except the group of university students (P = 0.028), were in Hardy-Weinberg equilibrium (by a Markov chain method in GENEPOP). Furthermore, we compared the genotypes among individuals with SARS with different prognoses. If L-SIGN homozygosity is a protective factor against infection, it may also be associated with better prognosis after acquiring the infection. Therefore, we also examined whether homozygotes had a better prognosis by classifying individuals with SARS who had an uneventful recovery versus those who had severe disease and were admitted to the intensive care unit for mechanical ventilation support (an approach similar to that reported previously[5]). However, we did not detect any significant association (P = 0.9, **Supplementary Table 1** online).

Sample size is the main limitation of both studies. However, these two samples already represent the few 'large' collections of individuals with SARS available for genetic study. To estimate the size of an overall effect, we performed a meta-analysis of the two data sets together by the Mantel-Haenszel test using control groups in Hardy-Weinberg equilibrium (two groups of controls in this study and random controls from Chan *et al.*; total n = 1,468; 462 affected individuals and 1,006 controls). The combined odds ratio was not significant (combined OR = 0.84; 95% confidence interval: 0.66–1.06, P = 0.14).

The difference in the results between the two studies was basically accounted for by a difference in the homozygote proportions in the controls (45.4% in this study versus 55.0% in Chan *et al.*), while the homozygote proportions among individuals with SARS are almost identical (46.9% here versus 46.3% in Chan *et al.*). The reason for the discrepancy in the homozygote proportions in the 'control' groups is not clear. However, a subpopulation difference in allelic and genotypic frequencies exists between northern and southern Chinese. The seven-repeat allele was more prevalent in the Beijing sample (0.7 in Beijing versus 0.64 in Hong Kong; P = 0.05), which also largely accounted for the higher proportion of homozygotes (55.7% in Beijing versus 46.0% in Hong Kong; P = 0.02). Unrecognized subpopulation structure may confound genetic association studies. Results in the study by Chan *et al.* suggested that this confounding factor might be present. There were three groups of controls, including two groups of hospital controls (health care workers who worked in SARS wards and affected individuals attending various outpatient clinics) and a group of blood donor controls. Interestingly, genotype distributions from both groups of hospital-based controls deviated significantly (P < 0.0001) or marginally (P = 0.05) from Hardy-Weinberg equilibrium.

In addition, other yet-unknown mechanisms (such as alternative splicing of the neck region, which could interfere with formation of homotetramers among homozygotes) may account for the discrepancy between the two studies. Replication is an important approach to verify any significant genetic association findings[6,7], and additional association studies are required to establish the putative protective effect of L-SIGN homozygosity against SARS or other infections.

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Lack of support for an association between *CLEC4M* homozygosity and protection against SARS coronavirus infection

To the Editor:
In the January 2006 issue, Chan *et al.*[1] reported a significant association between severe acute respiratory syndrome (SARS) and a variable number of tandem repeats (VNTR) polymorphism in exon 4 of *CLEC4M* in a collection of individuals from Hong Kong. *CLEC4M* encodes L-SIGN ('liver/lymph node-specific ICAM-3 grabbing nonintegrin'), which serves as a receptor for many viruses, including SARS coronavirus (CoV)[2]. Individuals homozygous for *CLEC4M* tandem repeats were reported to be less susceptible to SARS CoV infection. The authors also showed that cells homozygous for *CLEC4M* repeats had a higher binding capacity for SARS CoV, higher proteasome-dependent viral degradation and a lower capacity for *trans* infection. Thus, both genetic and functional studies suggested that homozygosity for *CLEC4M* was associated with protection against SARS CoV infection.

It is important to bear in mind that association studies require replication in independent populations[3]. We therefore attempted to replicate the findings of Chan *et al.* by genotyping the VNTR polymorphism in three additional collections of case-control samples from northern China: (i) the 'Beijing community population', consisting of 339 individuals with SARS and 227 random controls recruited from the community[4]; (ii) the 'Beijing health care worker (HCW) population', consisting of 42 health care workers infected with SARS during the course of hospital duty and 40 health care workers who had worked in SARS wards but remained free of disease and were confirmed to be seronegative for SARS[5] and (iii) the 'Tianjin population', consisting of 60 individuals with SARS and 129 disease-free controls (including 85 random controls and 44 health care workers)[6]. The three collections of case-control samples and their ascertainment criteria have been described in detail previously (**Supplementary Methods** online)[4–6]. All groups except the individuals with SARS from the Beijing community were in Hardy-Weinberg equilibrium. We found no significant differences in allele, genotype and homozygote or heterozygote frequencies between affected individuals and controls in the three populations (**Table 1** and **Supplementary Table 1** online). Early reports

Table 1 Homozygote or heterozygote frequencies of the *CLEC4M* polymorphism in samples from northern China

