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Immunogenetics in SARS: a case-control study

Introduction

Severe acute respiratory syndrome (SARS) was first reported in Guangdong province, China, in November 2002. Subsequently, multiple outbreaks occurred globally in early 2003, affecting over 8000 individuals of whom 700 died.^{1,2} This highly infectious disease was caused by a novel coronavirus (SARS-CoV).³ The clinical course was unpredictable and marked by a high fatality rate.¹ Its clinical severity varied from a febrile condition with mild respiratory symptoms and radiological evidence of lung involvement to severe respiratory distress with extensive lung damage and recourse to assisted ventilation.¹ In addition, individual susceptibility to the infection by the SARS-CoV was also variable. In SARS families, the index patient usually had one or more close household contacts (sharing rooms, meals and toilet facilities) with other family members and could have spread the virus in the early phase of the disease. Although family members were exposed to the same household environments, not all of them contracted SARS. This suggests a differential susceptibility to infection among family members. The mechanism of this variation remains unclear.

The T-cell system is a critical effector pathway for killing viruses by our body defences. To facilitate effective killing of the correct viral targets, human leukocyte antigens (HLA), as cell surface proteins, participate in selection and establishment of an antigen specific T-cell repertoire and in subsequent activation of such T cells during initiation of immune responses. Classes I (HLA-A, -B, and -Cw) and II (HLA-DR and -DQ) of the HLA antigens are responsible for CD8- and CD4-T cell responses, respectively. They are highly polymorphic and different alleles specify different functional significances in immune responses. The polymorphism in this machinery may contribute to the variations of patient susceptibility to immune-mediated/controlled diseases.

We examined HLA genotypes of 90 serologically confirmed SARS patients and found a significant increase in HLA-B*0703 frequency and a decrease in HLA-DRB1*0301 frequency in the SARS patients compared to the local Chinese population.⁶ A similar study in Taiwan compared the HLA genotypes of 33 probable SARS patients with the normal population.⁵ No significant association was identified except when comparing a highly selected group of five patients admitted to the intensive care unit with non-SARS health care workers; the HLA-B46 allele was found associated with this small SARS patient group. However, association with HLA-B46 was not found in our study.

Environmental factors such as contact history with SARS patients, prevention practices and personal hygiene are also important determinants for SARS infection. Thus, stringent definition of close contacts is critical for further assignment to susceptible and resistant groups.

Methods

Study design

A case-control study was conducted from April 2005 to April 2007 in 536 SARS index patients and contacts recruited from the Hong Kong Department of Health SARS database (817 SARS patients and 936 contacts), which was established for contact tracing during the SARS outbreak. Verbal informed consent was obtained from each participant over the phone in collaboration with The University of

Key Messages

1. Human leukocyte antigen (HLA) genotypes from 102 SARS patients (susceptible) and 108 SARS contacts (resistant) were obtained.
2. Allelic frequencies of the Class I (HLA-A, -B, and -Cw) and Class II (HLA-DR and -DQ) genes from these genetically unrelated subjects were compared.
3. A significantly higher frequency of DRB4*01010101 was found in the SARS-susceptible than SARS-resistant group. In contrast, significantly higher frequencies of HLA-B*1502 and HLADRB3*030101 were found in the SARS-resistant than SARS-susceptible group. However, none of these associations was significant after Bonferroni correction. Further, analysis of 10/36 genetically related families did not reveal any HLA alleles associated with SARS susceptibility or resistance.
4. We could not confirm previous findings of an HLA association with susceptibility or resistance to SARS. The significance of these associations needs to be validated by further independent studies.

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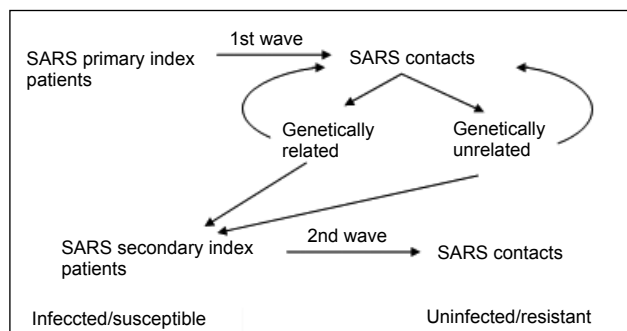


