Original Article

# Study of the association between −403G/A and −28C/G RANTES gene polymorphisms and asthma in Lebanon

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**Abstract:**

**CONTEXT:** Asthma is a complex inflammatory condition often associated with bronchial hyper reactivity and atopy. Genetic and environmental factors are implicated in the etiopathogenesis of asthma. Regulated upon Activation Normal T- cell Expressed and Secreted (RANTES) is a CC chemokine responsible for the recruitment of inflammatory cells, suggesting a possible role for this chemokine in asthma. Both -403A and -28G alleles of the RANTES promoter region were found to be associated with asthma/atopy in some but not all studies.

**AIM:** The purpose of this study was to investigate the genetic influence of -403A and -28G alleles of the RANTES promoter region on the development of asthma in Lebanon.

**SETTINGS AND DESIGN:** This case control study was conducted at Makassed Hospital, Beirut on 40 asthmatic patients and 38 healthy controls.

**METHODS:** RANTES gene polymorphisms -403G/A and -28C/G alleles were genotyped using PCR-RFLP.

**RESULTS:** No significant differences in allele or genotype frequencies for the RANTES gene polymorphisms between asthmatic patients and controls were found. The difference of the -403 GA genotype frequency between patients and controls was not statistically significant; (OR=0.8, 95% CI=0.2–2.3, *P*=0.8). Similarly, the difference of the A-allele frequencies between patients and controls was not significant (OR=0.824, CI=0.3–2.2, *P*=0.7).

**CONCLUSIONS:** Our data show that RANTES gene promoter polymorphisms are not associated with asthma susceptibility in the Lebanese population.

**Key words:**

Asthma, Lebanon, polymorphism, RANTES

Asthma is a complex, multifactorial disease of the lungs with an increasing prevalence worldwide.[1] Both genetic factors[2] and environmental factors such as viruses,[3] allergens,[4] and occupational exposures[5] are indicated in the disease aetiology.

Regulated upon Activation Normal T-cell Expressed and Secreted (RANTES) is a C-C chemokine, which can produce chemotaxis and activation of inflammatory cells that are central to the airway inflammation characteristic of asthma.[6] Increased RANTES mRNA expression has been demonstrated in bronchial biopsy specimens derived from asthmatic patients,[7] and higher RANTES levels have been demonstrated in the bronchoalveolar lavage fluid of patients with active asthma.[8] The number of eosinophils recruited to the lung has been reported to be strongly associated with immunoreactive RANTES concentration.[9]

Previous linkage studies have found that human chromosome 17q,[10,11] where the gene encoding RANTES is located, contains loci linked with asthma. It has been demonstrated that 2 functional polymorphisms in the proximal promoter region of the RANTES gene (−28C to G and −403G to A) increase transcriptional activity of RANTES gene.[12,13] In human cell lines, the −28G and the −403A were shown to increase promoter activity of RANTES in comparison with the more frequent −28C and −403G, suggesting that these polymorphisms increase RANTES expression.[12,13] The known biological activity and contribution of the chemokine pathway to allergic inflammation supports an important role for RANTES in the expression of atopy and atopic asthma.[14] Increased production of RANTES would be expected to increase the level of inflammatory cell recruitment, as it is one of the major chemoattractant proteins for eosinophils[11] into asthmatic airways after allergen challenge.[15,16]

The role of this particular pathway to immune regulation is also supported by observations that the GATA transcription motif in position -403 of the chemokine RANTES gene is associated with a number of inflammatory-mediated diseases including rheumatoid arthritis,[17] childhood atopic dermatitis[12] and in delaying the progression of HIV infection.[18]

Through the use of genome-wide searches,

several candidate genes for asthma and atopy have been described;[11,10,19] one of these is RANTES. The gene encoding RANTES is located on chromosome 17q11.2–q12[20] that has been shown to be in linkage with asthma in several studies.[11,10] Two common single nucleotide polymorphisms (SNPs) consisting of G to A exchange at position -403 in the promoter of RANTES gene and C to G exchange at position -28, have been associated with asthma,[21-23] atopy and elevated levels of total IgE.[24] A significant higher constitutive transcriptional activity of the -403 A and -28 G expressing promoter was detected respectively in atopic dermatitis children[12] and human immunodeficiency (HIV) disease.[13]

In this study, we investigate the possible association of the -403A and -28G alleles with asthma susceptibility in Lebanese patients.

