Short Communication

Relationship between *RANTES* Polymorphisms and Respiratory Syncytial Virus Bronchiolitis in a Japanese Infant Population

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SUMMARY: Respiratory syncytial virus (RSV) is the most important virus associated with bronchiolitis in infants and young children. The regulated upon activation, normal T-cell expressed and secreted protein (RANTES, also known as CCL5) appears to be a key player in the etiology of RSV-infected airway inflammation. In this study, we genotyped three single-nucleotide polymorphisms in the *RANTES* gene: −403G/A, −28C/G, and In1.1T/C in 59 infants with severe RSV bronchiolitis and 201 control subjects. The frequencies of the −403G/A + A/A, −28C/G + G/G, and In1.1T/C + C/C genotypes were significantly lower in patients with severe RSV bronchiolitis than in control subjects, and the frequencies of the −403A, −28G, and In1.1C alleles were significantly lower in RSV patients than in control subjects. The present results suggest that *RANTES* polymorphisms may confer risk for severe RSV bronchiolitis.

Respiratory syncytial virus (RSV) is the most important pathogen causing lower respiratory tract infection in infants and young children (1–3). Bronchiolitis is an important disease in infancy and early childhood, and the development of severe bronchiolitis is closely related to RSV infection. Previous studies have implicated cellular immunity in airway inflammation after RSV infection (4,5). Multiple proinflammatory cytokines and chemokines released by alveolar macrophages and epithelial cells are involved in the activation of cellular immunity after RSV infection (6). Regulated upon activation, normal T-cell expressed and secreted protein (RANTES, also known as CCL5) is a chemokine that attracts monocytes, eosinophils, basophils, and memory T lymphocytes (7–11). RANTES is generated by macrophages, CD8$^+$ T lymphocytes, and epithelial cells (12–15).

The human *RANTES* gene is composed of three exons and two introns (16), and in the *RANTES* gene, three single-nucleotide polymorphisms (SNPs) have been characterized: −403G/A (rs2107538), −28C/G (rs2280788), and In1.1T/C (rs2280789) (17–19). SNPs −403G/A and −28C/G are located in the promoter region of the human *RANTES* gene, and In1.1T/C is located in intron 1. Thus far, a few studies have reported on the association between these *RANTES* SNPs and RSV bronchiolitis (12,20,21), with conflicting results. Tian et al. compared the allele frequency and genotype of *RANTES* −403G/A in an RSV bronchiolitis group to a control group and failed to find a significant differ-

ence (20). Zhao et al. reported a significant association between *RANTES* −28C/G and RSV bronchiolitis (21). Amanatidou et al. examined −403G/A, −28C/G, and In1.1T/C and found no significant association between these SNPs and RSV broncioliitis when tested separately; however, there was a significant difference in the frequency of the genotype combination −28C/C + −403G/A + In1.1T/T between patients and control subjects (12).

The purpose of the present study was to survey the association between genetic variation in the *RANTES* gene and RSV bronchiolitis in a Japanese infant population.

A total of 59 infants who had been hospitalized with severe RSV bronchiolitis at Chiba University Hospital, Asahi Central Hospital, Chiba Children's Hospital, Shimosizu Hospital (Chiba, Japan), and Ono Pediatric Clinic (Nagasaki, Japan) were recruited. The diagnosis of RSV bronchiolitis was established on the basis of wheezing and the presence of RSV antigen in nasopharyngeal secretion specimens. Exclusion criteria included prematurity, chronic respiratory disease, previous wheezing episodes, cardiac disease, and age >24 months. The mean age of the patients (± standard error of the mean [SEM]) was 5.32 ± 0.81 months (range, 1–19 months).

The control subjects were 201 children who had never had a wheezing episode and were selected from 411 children recruited at an elementary school affiliated with Chiba University. This study was approved by the Ethics Committee on Human Research at Chiba University. Informed consent was obtained from the parents or guardians of all subjects.

