °C.

Statistical analysis

To assess the association of HLA variations with SARS infection, the frequencies of HLA class I and II alleles were estimated by direct counting in both the patient and the control groups. The frequencies of HLA alleles (HLA antigens) in patients with SARS and healthy controls were each tested for deviation from Hardy–Weinberg equilibrium, and compared using a χ^2 test (continuity correction for the χ^2 test was used where a small number of alleles was observed ($E < 5$) and a two-tail Fisher's exact test was used to calculate the *P*-value when $E < 1$. Corrected *P*-values (*Pc*) were obtained by multiplying the number of antigens or alleles (Edward, 1974: reference not listed) at each locus: A ($n = 15$), B ($n = 32$) and DRB1 ($n = 13$). A *Pc* value < 0.05 was considered statistically significant. Odds ratios (OR) rather than relative risks (RR) were quoted since ORs are most commonly used in HLA association studies. The values were obtained from standard contingency table analysis using Haldane's modification of Woolf's method (Haldane, 1956). An OR value > 1.0 implies that subjects carrying the antigen are at an increased risk of a disease (a positive association), while an OR value < 1.0 implies that subjects carrying the antigen are at a lower risk of a disease than those not carrying the antigen (a negative association).

To assess the association of HLA alleles with the severity of our SARS cases, SARS patients were divided into assisted ventilation (mask and intubation) ($n = 30$) and non-assisted ventilation groups ($n = 65$). The patients were also grouped according to the occurrence of lymphocytopenia ($< 1 \times 10^9$ L^{-1}) ($n = 63$). Four grades were scored to indicate the area of lung infiltration as indicated

by the chest radiographs: grade 1 indicated 1/4 (n = 25), 2 for 2/4 (n = 58), 3 for 3/4 (n = 10) and 4 for 4/4 (n = 2). The association between lymphocytopenia and HLA variation was analysed using a one-way ANOVA using the SNK test for multiple comparisons. Mordridit score analysis was applied when comparing associations between lung infiltrates and HLA. All analyses were performed using SPSS 11.0. All probabilities were two tailed with a P-value < 0.05 considered statistically significant.

Results

The allelic frequencies of HLA-A, -B and -DRB1 variants in the 95 SARS patients and 403 healthy control subjects are listed in Tables 1–3, respectively. The Fisher's exact test showed that in SARS patients the frequencies of HLA-A23 [2.1%, P = 0.0361, OR, 21.6 (95%CI, 0.39–36.90)], -A34 [2.1%, P = 0.0361, OR, 21.6 (95%CI, 0.39–36.90)], -B60 [37.9%, P = 0.0061, OR, 1.9 (95%CI, 0.23–2.13)] and HLA-DRB1*12 [32.6%, P = 0.046, OR, 1.6 (95%CI, 0.20–2.01)] were higher than the control group, whereas the frequencies of HLA-A33 [12.6%, P = 0.02, OR, 0.5 (95%CI, 0.39–1.37)], HLA-B58 [11.6%, P = 0.027, OR, 0.5 (95%CI, 0.07–1.41)] and HLA-B61 [1.05%, P = 0.0176, OR, 0.2 (95%CI, 0.01–2.05)] were lower. However, after correction of the P-value by multiplying the number of alleles at each locus, all statistical significance was lost. With regard to the association of HLA polymorphism with severity of SARS, the auxiliary ventilation, areas of lung infiltrate and lymphocytopenia all seemed to occur randomly with HLA-A, -B, and -DRB1 alleles, since none of the associations tested were significant (data not shown).

No deviation from Hardy–Weinberg equilibrium was observed when the frequencies of HLA alleles were tested in the SARS patients or the healthy controls (P > 0.05).

Table 1. Allelic frequencies of human leucocyte antigen (HLA)-A in SARS patients and healthy controls

HLA-A	SARS		Normal				
Allele	*n*	%	*n*	%	OR (95%CI)	*P*	*Pc*
1	5	5.26	11	2.73	2.07 (0.50–3.75)	0.349	
2	49	51.58	207	51.36	1.01 (0.64–1.56)	0.97	
3	2	2.11	17	4.22	0.59 (0.23–2.76)	0.503	
11	54	56.84	207	51.36	1.24 (0.70–1.72)	0.336	
23	2	2.11	0	0	21.58 (0.39–36.90)	0.0361	ns
24	25	26.32	103	25.56	1.05 (0.62–1.69)	0.879	
26	4	4.21	13	3.23	1.42 (0.41–3.32)	0.872	
29	2	2.11	7	1.74	1.41 (0.30–4.46)	1	
30	7	7.37	24	5.96	1.31 (0.49–2.58)	0.608	
31	4	4.21	14	3.47	1.32 (0.40–4.21)	0.968	
32	0	0	7	1.74	0.28 (0.07–4.63)	0.418	
33	12	12.63	95	23.57	0.48 (0.39–1.37)	0.02	
34	2	2.11	0	0	21.58 (0.39–36.90)	0.0361	ns
68	1	1.05	7	1.74	0.83 (0.19–4.43)	0.981	
74	0	0	2	0.50	0.84 (0.10–9.01)	1	