## Methods

### Study subjects

A case-control study including 40 patients with asthma and 38 healthy unrelated individuals were recruited for this study. All patients attended Makassed Hospital, Beirut. Asthma was diagnosed according to the criteria of the American Thoracic Society 1995.[25] All patients completed an asthma assessment questionnaire.

The participants reported whether they are current smokers, ex-smokers or whether they never smoked. Those who smoked more than 5 packs per year were excluded from the study. Controls consisted of 38 healthy Lebanese unrelated individuals; they were recruited from Beirut Arab University for this study, all showed no clinical evidence or family history of asthma or other conditions. All subjects signed an informed consent before enrolment in the study. The clinical characteristics of recruited subjects are summarized in [Table 1]. EDTA blood samples were collected from both cases and controls after consent. The study was approved by the local research committee.

Genomic DNA was extracted from whole blood through use of a GFX Genomic Blood DNA Purification Kit (Amershan Bioscience Co, UK) for all study subjects.

**Table 1: Demographic and clinical characteristics of the study population**

| Clinical parameters | Patients with asthma (*n*=40) (%) | Healthy control subjects (*n*=38) (%) | *P* |
|---|---|---|---|
| Age in yrs | 32.6 (±13.8) | 23.4 (±7.8) | 0.0005 |
| Females | 62.5 | 47.4 | 0.18 |
| Current smokers | 3 (7.5) | None | |
| Exsmokers | 3 (7.5) | | |
| Age at onset in yrs (range, 2 months-53 years) | 17.2 (±14.9) | N/A | |
| Atopic patients (%) | 26 (65) | | |
| Total serum IgE in IU/mL | 618.5 (±924) | | |
| Log IgE | 2.8 (±2.96) | | |
| Eosinophil count % | 5.1 (±4) | | |
| $FEV_1$% predicted | 87.6 (±22.7) | | |

Data are presented as mean (±SD) N/A not applicable

Spirometry was done at Makassed Hospital pulmonary laboratory via Collins® machine which is calibrated weekly. The subjects had at least 3 trials and the highest forced expiratory volume in one second ($FEV_1$) and forced vital capacity (FVC) were used in the analysis. Data on the pulmonary functions were expressed as percentage of the predicted value (% predicted), using predicting equations based on age, sex, and height. Participants who were taking medications for asthma were asked not to use an inhaled short-acting bronchodilator for 6 hours and long acting $\beta_2$-agonist for 24 hours before the test.

Bronchodilator reversibility was determined using a standard dose of $ß_{2-}$ agonist (salbutamol). Spirometry was performed after 15 minutes and reversibility calculated as percent change of $FEV_1$ and/or FVC. Reversibility was considered significant if there was an increase of ≥12% and/or >200 cc in $FEV_1$ or FVC.

All laboratory tests were carried out at Makassed Hospital. Total serum IgE levels were determined by means of Unicap 100, IgE levels were defined as high when total serum IgE exceeded 100 IU/ml; atopy was defined as a personal history of allergy, seasonal rhinitis, eczema, or allergic conjunctivitis in addition to positive skin prick test to at least 1 allergen (wheal diameter 3 mm greater than saline solution) and/or total serum IgE level >100 IU/ml.

The white blood cell counts and the eosinophil cell counts were measured by means of Beckman Coulter.

### Determination of RANTES genotypes

RANTES promoter -403 and -28 polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described by Hajeer *et al*.[26,27] Genomic DNA was amplified using the forward (5′-ACA GAG ACT CGA ATT TCC GGA-3′) and reverse (5′-CCA CGT GCT GTC TTG ATC CTC-3′) primers for -28C/G; forward (5′-GCC TCA ATT TAC AGT GTG-3′), and reverse (5′-TGC TTA TTC ATT ACA GAT GTT-3′) primers for -403G/A.