	VNTR polymorphism[a]	
	Heterozygotes	Homozygotes
Beijing community population		
All cases (*n* = 339)	139 (41.0%)	200 (59.0%)
Random controls (*n* = 227)	110 (48.5%)	117 (51.5%)
OR (95% c.i.)	1.29 (0.89–1.87)	
P value	0.19	
Cases with comorbid conditions (*n* = 52)	20 (38.5%)	32 (61.5%)
Random controls (*n* = 227)	110 (48.5%)	117 (51.5%)
OR (95% c.i.)	1.36 (0.60–3.07)	
P value	0.46	
Cases without comorbid conditions (*n* = 287)	119 (41.5%)	168 (58.5%)
Random controls (*n* = 227)	110 (48.5%)	117 (51.5%)
OR (95% c.i.)	1.26 (0.86–1.85)	
P value	0.23	
Severe cases (*n* = 19)	8 (42.1%)	11 (57.9%)
Mild cases (*n* = 268)	111 (41.4%)	157 (58.6%)
OR (95% c.i.)	1. 00 (0.38–2.59)	
P value	1. 00	
Beijing HCW population		
HCW cases (*n* = 42)	14 (33.3%)	28 (66.7%)
HCW controls (*n* = 40)	18 (45.0%)	22 (55.0%)
OR (95% c.i.)	1.51 (0.58–3.99)	
P value	0.40	
Tianjin population		
All cases (*n* = 60)	33 (55.0%)	27 (45.0%)
All controls (*n* = 129)	72 (55.8%)	57 (44.2%)
OR (95% c.i.)	1.18 (0.60–2.34)	
P value	0.63	

OR, odds ratio; c.i., confidence interval; VNTR, variable number tandem repeat; HCW, health care worker.
[a]The heterozygotes are used as the reference group, and all ORs and *P* values are adjusted for age and gender. Primer sequences used for genotyping are listed in **Supplementary Table 2** online.

have shown that some nongenetic factors, such as comorbid conditions (including diabetes mellitus, hypertension, heart disease, tuberculosis, asthma and malignancy), are risk factors for the development of SARS[7,8] and may confound the contribution of genetic factors to this disorder. However, after stratification by comorbid conditions, the association remained nonsignificant in our Beijing community population (**Table 1**). Of the 287 affected individuals without comorbid conditions, 19 were individuals with severe SARS who were identified by their admission to intensive care units or by their death, and the remaining 268 were individuals with mildly symptomatic SARS. To account for this, we assessed whether there was an association between homozygosity for *CLEC4M* tandem repeats and severity of SARS, but we found none (**Table 1**). This result may be due to the limited number of individuals with severe SARS in the current study and will require confirmation in additional studies.

Collectively, the results in our three collections of case-control samples from northern China are not supportive of the findings of significant association between the VNTR polymorphism and SARS risk reported by Chan *et al.*[1]. There are several possible reasons for the inconsistent results. First, inadequate power may be an explanation of our negative results. However, the sample size in our Beijing community population (with 339 affected individuals and 227 controls) is approximately similar to that used by Chan *et al.* (with 285 affected individuals and 380 random controls), and this sample size had power >0.94 to replicate the effects by Chan *et al.* (calculated by the genetic power calculator[9]). Additionally, just by using our Beijing community sample set, we have successfully confirmed the positive associations between the mannose-binding lectin polymorphisms and SARS risk[4] that were observed previously in a case-control sample from Hong Kong[10]. Furthermore, the consistency of the negative associations in our Beijing HCW population and our Tianjin population strengthens our results.

Second, there may be a small, population-specific difference in the contribution of *CLEC4M* polymorphism to SARS susceptibility. This might occur if there were population differences in linkage disequilibrium pattern or allele frequencies of *CLEC4M*. Indeed, the homozygote or heterozygote frequencies in individuals with SARS in our Beijing community or Beijing HCW populations were significantly different from those reported for affected individuals in Hong Kong community or Hong Kong HCW populations ($P = 0.0066$ and $P = 0.017$, respectively; **Supplementary Table 1**). Additionally, the difference in allele frequency between Hong Kong outpatient controls and Tianjin random controls and the differences in homozygote or heterozygote frequencies between Hong Kong HCW controls and Tianjin HCW controls were also significant ($P = 0.0064$ and $P = 0.017$, respectively; **Supplementary Table 1**). Furthermore, there was also a significant difference in the genotype and homozygote or heterozygote frequency between the Chinese population and Europeans[1].

Another possibility is that Chan *et al.* unwittingly neglected to account for the potential confounding factors that may distort the contribution of *CLEC4M* VNTR polymorphism to SARS susceptibility. Although Chan *et al.* took stringent precautions to stratify their samples by 'health care worker', population samples may still differ by many other factors that depend on setting and context of recruitment, such as age of the subjects at SARS onset, sex of the subjects and any comorbid conditions. Unfortunately, Chan *et al.* do not provide data with regard to such important information on confounding factors. Last, the initial findings of Chan *et al.* may not represent real associations and might be false positives. In genetic association studies of common diseases, there is a very low prior probability of detecting a true association result when accounting for statistical adjustment for multiple comparisons. Indeed, the genetic associations presented by Chan *et al.* were marginally statistically significant ($P = 0.027$, 0.045 and 0.031, when comparing all SARS samples to random controls,

community SARS to outpatient controls and HCW SARS to HCW controls, respectively).