Peripheral blood was collected in tubes containing ethylenediaminetetraacetic acid. DNA was extracted from blood with a QIAamp DNA Blood Kit (Qiagen, Valencia, Calif., USA) or from buccal cells with a Buc-

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calAmp DNA Extraction Kit (Epicentre Biotechnologies, Madison, Wis., USA), according to the manufacturers' instructions. Genomic DNA was amplified with an Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare UK, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions. *RANTES* gene polymorphisms in In1.1T/C (rs2280789) were genotyped with a Taqman system (Applied Biosystems, Foster City, Calif., USA), while genotyping of −403G/A (rs2107538) and −28C/G (rs2280788) was performed using the SNaPshot method (Applied Biosystems).

Statistical comparisons between patients and control subjects were performed using the contingency χ^2 test or unpaired Student's *t* test. Hardy-Weinberg equilibrium was assessed with a χ^2 goodness of fit test. Strength of linkage disequilibrium (LD) and haplotype frequencies were estimated with SNPAlyze Pro software (version 7.0; Dynacom, Mobara, Japan). All statistical analyses were performed with SPSS Statistics software (version 17.0; SPSS Japan, Tokyo, Japan) unless otherwise stated. *P* values of less than 0.05 were considered significant unless otherwise stated.

We investigated the characteristics of the 59 patients and 201 control subjects. The following were considered risk factors for the development of RSV bronchiolitis: sex, birth weight, the presence of older siblings, breast-feeding, day care attendance during infancy, and parental smoking during infancy. None of these factors differed significantly between the patients and control subjects (data not shown).

The genotypes and allele frequencies of the three SNPs in the patients and control subjects are shown in Table 1. In the control subjects, all the genotypes were in Hardy-Weinberg equilibrium. The −403A, −28G,

and In1.1C alleles were minor alleles. In a dominant model, the frequencies of −403G/A + A/A, −28C/G + G/G, and In1.1T/C + C/C were significantly lower in patients than in control subjects.

The LD status was evaluated for the 201 control subjects; the r^2 values between −403G/A and −28C/G, −403G/A and In1.1T/C, and −28C/G and In1.1T/C were 0.297, 0.948, and 0.313, respectively. Because the LD between −403G/A and In1.1T/C was strong, the genotype results for these two SNPs were nearly identical (Table 1).

We then analyzed the association between the three SNP haplotypes and RSV (Table 2). The estimated frequencies of the H4 haplotype (−403A, −28C, and In1.1T), H5 haplotype (−403G, −28C, and In1.1C), and H6 haplotype (−403G, −28G, and In1.1T) were very low (H4 haplotype [patients, 0.026; control subjects, 0.012]; H5 haplotype [patients, 0.017; control subjects, 6.24×10^{-22}]; H6 haplotype [patients, 9.24×10^{-3}; control subjects, 4.79×10^{-9}]) and were nearly undetectable in the actual samples. We therefore excluded the H4, H5, and H6 haplotypes from the association analysis. Among the three remaining haplotypes, the frequency of H3 (−403A, −28G, and In1.1C) was significantly lower in patients than in control subjects (*P* = 0.0156). The frequency of H1 (−403G, −28C, and In1.1T) was higher (*P* = 0.0443) by 0.100 in patients than in control subjects. However, this difference was not significant after Bonferroni correction. The frequency of the H2 haplotype was similar between the two groups (*P* = 0.2081).

In this study, we examined the association of three SNPs in the *RANTES* gene with RSV bronchiolitis in a population of Japanese infants. The frequencies of genotypes containing −403A, −28G, and In1.1C were

Table 1. Distribution of genotype in infants with severe respiratory syncytial virus (RSV) bronchiolitis and control subjects

	Infants with severe RSV bronchiolitis (*n* = 59)	Control subject (*n* = 201)	*P*	OR (95% CI)
RANTES −403 G/A				
G/G	34 (57.6)	80 (39.8)		
G/A + A/A	25 (42.4)	121 (60.2)	0.017*	0.49 (0.27–0.88)
Allele				
G	88 (74.6)	249 (61.9)		
A	30 (25.4)	153 (38.1)	0.012*	0.56 (0.35–0.88)
RANTES −28 C/G				
C/C	52 (88.1)	143 (71.1)		
C/G + G/G	7 (11.9)	58 (28.9)	0.010*	0.33 (0.14–0.77)
Allele				
C	109 (92.4)	340 (84.6)		
G	9 (7.6)	62 (15.4)	0.032*	0.45 (0.22–0.94)
RANTES In1.1 T/C				
T/T	34 (57.6)	83 (41.3)		
T/C + C/C	25 (42.4)	118 (58.7)	0.037*	0.52 (0.29–0.93)
Allele				
T	89 (75.4)	254 (63.2)		
C	29 (24.6)	148 (36.8)	0.015*	0.56 (0.35–0.89)

Data are presented as number (%) of subjects unless otherwise indicated.
CI, confidence interval; OR, odds ratio. *Statistically significant.