Since neither the wild-type nor the mutant allele of -403 polymorphism abolishes or introduces a restriction enzyme site, a single base change was introduced at the 3′ end of the reverse primer (C→G, underlined) at position -396 relative to the transcription site, thus creating an enzyme site that can be used to differentiate between wild-type and mutant alleles.

The PCR reaction was performed in a final volume of 25 µl containing 2 µl (100 ng) genomic DNA, 6.3 pmol of each primer, 1.5 mM of $MgCl_2$ (Fermentas), 1x NH4 Buffer (Fermentas), 0.2 mM dNTPs (ABgene), and 1 U *Taq* polymerase (Fermentas). PCR cycles were as follows: Initial denaturation at 95°C for 2 minutes followed by 35 cycles each of denaturation at 95°C for 40 seconds, annealing at 50°C for 40 seconds and extension at 72°C for 40 seconds. A final extension step was carried out at 72°C for 5 minutes.

PCR products were visualized on 2% agarose gel stained with ethidium bromide (Amresco). For the -403G/A polymorphism the PCR product length generated is 135 bp, while for the -28C/G polymorphism, the PCR product length generated is 187 bp.

Enzyme digestion (RFLP) was carried out as described previously;[28,29] in a 15 µl final volume using 5 µl of PCR product and: 3 U of *MnlI* (Fermentas) enzyme for RANTES-28C/G, the reactions were incubated at 37°C overnight, the wild- type allele was detected as five bands (114, 27, 20, and two 13 bp), while heterozygous individuals with CG genotype, three bands were revealed (134, 27, and two 13 bp) or 4 U of *MaeIII* (Roche Diagnostics GmbH) enzyme for RANTES -403G/A, the reactions were incubated at 55°C overnight; Digestion with *Mae*III yields 112 and 23 bp fragments when G allele is present, while the mutant A allele was detected as a band of 135 bp. The digested products were subjected to electrophoresis on 3% agarose gel stained with ethidium bromide.

### DNA sequencing

To confirm the genotyping results obtained in PCR-RFLP of the promoter region of the RANTES gene, four random samples were examined by DNA sequencing using an automated fluorescent labelling system. DNA sequences of the PCR products were determined using Applied Biosystems model 3130 sequencer (Hitachi Applied Biosystems, Genetic Analyzer). All sequences matched the RFLP patterns.

### Statistical methods

Cases and controls were compared regarding age using Wilcoxon-Rank-Sum-Test and sex using Chi-Square test to assess whether cases differ significantly in age and sex from controls. The association of the mutant allele with asthma was assessed by calculating the odds ratio (OR) and 95% confidence interval (95% CI). Chi-square and Fisher's Exact test were used to test for the difference in RANTES genotypes and alleles between patients and controls. The statistical significances of the traits values (blood eosinophil counts, total serum IgE levels, and $FEV_1$% predicted) between genotypes were compared by means of the t- independent test. Statistical significance was defined as $P \leq 0.05$. Calculations were performed with the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

## Results

The distribution of RANTES polymorphisms in the study group are shown in [Table 2]. Results of DNA genotyping in asthmatic patients showed that -28CC genotype is the only genotype detected with 100% frequency in the absence of the two other genotypes. In the control population, the CC genotype was the most frequent (97.4%), followed by the CG genotype (2.6%), and the GG genotype was absent. The frequency of the C-allele was 98.7% and that of the G-allele was 1.3%.

The frequencies of RANTES -403 GG, GA and AA genotypes among asthmatic patients were 75%, 25% and 0% respectively. The frequency of the G- allele was 87.5% and that of the A-allele was 12.5%. In control subjects, the distribution of RANTES-403 GG, GA and AA genotypes was 78.9%, 21.1%, and 0%, respectively. The allele frequencies were 89.5% and 10.5% for the G and A alleles, respectively.

Statistical analysis showed no significant difference in the -28CG and -28CC genotype frequency between patients and control [Table 2]. In addition, the frequency of the -28G allele, as well as the CG genotype, showed no statistical difference between patients and controls [Table 2].