n, number of individuals carrying a given allele; *P*, *P*-value; *Pc*, corrected *P*-value; OR, odds ratio; ns, not significant.

Table 2. Allelic frequencies of human leucocyte antigen (HLA)-B in SARS patients and healthy controls

HLA-B	SARS		Normal				
Allele	*n*	%	*n*	%	OR (95%CI)	*P*	*Pc*
7	1	1.05	13	3.23	0.46 (0.16–3.19)	0.419	
8	1	1.05	7	1.74	0.84 (0.19–4.43)	0.981	
13	20	21.05	86	21.34	1.00 (0.58–1.71)	0.951	
14	1	1.05	1	0.25	4.26 (0.26–13.49)	0.345	
16	9	9.47	38	9.43	1.04 (0.49–2.12)	0.989	
18	0	0	4	0.99	0.46 (0.08–6.21)	1	
27	2	2.11	16	3.97	0.63 (0.23–2.85)	0.568	
35	7	7.34	18	4.47	1.77 (0.54–3.02)	0.366	
37	3	3.16	6	1.49	2.31 (0.41–5.02)	0.503	
44	1	1.05	18	4.47	0.33 (0.14–2.70)	0.206	
45	0	0	3	0.74	0.60 (0.09–7.24)	1	
46	18	18.95	99	24.57	0.73 (0.50–1.51)	0.245	
47	0	0	2	0.50	0.84 (0.10–9.01)	1	
48	5	5.26	16	3.97	1.43 (0.45–3.04)	1	
50	0	0	4	0.99	0.46 (0.08–6.21)	1	
51	11	11.58	47	11.66	1.02 (0.51–1.98)	0.982	
52	3	3.16	17	4.22	0.84 (0.31–2.80)	0.855	
53	1	1.05	0	0	12.81 (0.21–33.73)	0.191	
54	5	5.26	16	3.97	1.43 (0.45–3.04)	0.779	
55	4	4.21	15	3.72	1.23 (0.39–3.07)	1	
56	0	0	11	2.73	0.18 (0.06–3.69)	0.215	
57	0	0	5	1.24	0.38 (0.08–5.52)	0.589	
58	11	11.58	87	21.59	0.49 (0.38–1.41)	0.027	ns
60	36	37.89	97	24.07	1.93 (0.83–2.13)	0.0061	ns
61	1	1.05	31	7.69	0.19 (0.11–2.05)	0.0176	ns
62	17	17.89	48	11.91	1.63 (0.68–2.24)	0.119	
63	0	0	1	0.25	1.40 (0.10–12.92)	1	
67	1	1.05	5	1.24	1.15 (0.21–5.35)	1	
71	1	1.05	7	1.74	0.84 (0.19–4.43)	0.981	
72	1	1.05	0	0	12.81 (0.27–33.73)	0.191	
75	10	10.53	49	12.16	0.88 (0.47–1.89)	0.658	
76	0	0	1	0.25	1.40 (0.10–12.92)	1	

n, number of individuals carrying a given allele; *P*, *P*-value; *Pc*, corrected *P*-value; OR, odds ratio; ns, not significant.

Table 3. Allelic frequencies of human leucocyte antigen (HLA)-DRB1 in SARS patients and healthy controls

HLA-DRB1	SARS		Normal				
Allele	*n*	%	*n*	%	OR (95%CI)	*P*	*Pc*
1	2	2.11	5	1.24	1.94 (0.33–5.43)	0.873	
3	13	13.68	74	18.36	0.72 (0.47–1.61)	0.28	
4	20	21.05	89	22.08	0.95 (0.57–1.68)	0.827	
7	11	11.58	50	12.41	0.95 (0.50–1.92)	0.825	
8	14	14.74	48	11.91	1.30 (0.60–2.10)	0.453	
9	22	23.16	104	25.81	0.88 (0.56–1.59)	0.593	
10	5	5.26	11	2.73	2.07 (0.50–3.75)	0.349	
11	6	6.32	45	11.17	0.57 (0.34–1.80)	0.161	
12	31	32.63	92	22.83	1.64 (0.77–2.01)	0.046	ns
13	6	6.32	45	11.17	0.57 (0.34–1.80)	0.161	
14	12	12.63	63	15.63	0.80 (0.48–1.73)	0.462	
15	19	20.00	114	28.29	0.64 (0.48–1.41)	0.1	
16	12	12.63	35	8.68	1.55 (0.62–2.38)	0.237	

n, number of individuals carrying a given allele; *P*, *P*-value; *Pc*, corrected *P*-value; OR, odds ratio; ns, not significant.

