



# ***RANTES* G-401A polymorphism is associated with allergen sensitization and FEV<sub>1</sub> in Chinese children**

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**Summary** G-401A polymorphism in *RANTES* promoter was associated with near-fatal asthma and atopic dermatitis in children. We studied whether gain-of-function mutations in *RANTES* gene were associated with asthma and atopy-related traits in Chinese children. Plasma total and aeroallergen-specific IgE concentrations were measured using micro-particle immunoassay and fluorescent enzyme immunoassay, respectively. Restriction fragment length polymorphism was used to genotype *RANTES* G-401A and C-28G. One hundred and twenty-nine asthmatic children and 66 controls were recruited. Their mean logarithmic plasma total IgE concentrations were 2.53 and 1.98, respectively ( $P < 0.0001$ ). *RANTES* G-401A was not associated with physician-diagnosed asthma ( $P = 0.408$ ). However, *RANTES* -401A allele was significantly associated with IgE sensitization to cat (odds ratio 2.35; 95% CI 1.15–4.77;  $P = 0.010$ ). Those homozygous for -401A had higher plasma cat-specific IgE levels ( $P = 0.034$ ). Subjects having -401A were also more likely to have mold-specific IgE (odds ratio 3.82; 95% CI 1.24–12.14;  $P = 0.007$ ). On spirometry, those with -401A/A had lower forced expiratory volume in 1-s (FEV<sub>1</sub>;  $P = 0.044$ ). *RANTES* C-28G was not associated with any outcome in this study. In conclusion, the gain-of-function mutation at -401 of *RANTES* promoter is associated with sensitization to cat and mold allergens and FEV<sub>1</sub> in Chinese children.

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## **Introduction**

Regulated on activation, normal T cell expressed and secreted (*RANTES*) is one of the most extensively studied C–C chemokines in allergic inflammation.<sup>1</sup> Elevated *RANTES* level was detected in bronchoalveolar lavage fluid of patients with acute

asthma and following allergen challenge.<sup>2–4</sup> Genome-wide screen in African Americans also linked asthma to the C–C chemokine gene cluster on chromosome 17p12–17q11.2.<sup>5</sup> Two mutations at -401 and -28 in *RANTES* resulted in increased transcriptional activity of the mutant promoter.<sup>6–9</sup> In Caucasian adults, *RANTES* G-401A was associated with asthma, skin test positivity and forced expiratory volume in 1-s (FEV<sub>1</sub>).<sup>10</sup> This polymorphism was also associated with atopic dermatitis in German children.<sup>9</sup> This study aims at investigating

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the relation between *RANTES* promoter polymorphisms and various asthma and atopy phenotypes in Chinese children.

## Patients and methods

### Subjects

This study recruited unrelated Chinese children aged 5–18 years with asthma diagnosed according to American Thoracic Society criteria.<sup>11</sup> Briefly, these patients had  $\geq 6$ -month history of recurrent cough, dyspnea or wheezing that was relieved by bronchodilator and reversibility and/or hyperreactivity on pulmonary function studies. These asthmatic patients were recruited from pediatric clinics of a university teaching hospital. Non-allergic controls were recruited among those referred for assessment of minor complaints. All subjects were free from infection for four weeks before study. Clinical Research Ethics Committee of our university approved this study.

### Clinical assessments

Following written consent, plasma samples were collected from subjects for total IgE by micro-particle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL, USA), and specific IgE to locally relevant aeroallergens<sup>12</sup> (*D. pteronyssinus*, cat, dog, cockroaches and mixed molds) by fluorescent enzyme immunoassay (AutoCAP system, Pharmacia Diagnostics AB, Uppsala, Sweden). Atopy is defined by  $\geq$  one aeroallergen-specific IgE. All asthmatics also underwent simple spirometry, and results were compared with local references.<sup>13</sup>

### Genotyping *RANTES* polymorphisms

*RANTES* G-401A and C-28G were genotyped by restriction fragment length polymorphism as described.<sup>6,7</sup> Briefly, the primers for G-401A were (F) 5'-GCC TCA ATT TAC AGT GTG-3' and (R) 5'-TGC TTA TTC ATT ACA GAT GTT-3', and those for C-28G were (F) 5'-ACA GAG ACT CGA ATT TCC GGA-3' and (R) 5'-CCA CGT GCT GTC TTG ATC CTC-3'. Fifty nanograms genomic DNA was amplified with 25 pmol of each primer and 1 unit Taq polymerase (MBI Fermentas, Amherst, NY, USA) or 0.75 unit Ampli Taq Gold (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction consisted of 95°C for 5 min, 38 cycles of 94°C for 45 s, 53°C for 45 s and 72°C for 45 s, and 72°C for 7 min. *Mae*III cut the product into two bands (112 and 23 bp) for -401G, whereas *Mn*I

digested the product into three bands (126, 27 and 20 bp) for -28C. Genotypes of 8 random samples from each of G-401A and C-28G were confirmed by direct sequencing using BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

### Statistical analysis

The demographic and clinical data were analyzed using Student *t*-test,  $\chi^2$  or Fisher exact test as appropriate. The distribution of *RANTES* genotypes or alleles between patients and controls were analyzed by  $\chi^2$  or Fisher exact test. Plasma IgE concentrations and FEV<sub>1</sub> between different *RANTES* genotypes were analyzed by analysis of variance or Kruskal–Wallis test. *P*-values less than 0.05 were considered significant Fig. 1.