Fig 1. SARS families: human leukocyte antigen markers for differential susceptibilities with similar exposures

Hong Kong SARS research team. Of 433 saliva specimens collected, only 300 subjects including 176 from 36 genetically related ($n=90$) and 36 genetically unrelated ($n=86$) families completed relationship information or had adequate DNA extracted for HLA typing. The SARS index patients were considered as cases (susceptible) and contacts as controls (resistant) [Fig 1]. SARS was serologically confirmed and defined in accordance with the World Health Organization criteria.¹ A close contact was defined as a person who had cared for, lived with (in the same household), or came into direct contact with body fluids of the SARS patients within 10 days before hospital admission. The HLA allelic frequencies between these susceptible and resistant groups were compared for identification of positive and negative HLA risk markers by exclusion of all genetically related members. Genetically related family units within index contact pairs were also pulled out for family analysis with the aim to cross validate any positive observations.

Genetically unrelated case-control analysis

To prevent potential bias, all 90 cases and contacts from the 36 genetically related families were excluded. Thus, to identify HLA susceptible and resistant alleles, 102 SARS patients and 108 contacts were included for comparison of HLA allelic frequencies for the statistical significance of any association.

Genetically related familial analysis

Of 72 families recruited, only 36 were genetically related (Fig 2). The members ($n=86$) of the other 36 genetically unrelated families were husband and wife, co-inhabitants (boyfriends and girlfriends) or in-laws (not shown). The family relationship of the recruited subjects ($n=90$) among the 36 genetically related families is shown (Fig 2). Among these 36 related families, 10 were nuclear with both parents and ≥ 1 children (Fig 2). They were analysed statistically using the general family-based association test (FBAT), defined by $U = \sum T_{ij} (X_{ij} - E(X_{ij}|S_i))$ (1), where i indexes pedigree, j indexes non-founders in the pedigree, and summation is over all i and j ; T_{ij} is a coding function for the trait of interest, and X_{ij} is a coding function for the genotype. The coded genotype was chosen to reflect the selected mode of inheritance; for example, additive, dominant

and recessive. Under the null hypothesis, the expected marker score, $E(X_{ij}|S_i)$, was computed conditionally on the sufficient statistic S_i , which is denoted by S . Under the null hypothesis, Mendel's laws determine which marker alleles are transmitted to the affected offspring. The FBAT compares the observed number of alleles that are transmitted with those expected in Mendelian transmissions. The assumption of Mendelian transmissions is all that is needed to ensure valid results of the FBAT approach. An excess of alleles of one type among the affected indicates that a disease-susceptibility locus for a trait of interest is linked and associated with the marker locus.

Human leukocyte antigen typing

DNA was extracted from the saliva specimens from all SARS patients and contacts. Sequencing based typing (SBT) of HLA-A, -B, -Cw, DRB and DQB1 was performed according to the protocols established by the International Histocompatibility Working Group (IHWG). Sequencing data were analysed using the SBTengine software (Genome Diagnostics, Netherlands). Dynal AllSet+ SSP kit was used for DQA1 typing (Invitrogen, UK). The SBT method based on direct sequencing of the specific HLA locus after PCR-amplification has been established as one of the most reliable and sensitive HLA genotyping techniques. Locus-specific PCR amplification was first performed with primer sets as suggested by IHWG, using Taq polymerase (Promega) on 9700 thermal cycler (Applied Biosystems). Specific PCR products were excised from gels after electrophoresis and purified by gel DNA extraction kit (Qiagen). Sequencing reactions were then performed using BigDye 3.1 reagent (Applied Biosystems) and resolved on a 3130 sequencer (Applied Biosystems). Finally, the sequencing data were analysed by sequence alignment and database matching using the SBTengine software (Genome Diagnostics).