Table 4. Human leucocyte antigen (HLA) alleles reported suspect being associated with SARS in three populations

	SARS		Control				
HLA allele Population	*n* (*N*)	%	*n* (*N*)	%	OR (95%CI)	*P*	*Pc*
B*4601/B46							
Taiwan	15 (66)	22.72	52 (380)	13.68	1.86 (1.02–3.54)	0.06	
(A)							
(B)	15 (66)	22.72	25 (202)	12.38	2.08 (1.04–4.24)	0.04	ns
(C)	6 (10)		25 (202)	12.38	10.62 (2.80–40.26)	0.0008	0.0279
(D)	6 (10)		9 (56)	16.07	7.83 (1.83–33.47)	0.007	ns
Hong Kong	27 (83)	32.5	5125 (18 774)	27.3	1.28 (0.81–2.04)	0.3229	
Guangzhou	18 (95)	18.95	99 (403)	24.57	0.73 (0.50–1.51)	0.245.	
B*1301/B13							
Taiwan	1 (66)	1.515	34 (380)	8.947	0.16 (0.02–0.97)	0.02	ns
Hong Kong	20 (83)	24.1	3298 (18 774)	17.6	1.49 (0.90–2.47)	0.1466	
Guangzhou	20 (95)	21.05	86 (403)	21.34	1.00 (0.58–1.71)	0.951	
B*0703/B7							
Taiwan	NA		NA				
Hong Kong	9 (83)	10.8	544 (18 774)	2.9	4.08 (2.03–8.18)	0.00072	
Guangzhou	1 (95)	1.05	13 (403)	3.23	0.46 (0.16–3.19)	0.419	
DRB1*0301							
Taiwan	NA		NA				
Hong Kong	1 (79)	1.3	42 (250)	16.8	0.06 (0.01–0.47)	0.00008	
Guangzhou	13 (95)	13.68	74 (403)	18.36	0.72 (0.47–1.61)	0.28	

In Taiwan study, HLA allele frequencies were estimated by direct counting. For HLA–B46 association: (A) 33 cases of probable SARS patients compared with 190 healthy unrelated controls; (B) 33 cases of probable SARS patients with 101 high risk non-infected health care workers; (C) 5 cases of 'severe cases' (deceased or intubated) probable SARS patients with 101 high risk non-infected health care workers; (D) 5 cases of 'severe cases' (deceased or intubated) probable SARS patients with 28 'excluded fever patients'. For the other alleles listed, 33 cases of probable SARS patients compared with 190 healthy unrelated controls.

n, number of individuals carrying a given allele; *N*, number of cases tested; *P*, *P*-value; *Pc*, corrected *P*-value; OR, odds ratio; ns, not significant; NA, not available.

Discussion

Classical HLA classes I and II molecules are essential to present antigenic peptide (including viral peptide) for T-cell recognition and thus initiate an adaptive immune response. To assist the survival of a population, the genetic cluster of HLA loci manifests a striking level of polymorphism to protect the host from any pathogen mutation eluding host immune surveillance. Antigenic peptides binding to different alleles of HLA molecules vary in their affinity, with the HLA allotype thus affecting the host immune response to a given antigen.

Studies have indicated that HLA variations are associated with susceptibility or resistance to a wide range of infections, including malaria, tuberculosis, leprosy, HIV and hepatitis virus persistence (De Vries *et al.*, 1979; Hill, 1998; Liu *et al.*, 2003). SARS has been shown to be caused by infection with SARS-CoV, with major lung involvement and consequent high infectivity and mortality. Although many questions remain to be answered, immunological mechanisms are believed to be involved in the development of the disease. As previously noted, in the 2002–03 SARS epidemic, the disease was mostly confined to South-East and East Asian populations, suggestive of greater susceptibility to SARS-CoV.