Thus, we did not find any significant differences in allele, genotype and homozygote or heterozygote frequencies between cases and controls in our three independent populations of northern Chinese. Although the biological plausibility of L-SIGN and the functional evidence of the VNTR polymorphism in the original report remain interesting, we urge that the association between *CLEC4M* polymorphism and SARS be investigated in other subpopulations of ethnic Chinese origin (for example, Taiwanese or Guangdong Chinese) or in those of different ancestry, such as Europeans.

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Chan *et al.* reply:

Tang *et al.* and Zhi *et al.* report that in contrast to our findings[1], they were unable to find association between homozygosity or heterozygosity of the *CLEC4M* (L-SIGN) exon 4 tandem repeat polymorphism and SARS CoV susceptibility in the Chinese population. Their data cannot conclusively negate our findings for the reasons below.

We agree with Tang *et al.* that the difference between their analysis and ours is largely accounted for by the difference in the percentage of homozygosity in controls. Tang's controls included neonatal cord blood, healthy elderly individuals aged >70 years, local university students and control samples collected in Beijing. The homozygosity and heterozygosity distribution of their Beijing controls is very similar to that of our Hong Kong random controls. However, the former three control groups collected in Hong Kong are poorly matched by age for comparison with individuals with SARS and our random controls (**Supplementary Table 1** online), and such an age distribution clearly excludes the population aged 25–69 years, which makes up the largest proportion (>70%) of SARS-infected individuals during the Hong Kong outbreak in 2003 (ref. 2). Age and gender are well-known confounding factors in any case-control study, and for SARS, individuals aged ≥65 years and <18 years are actually associated with a lower risk[3]. Thus, it is surprising that Tang *et al.* chose these specific control groups at extreme age ranges, given that there are no obvious obstacles for recruiting appropriate age-matched controls in Hong Kong. They also failed to perform logistic regression accounting for age differences, which should have been incorporated in their statistical analysis. The age and gender distribution of the SARS and control populations in our study, in contrast, is more properly matched, and by logistic regression, our results remain statistically significant (**Table 1** and **Supplementary Table 1**). Our results remain consistent after accounting for comorbid conditions (**Table 1**).

Tang *et al.* also used control groups differing widely in age to determine if age had any effect on genotype frequencies. However, their data *per se* already showed a significant difference in overall genotype distribution, when comparing neonates versus elderly individuals versus university students ($P = 0.029$, χ^2 test). The difference in genotype between neonates and university students is also significant ($P = 0.009$ by CLUMP), suggesting that age-related selection may exist for *CLEC4M* genotypes. Indeed, age-dependent variation of allele and genotype frequencies has been reported for other genes[4,5].

Tang *et al.* also claim to show a subpopulation difference in allele and genotype frequencies between northern and southern Chinese. They note that "the seven-repeat allele was more prevalent in the Beijing sample (0.7 in Beijing versus 0.64 in Hong Kong, $P = 0.05$), which also largely accounted for the higher proportion of homozygotes (55.7% in Beijing versus 46.0% in Hong Kong, $P = 0.02$)." It seems these quoted Hong Kong figures refer to frequencies from their cord blood group alone. Such a comparison is difficult to justify, again because age is seriously mismatched. It should be noted that there is no difference in either the seven-repeat allele frequency or the proportion of homozygotes between the Hong Kong random controls of Chan *et al.*[1] and the Beijing controls of Tang *et al.* (**Supplementary Table 2** online). Barreiro *et al.*[6,7] investigated the *CLEC4M* homozygote and heterozygote distribution of different ethnic groups and reported that the proportion of homozygotes in East Asians, consisting predominantly of Chinese, was 53% (**Supplementary Table 3** online), a figure similar to the Hong Kong random controls of Chan *et al.* and the Beijing controls of Tang *et al.* There is also no significant difference in allele or genotype frequencies of these two control populations from the East Asian samples of Barreiro *et al.*[7] (**Supplementary Table 2**). When these results are taken together, little subpopulation structure is observed for *CLEC4M* homozygote and heterozygote distribution between the northern and southern Chinese populations.

Finally, given the apparent absence of subpopulation structure for *CLEC4M* homozygote and heterozygote distribution in the Chinese population, we performed a meta-analysis of our data set and that of Tang *et al.* by the Mantel-Haenszel test using all control groups that are in Hardy-Weinberg equilibrium: our random controls and outpatient controls ($n = 670$), and cord blood, healthy elderly individuals and Beijing controls ($n = 827$) of Tang