Results

Comparison between case (susceptible) and control (resistant) groups

A total of 102 SARS patients and 108 contact non-SARS subjects were included. The SARS group consisted of 64 males and 38 females aged 13 to 88 (mean, 42) years. The contact non-SARS group consisted of 62 males and 46 females, with age information unavailable. We compared the HLA frequencies between the two groups. A significantly higher frequency of DRB4*01010101 was found in the SARS than contact group (50.98% vs 30.56%, $p=0.0031$, $P_c=0.093$, odds ratio [OR]=2.364, 95% confidence interval [CI]=1.344 to 4.156), whereas significantly higher frequencies of HLA-B*1502 (25.93% vs 9.80%, $p=0.0037$, $P_c=0.2035$, OR=0.3106, 95% CI=0.1421 to 0.6788) and HLA-DRB3*030101 (27.78% vs 14.71%, $p=0.0282$, $P_c=0.846$, OR=0.4483, 95% CI=0.2246 to 0.8948) were observed in the contact than SARS group (Table 1). However, none of these associations was significant after the Bonferroni correction. In addition, in contrast to our

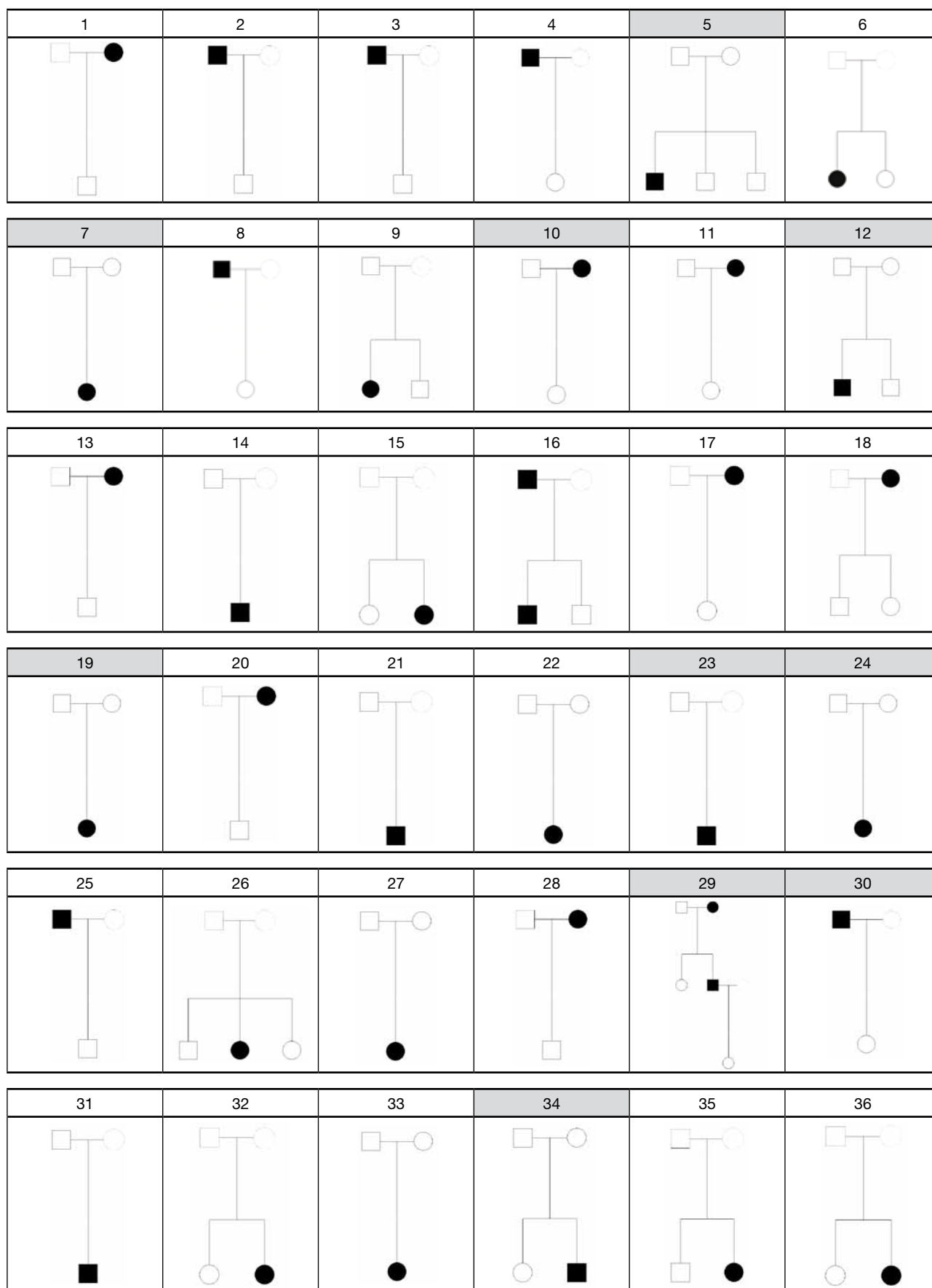


Fig 2. Summary of the pedigrees of the 36 genetically related families

Black boxes/circles indicate SARS index patients, whereas open boxes/circles denote resistant contacts