Guangdong province, where the SARS epidemic started, has a population of 85.2 million. The first case of SARS that fulfilled the WHO case definition was reported in Foshan, a city about 20 km from Guangzhou (formerly Canton), the provincial capital (Zhong *et al.*, 2003; Xu *et al.*, 2004). Phylogenetic studies have indicated that the populations of Guangdong, Hong Kong and Taiwan populations largely share common origins (Chu *et al.*, 1998). For example, over 98% of the Hong Kong population are Chinese, and the majority have ancestral origins in neighbouring Guangdong province and are of Han Chinese ethnicity (Chang & Hawkins, 1997). The present-day population of Taiwan comprises indigenous Taiwanese groups, and people of Minnan and Hakka ethnicity who are the descendants of settlers from the provinces of Fujian and Guangdong during the last few centuries (Lin *et al.*, 2001). It is of interest that the SARS cases in Taiwan mainly consisted of southern Chinese origin Taiwanese rather than the indigenous peoples (Lin *et al.*, 2003).

Similarities in the genetic profiles of these three populations, Guangdong, Hong Kong and Taiwan offers the opportunity of identifying possible associations between HLA alleles and SARS infection. Hypothetical SARS-associated HLA alleles among the three populations are listed in Table 4, and it is obvious that the SARS-associated HLA alleles that have been reported in any single population could not be verified in either of the other two populations. It also seems that the Guangzhou population was more susceptible to the SARS epidemic irrespective of their HLA profiles.

The mutation of SARS-CoV could give rise to variations and discrepancies in the results of the HLA association studies in the three populations, and large-scale studies are essential for the demonstration of an association of HLA variation with susceptibility to a disease. On the other hand, mutation of SARS-CoV might result in changes of its T-cell receptor epitope, which would require a specific HLA allele(s) for presentation. Because of the poor fidelity of RNA-dependent RNA polymerase, genetic variation typically gives rise to a heterogeneous virus pool in RNA populations, including coronaviruses. This feature makes viruses highly adaptable and contributes to difficulties in preventing and controlling viral diseases. The virus isolated from the Guangzhou patients is the prototype of the SARS-CoV, with a reported moderate mutation rate, that subsequently was found in Hong Kong and countries around the world (Zhao *et al*., 2004). The Guangzhou SARS-CoV has the highest homology (> 99%) with the sequences of the SARS-CoV that were isolated from SARS cases in Hong Kong, Beijing, Singapore, Canada and the USA, suggesting that all of these SARS-CoV are closely related (Zhong *et al*., 2003), therefore mutation of SARS-CoV could not be considered as a major contributory factor in the lack of HLA association observed in the present study.

During the SARS episode in Guangdong, six of the 95 SARS patients died of the disease. No samples from these deceased patients were available for our study. However, a history of lymphocytopenia, lung involvement and auxiliary ventilation were considered as surrogate measures of the severity of the disease, and no significant association of HLA variations with these parameters was observed among the patients, who instead displayed a random distribution of HLA-A, -B and -DRB1 alleles.

In conclusion, the confined regional spread of the 2002–03 SARS epidemic appears to have been mostly dependent on factors such as relative population density, the frequency and extent of travel of affected individuals, and the rapid introduction and uptake of protective measures, rather than to HLA variation in the population.

Acknowledgements

This work was supported by the Grants of 100-Chinese Merit Scientist Program, Chinese Academy of Sciences, China Postdoctoral Science Foundation (20060390536), Major State Basic Research Program of China (2001CB510008, 2005CB522804, 1006AA02Z434), National Natural Science Foundation, China (30472000) and Beijing Natural Science Foundation (KZ200610025014). We thank Professor Alan H Bittles of Edith Cowan University, Australia, for kind comments and language editing of the manuscript.

References

Bunce, M., O'Neill, C.M., Barnardo, M.C., Krausa, P., Browning, M.J., Morris, P.J. & Welsh, K.I. (1995) Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens*, **46**, 355.

Chan, K.C., Tang, N.L., Hui, D.S., Chung, G.T., Wu, A.K., Chim, S.S. *et al*. (2005) Absence of association between angiotensin converting enzyme polymorphism and development of adult respiratory distress syndrome in patients with severe acute respiratory syndrome: a case control study. *BMC Infectious Diseases*, **5**, 26.

Chan, V.S., Chan, K.Y., Chen, Y., Poon, L.L., Cheung, A.N., Zheng, B. *et al*. (2006) Homozygous L-SIGN (CLEC4M.) plays a protective role in SARS coronavirus infection. *Nature Genetics*, **38**, 38.

Chang, Y.W. & Hawkins, B.R. (1997) HLA class I and class II frequencies of a Hong Kong Chinese population based on bone marrow donor registry data. *Human Immunology*, **56**, 125.