**Table 2: Distribution of genotypes and alleles in controls and asthmatic patients for the RANTES promoter polymorphism at positions -403G/A and -28C/G**

| RANTES | Controls *n*=38 | | Asthmatics *n*=40 | | *P* value | OR |
|---|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | | |
| -403G/A Genotype | | | | | | |
| GG | 30 | 78.9 | 30 | 75 | 0.8 | 0.8 |
| GA | 8 | 21.1 | 10 | 25 | | |
| AA | 0 | 0 | 0 | 0 | | |
| -403G/A Allele | | | | | | |
| -403G | 68 | 89.5 | 70 | 87.5 | 0.7 | 0.8 |
| -403A | 8 | 10.5 | 10 | 12.5 | | |
| -28C/G Genotype | | | | | | |
| CC | 37 | 97.4 | 40 | 100 | 0.5 | |
| CG | 1 | 2.6 | 0 | 0 | | |
| GG | 0 | 0 | 0 | 0 | | |
| -28C/G Allele | | | | | | |
| -28C | 75 | 98.7 | 80 | 100 | 0.3 | |
| -28G | 1 | 1.3 | 0 | 0 | | |

RANTES = Regulated upon activation normal T-cell expressed and secreted

Furthermore, the difference of the -403 GA genotype frequency between patients and controls was not statistically significant; 25% for patients and 21.1% for controls (OR=0.8, 95% CI=0.2–2.3, *P*=0.8) [Table 2]. Similarly, the difference of the A-allele frequencies between patients and controls was not significant; 12.5% for patients and 10.5% for controls (OR=0.824, CI=0.3-2.2, *P*=0.7).

Moreover, there was no significant difference in levels biological markers (serum IgE, eosinophils count, and $FEV_1$% predicted) between asthmatic patients with -403G/G and G/A RANTES genotypes (data not shown).

## Discussion

It has been demonstrated that two functional polymorphisms in the proximal promoter region of the RANTES gene (-28C to G and -403G to A) increase transcriptional activity of RANTES gene and RANTES expression in the human body.[12,13] Increased production of RANTES would be expected to increase the level of inflammatory cell recruitment, as it is one of the major chemo-attractant proteins for eosinophil[9] and other cells into asthmatic airways after allergen challenge.[17,16] It is therefore quite likely that these polymorphisms are possible loci involved in asthma susceptibility.

Results obtained from this study showed no significant association between the RANTES -403A allele or the -28G allele and asthma in the Lebanese population. According to these results, these polymorphisms in the promoter region of the RANTES gene do not influence significantly the development of asthma.

These results were consistent with reports from Korean,[29] Japanese,[21] Hungarian,[30] Spanish,[31] and African American[32] populations where RANTES promoter polymorphism did not have a significant effect on the susceptibility to asthma.

On the contrary, some other studies suggested a positive association between RANTES polymorphisms and asthma susceptibility, or with different types of bronchial asthma. A study by Lachheb *et al*. on Tunisian children[33] suggested an association between alleles level of -28C/G and -403G/A promoter polymorphism and asthma. In addition, a study on Northern European Caucasians population[34] found that the -403A allele was associated with an increased susceptibility to both asthma and atopy.

In view of the plausible role of the -403G/A polymorphism in promoting allergic responses and its high allelic frequency in Caucasian populations, Abdulhadi *et al*.,[23] sought to confirm or refute the previous reported case–control study[32] using a family-based association tests (FBATs) and generation-specific case-control analyses. The -403G/A was transmitted with atopy and atopic asthma, although its contribution appears to relate more to atopy than asthma and BHR.

Several studies of the RANTES genotypes in the Asian populations have supported this association. In a Chinese cohort by Wang *et al*.,[35] the prevalence of the -28G allele was significantly different between asthmatic patients and controls (19.5% vs. 10.6%). Also, the levels of plasma RANTES and peripheral eosinophils were significantly different among the three genotypes but not the total levels of plasma IgE.

Our results were consistent with previous report in Korean children,[29] where no significant differences in clinical characteristics according to the genotypes of RANTES -403G/A polymorphism were observed. Also, in the Hungarian study,[30] no clinical or laboratory characteristics were associated with a given RANTES genotype.