Table 2. Frequency of *RANTES* −403/−28/In1.1 haplotypes in infants with severe respiratory syncytial virus (RSV) bronchiolitis and control subjects

Haplotype of *RANTES* −403/−28/In1.1		Infants with severe RSV bronchiolitis (118 Alleles)	Control subject (402 Alleles)	P[1]
H1	G-C-T	0.719	0.619	0.0443
H2	A-C-C	0.161	0.214	0.2081
H3	A-G-C	0.067	0.154	0.0156*

[1]: Significant P value after Bonferroni correction for three haplotypes is 0.0167 (0.05/3).
*Statistically significant.

significantly lower in patients than in control subjects, suggesting that these *RANTES* polymorphisms are associated with the risk of developing RSV bronchiolitis. The present results differ from those of several previous studies (12,20,21), and the reasons for these differences are not clear but may include the following. First, the patients in the present study may differ from those in other studies. We applied strict selection criteria for RSV bronchiolitis; in order to exclude preexisting asthma as much as possible, we recruited only patients who had experienced their first wheezing episode during the RSV infection. Second, our case-control data were adjusted for risk factors (sex, birth weight, the presence of older siblings, breast-feeding, day care attendance during infancy, and parental smoking during infancy).

To clarify the functional significance of the SNPs in *RANTES*, several studies have compared the transcriptional activity of different alleles by luciferase assay (19,20,22,23). Taking into consideration our results and those of previous studies, the relationship between RSV bronchiolitis and *RANTES* polymorphisms may be as follows. If we take into account only the *RANTES* promoter region, −403A reported by Tian et al. (20) and −28G reported by Liu et al. (23) are associated with increased promoter activity. Because our patients showed a lower frequency of −403A and −28G, we can speculate that individuals with higher RANTES production may be less susceptible to severe RSV bronchiolitis. In fact, there are a few studies that agree with this hypothesis (24,25).

If we also take the intron sequence into consideration, based on the results of An et al. (19) and Tian et al. (20), the A-C-T and A-G-T haplotypes are thought to be associated with higher promoter activity than the other haplotypes. However, the estimated frequencies of A-C-T and A-G-T haplotypes were very low in our subjects; therefore, any relationship between RSV bronchiolitis and these two haplotypes can be neglected. In our study, the frequency of the C allele (lower transcriptional activity) in In1.1T/C was lower in patients than in control subjects. In other words, the frequencies of the A-G-C and A-C-C haplotypes, which correspond to lower transcriptional activity, were lower in patients than in control subjects. This suggested that individuals with lower RANTES production might be less susceptible to severe RSV bronchiolitis. There are many studies that are compatible with this hypothesis (26–28). It is generally accepted that RANTES recruits memory T cells, monocytes, eosinophils, and basophils and is implicated in airway inflammation (29,30). Considering these data

and the expression data of An et al. (19), our results support the notion that individuals with higher RANTES expression are more susceptible to severe RSV bronchiolitis. However, there are few reports focusing on the relationship between disease severity and RANTES concentration in airway tissues (25). In addition to RANTES expression, there are other factors that are important in the pathophysiology of severe RSV bronchiolitis, including the antigenicity of RSV and host immune conditions, among others (31). A study evaluating the relative importance of RANTES expression and the interaction between RANTES expression and the aforementioned factors would be an interesting next step.

In conclusion, our results show an association of *RANTES* gene polymorphisms with risk for severe RSV bronchiolitis. Because the high-risk alleles in this study differ from those in previous studies, further analyses are needed to clarify the relationship between *RANTES* polymorphisms and RSV bronchiolitis.

Conflict of interest None to declare.

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