Table 1. Associations of human leukocyte antigen genes with SAR

	No. (%) of SARS (n=102)	No. (%) of contacts (n=108)	P value	P value (corrected)	Odds ratio	95% CI
B*1502	10 (10)	28 (26)	0.004	0.204	0.311	0.142 to 0.679
DRB3*030131	15 (15)	30 (28)	0.028	0.448	0.448	0.225 to 0.895
DRB4*01010101	52 (51)	33 (31)	0.003	0.093	2.364	1.344 to 4.156

Table 2. Data analysis of the 12 alleles with threshold frequencies by family-based association test (FBAT)

Allele	Freq	HW	Freq parent	HW parents	Nbr info fam	P value (FBAT)	P value (FBATI)	Power (FBAT)	Power (FBATI)	P value (Wald)	P value (Waldl)	Affection Status
A*020301	0.385	0.079	0.472	0	9	0.564	1	0.051	0.05	1	1	No SARS
A*110101	0.615	0.079	0.528	0	9	0.564	1	0.051	0.05	1	1	No SARS
Cw*0403	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS
Cw*040101	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS
B*1521	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS
B*1527	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS
DRB1*0404	0.500	0.002	0.481	0	9	1.000	1	0.050	0.05	1	1	No SARS
DRB1*140101	0.500	0.002	0.519	0	9	1.000	1	0.050	0.05	1	1	No SARS
DRB4*01010101	0.500	0.002	0.557	0	9	1.000	1	0.050	0.05	1	1	No SARS
DRB3*0201	0.500	0.002	0.443	0	9	1.000	1	0.050	0.05	1	1	No SARS
DQB1*030201	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS
DQB1*0502	0.500	0.002	0.500	0	9	1.000	1	0.050	0.05	1	1	No SARS

previous observations, no HLA-B*0703 was identified in either the SARS or contact group, and HLA-DRB1*0301 was observed in equal frequency in both groups.

Analysis of genetically related family units

Ten nuclear families from the 36 genetically related families were analysed using the general FBAT. From this calculation, 12 HLA alleles with significant threshold frequency were further analysed but none was found to be significantly associated (Table 2).

Discussion

Clinical outcome of viral infections is an interactive result of the virus and the host immunogenetic response; HLA is one important immunogenetic determinant that may attribute to the diversity of responses and outcomes.

Our previous exploratory HLA study had revealed a positive association of HLA-B*0703 and negative association of HLA-DRB1*0301 with SARS group. Coinheritance of HLA-B*0703 with HLA-B60 was found to confer an even higher risk for SARS.⁴ There were several limitations of the aforementioned study. First, comparison of the HLA genotypic frequencies was made between the SARS group and the normal population, which had not been exposed to SARS virus. Second, the normal population data for comparison were published in 1997 based mainly on a low-resolution platform including serotyping. Third, the sample size was relatively small. Our current study entailed a number of improvements. First, the control group could actually be viewed as SARS resistant, unlike the normal population, as they were exposed to the SARS virus and the circumstances of exposure were similar to those of the index SARS patients. Thus, more subtle associations if present could be detected. Second, a higher-resolution

platform was employed and the associations if found would yield more refined information. Third, HLA-Cw was also typed for the first time, for potential association with this set of Class I genes.

Our study could not confirm the previous findings of HLA associations using an additional independent SARS cohort with improved design and typing methods. Thus, at this stage the previous findings remain not validated. The reason for the discrepancy may hinge on the sampling and the typing method.

Although positive and negative associations were found with one and two specific HLA genotypes respectively, their significance needs to be validated by further independent studies. Our current findings do not suggest a strong involvement of HLA with the genetic susceptibility to SARS, at least when based on the HLA genes that we studied. Negative findings were also reported in a study performed in China. Other HLA genes or alternative immunogenetic response genes may play a role in this aspect and deserve exploration.

Acknowledgement

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