RANTES genotype was shown to be associated with increasing severity of airway obstruction in a previous study by Fryer *et al*.[34] However, a meta-analysis comprising several studies[21,22,29-32,37] was carried out by Zhang *et al*.,[36] and confirmed the absence of significant associations of the -403G/A and -28C/G polymorphisms with asthma.

According to our results, these polymorphisms in the promoter region of the RANTES gene do not influence significantly the development of asthma, atopy, or the eosinophil count. It can be assumed that these more inducible RANTES genes do not have a detectable effect on the investigated parameters or that, more simply, in those cells (e.g., airway epithelial cells) that participate in the pathomechanism of these diseases, other cis-regulating elements are responsible for the expression of RANTES.

The main weakness of this study relates to the sample size of both cases and controls. There has not been any report on allele frequencies of RANTES G-403A and C-28G in the Lebanese population at the time of this study, and this made sample size estimation difficult. This study may thus be underpowered on detecting any association between G-403A and rare outcomes. A further study with a larger population size is thus needed.

Our results showed that RANTES gene promoter polymorphisms are not associated with asthma susceptibility in the Lebanese population.

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## References

1. Peat JK, Li J. Reversing the trend: Reducing the prevalence of asthma. J Allergy Clin Immunol 1999;103:1-10.
2. Cookson W. The alliance of genes and environment in asthma and allergy. Nature 1999;402:B5-11.
3. Stein RT, Martinez FD. Respiratory syncytial virus and asthma: Still no final answer. Thorax 2010;65:1033-4.
4. Halonen M, Stern DA, Lohman C, Wright AL, Brown MA, Martinez FD. Two subphenotypes of childhood asthma that differ in maternal and paternal influences on asthma risk. Am J Respir Crit Care Med 1999;160:564-70.
5. Venables KM, Chan-Yeung M. Occupational asthma. Lancet 1997;349:1465-9.
6. Nelson PJ, Kim HT, Manning WC, Goralski TJ, Krensky AM. Genomic organization and transcriptional regulation of the RANTES chemokine gene. J Immunol 1993;151:2601-12.
7. Ying S, Meng Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, *et al*. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (Intrinsic) asthmatics. J Immunol 1999;163:6321-9.
8. Alam R, York J, Boyars M, Stafford S, Grant JA, Lee J, *et al*. Increased MCP-1, RANTES, and MIP-1alpha in bronchoalveolar lavage fluid of allergic asthmatic patients. Am J Respir Crit Care Med 1996;153:1398-404.
9. Teran LM, Noso N, Carroll M, Davies DE, Holgate S, Schroder JM. Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. J Immunol 1996;157:1806-12.
10. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). Nat Genet 1997;15:389-92.
11. Dizier MH, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, *et al*. Genome screen for asthma and related phenotypes in the French EGEA study. Am J Respir Crit Care Med 2000;162:1812-8.
12. Liu H, Chao D, Nakayama EE, Taguchi H, Goto M, Xin X, *et al*. Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. Proc Natl Acad Sci U S A 1999;96:4581-5.
13. Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, *et al*. Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J Immunol 2000;164:1612-6.
14. Conti P, Barbacane RC, Reale M. Chemokines in inflammatory states. Allergy Asthma Proc 1999;20:205-8.
15. Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. Am J Respir Crit Care Med 1999;160:640-7.
16. Kameyoshi Y, Dorschner A, Mallet AI, Christophers E, Schroder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. J Exp Med 1992;176:587-92.
17. Makki RF, al Sharif F, Gonzalez-Gay MA, Garcia-Porrua C, Ollier WE, Hajeer AH. RANTES gene polymorphism in polymyalgia rheumatica, giant cell arteritis and rheumatoid arthritis. Clin Exp Rheumatol 2000;18:391-3.
18. Gonzalez E, Dhanda R, Bamshad M, Mummidi S, Geevarghese R, Catano G, *et al*. Global survey of genetic variation in CCR5, RANTES, and MIP-1alpha: Impact on the epidemiology of the HIV-1 pandemic. Proc Natl Acad Sci U S A 2001;98:5199-204.

19. Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, *et al*. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. J Allergy Clin Immunol 2002;109:498-506.
20. Donlon TA, Krensky AM, Clayberger C. Localization of the human T lymphocyte activation gene 519 (D2S69E) to chromosome 2p12—q11. Cytogenet Cell Genet 1990;53:230-1.
21. Hizawa N, Yamaguchi E, Konno S, Tanino Y, Jinushi E, Nishimura M. A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. Am J Respir Crit Care Med 2002;166:686-90.
22. Yao TC, Kuo ML, See LC, Chen LC, Yan DC, Ou LS, *et al*. The RANTES promoter polymorphism: A genetic risk factor for near-fatal asthma in Chinese children. J Allergy Clin Immunol 2003;111:1285-92.
23. Al-Abdulhadi SA, Helms PJ, Main M, Smith O, Christie G. Preferential transmission and association of the -403 G–>A promoter RANTES polymorphism with atopic asthma. Genes Immun 2005;6:24-30.
24. Conti P, Pang X, Boucher W, Letourneau R, Reale M, Barbacane RC, *et al*. RANTES is a pro-inflammatory chemokine and chemoattracts basophil cells to extravascular sites. J Pathol 1997;183:352-8.
25. Navarro RP, Schaecher KL, Rice GK. Asthma management guidelines: Updates, advances, and new options. J Manag Care Pharm 2007;13 (6 Suppl D): S3-11; quiz S12-3.
26. Hajeer AH, al Sharif F, Ollier WE. A polymorphism at position –403 in the human RANTES promoter. Eur J Immunogenet 1999;26:375-6.
27. Al Sharif F, Ollier WE, Hajeer AH. A rare polymorphism at position -28 in the human RANTES promoter. Eur J Immunogenet 1999;26:373-4.
28. Maddox L, Schwartz DA. The pathophysiology of asthma. Annu Rev Med 2002;53:477-98.
29. Sohn MH, Kim SH, Kim KW, Jee HM, Park HS, Kim KE. RANTES gene promoter polymorphisms are associated with bronchial hyperresponsiveness in Korean children with asthma. Lung 2008;186:37-43.
30. Szalai C, Kozma GT, Nagy A, Bojszkó A, Krikovszky D, Szabó T, *et al*. Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity. J Allergy Clin Immunol 2001;108:375-81.
31. Muro M, Marin L, Torio A, Pagan JA, Alvarez-Lopez MR. CCL5/RANTES chemokine gene promoter polymorphisms are not associated with atopic and nonatopic asthma in a Spanish population. Int J Immunogenet 2008;35:19-23.
32. Moissidis I, Chinoy B, Yanamandra K, Napper D, Thurmon T, Bocchini J Jr, *et al*. Association of IL-13, RANTES, and leukotriene C4 synthase gene promoter polymorphisms with asthma and/or atopy in African Americans. Genet Med 2005;7:406-10.
33. Lachheb J, Chelbi H, Hamzaoui K, Hamzaoui A. Association between RANTES polymorphisms and asthma severity among Tunisian children. Hum Immunol 2007;68:675-80.
34. Fryer AA, Spiteri MA, Bianco A, Hepple M, Jones PW, Strange RC, *et al*. The -403 G–>A promoter polymorphism in the RANTES gene is associated with atopy and asthma. Genes Immun 2000;1: 509-14.
35. Wang LJ, Li YR, Chen JH, Cui TP, Wu JM. Polymorphism of regulated upon activation, normal T cell expressed and secreted promoter region -28 position in Chinese allergic asthmatic children. Zhonghua Jie He He Hu Xi Za Zhi 2004;27:394-7.
36. Zhang YG, Huang J, Zhang J, Li XB, He C, Xiao YL, *et al*. RANTES gene polymorphisms and asthma risk: A meta-analysis. Arch Med Res 2010;41:50-8.
37. Wang D, Bresnahan W, Shenk T. Human cytomegalovirus encodes a highly specific RANTES decoy receptor. Proc Natl Acad Sci U S A 2004;101:16642-7.


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