Chong, W.P., Ip, W.K., Tso, G.H., Ng, M.W., Wong, W.H., Law, H.K. *et al*. (2006) The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. *BMC Infectious Diseases*, **6**, 82.

Chu, J.Y., Huang, W., Kuang, S.Q., Wang, J.M., Xu, J.J., Chu, Z.T. *et al*. (1998) Genetic relationship of populations in China. *Proceedings of the National Academy of Sciences, USA*, **95**, 11763.

De Vries, R.R.P., Meera Khan, V.P., Bernini, L.F., van Loghem, E. & van Rood, J.J. (1979) Genetic control of survival in epidemics. *Immunogenetics*, **6**, 271.

Edward, J.H. (1974) HLA and disease, the detection of associations. *Immunogenetics*, **1**, 249.

Haldane, J.B. (1956) The estimation and significance of the logarithm of a ratio of frequencies. *Annals of Human Genetics*, **20**, 309.

Hamano, E., Hijikata, M., Itoyama, S., Quy, T., Phi, N.C., Long, H.T. *et al*. (2005) Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population. *Biochemical and Biophysical Research Communication*, **329**, 1234.

He, J., Feng, D., de Vlas, S.J., Wang, H., Fontanet, A., Zhang, P. *et al*. (2006) Association of SARS susceptibility with single nucleic acid polymorphisms of OAS1 and MxA genes: a case-control study. *BMC Infectious Diseases*, **6**, 106.

Hill, A.V. (1998) The immunogenetics of human infectious diseases. *Annual Review of Immunology*, **16**, 593.

Itoyama, S., Keicho, N., Hijikata, M., Quy, T., Phi, N.C., Long, H.T. *et al*. (2005) 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. *American Journal of Medical Genetics*, **136**, 52.

Lam, W.K., Zhong, N.S. & Tan, W.C. (2003) Overview on SARS in Asia and the World. *Respirology*, **8**, S2.

Lin, M., Chu, C.C., Chang, S.L., Lee, H.L., Loo, J.H., Akaza, T., Juji, T., Ohashi, J. & Tokunaga, K. (2001) The origin of Minnan and Hakka, the so called 'Taiwanese', inferred by HLA study. *Tissue Antigens*, **57**, 192.

Lin, M., Tseng, H.K., Trejaut, J.A., Lee, H.L., Loo, J.H., Chu, C.C. *et al*. (2003) Association of HLA class I with severe acute respiratory syndrome coronavirus infection. *BMC Medical Genetics*, **4**, 9.

Liu, C., Carrington, M., Kaslow, R.A., Gao, X., Rinaldo, C.R., Jacobson, L.P., Margolick, J.B., Phair, J., O'Brien, S.J. & Detels, R. (2003) Association of polymorphisms in human leukocyte antigen class I and transporter associated with antigen processing genes with resistance to human immunodeficiency virus type 1 infection. *Journal of Infectious Diseases*, **187**, 1404.

Marsh, S.G., Albert, E.D., Bodmer, W.F., Bontrop, R.E., Dupont, B., Erlich, H.A. *et al*. (2002) Nomenclature for factors of the HLA system, 2002. *Tissue Antigens*, **60**, 407.

Ng, M.H., Lau, K.M., Li, L., Cheng, S.H., Chan, W.Y., Hui, P.K., Zee, B., Leung, C.B. & Sung, J.J.Y. (2004) Association of

human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. *Journal of Infectious Diseases*, **190**, 515.

Peiris, J.S., Lai, S.T., Poon, L.L., Guan, Y., Yam, L.Y., Lim, W. *et al.* (2003) Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*, **361**, 1319.

World Health Organization (WHO) (2003) Case definitions for surveillance of severe acute respiratory syndrome (SARS). Available at: http://www.who.int/csr/sars/casedefinition/en/. Accessed 7 December 2004.

Xu, R.H., He, J.F., Evans, M.R., Peng, G.W., Field, H.E., Yu, D.W. *et al.* (2004) Epidemiologic clues to SARS origin in China. *Emerging Infectious Diseases*, **10**, 1030.

Zhao, Z., Li, H., Wu, X., Zhong, Y., Zhang, K., Zhang, Y., Boerwinkle Eric. Fu Y. (2004) Moderate mutation rate in the SARS coronavirus genome and its implications. *BMC Evolution Biology*, **4**, 21.

Zhong, N.S., Zheng, B.J., Li, Y.M., Poon, L.L.M., Xie, Z.H., Chan, K.H. *et al.* (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet*, **362**, 1353.