## Results

One hundred twenty-nine asthmatics and 66 controls, with mean (SD) ages of 9.9 (3.4) years and 10.5 (4.6) years ( $P = 0.35$ ), were recruited. Eighty-two (64%) patients and 41 (62%) controls were males ( $P = 0.84$ ). Table 1 summarizes the clinical characteristics of our patients. The mean (SD) log-transformed total IgE concentration was 2.53 (0.59) kIU/l in patients and 1.98 (0.78) kIU/l in controls ( $P < 0.0001$ ). Ninety-seven (75%) asthmatics and 29 (44%) controls had increased plasma total IgE (OR 3.87, 95% CI 1.97–7.63;  $P < 0.0001$ ), and 113 (88%) patients and 33 (50%) controls were atopic (OR 7.06, 95% CI 3.28–15.37;  $P < 0.0001$ ).

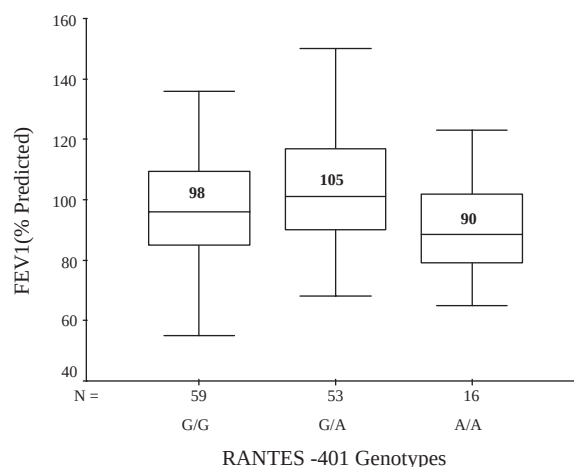


**Figure 1** Boxplots of percent FEV<sub>1</sub> versus *RANTES* G-401A genotypes. The median percent FEV<sub>1</sub> was provided for each of the three genotypes ( $P = 0.044$  by Kruskal–Wallis test).

**Table 1** Clinical characteristics and lung functions of our 129 asthmatic patients.

| Characteristics                                                                      | Results       |
|--------------------------------------------------------------------------------------|---------------|
| Demographic data                                                                     |               |
| Age at assessment in years, mean (SD)                                                | 9.9 (3.4)     |
| Male gender, <i>n</i> (%)                                                            | 82 (64)       |
| Coexisting physician-diagnosed allergic rhinitis*, <i>n</i> (%)                      | 104 (81)      |
| Coexisting physician-diagnosed atopic dermatitis*, <i>n</i> (%)                      | 45 (35)       |
| Details on asthma prophylaxis                                                        |               |
| Number of patients receiving inhaled corticosteroids, <i>n</i> (%)                   | 73 (57)       |
| Median (range) daily dose of inhaled beclomethasone dipropionate or equivalent in µg | 200(100–1000) |
| Median (interquartile range) FEV <sub>1</sub> , % predicted                          | 98 (85–111)   |

\*The diagnoses of allergic rhinitis and atopic dermatitis were made according to the criteria listed in references,<sup>14,15</sup> respectively.

**Table 2** The relationship between asthma or atopy phenotypes and *RANTES* G-401A.

| Groups                                         | -401 Genotypes |         |         |                  | -401 Alleles |                   |                  |
|------------------------------------------------|----------------|---------|---------|------------------|--------------|-------------------|------------------|
|                                                | G/G (%)        | G/A (%) | A/A (%) | <i>P</i> -value* | A (%)        | OR (95% CI)       | <i>P</i> -value* |
| <i>Allergen sensitization (positive RAST)†</i> |                |         |         |                  |              |                   |                  |
| Cat-sensitized                                 | 7 (35)         | 6 (30)  | 7 (35)  | 0.008            | 20 (50)      | 2.35 (1.15–4.77)  | 0.010            |
| With not cat-sensitized                        | 88 (51)        | 68 (39) | 18 (10) |                  | 104 (30)     | 1.0               |                  |
| Mixed mold-sensitized                          | 1 (13)         | 4 (50)  | 3 (38)  | 0.037            | 10 (63)      | 3.82 (1.24–12.14) | 0.007            |
| With not mixed mold-sensitized                 | 95 (51)        | 69 (37) | 22 (12) |                  | 113 (30)     | 1.0               |                  |
| All asthmatic patients                         | 60 (47)        | 53 (41) | 16 (12) | 0.408            | 85 (33)      | 1.26 (0.78–2.05)  | 0.322            |
| Asthmatics with ≥ 1 positive RAST              | 54 (48)        | 45 (40) | 14 (12) | 0.525            | 73 (32)      | 1.23 (0.75–2.02)  | 0.398            |
| With all control children                      | 37 (56)        | 21 (32) | 8 (12)  |                  | 37 (28)      | 1.0               |                  |
| Increased serum total IgE‡                     | 63 (50)        | 47 (37) | 16 (13) | 0.957            | 79 (31)      | 1.01 (0.63–1.62)  | 0.969            |
| With normal serum total IgE                    | 34 (49)        | 27 (39) | 8 (12)  |                  | 43 (31)      | 1.0               |                  |
| Atopic subjects (with ≥ 1 positive RAST)       | 72 (49)        | 55 (38) | 19 (13) | 0.874            | 93 (32)      | 1.11 (0.66–1.89)  | 0.677            |
| With non-atopic subjects                       | 25 (51)        | 19 (39) | 5 (10)  |                  | 29 (30)      | 1.0               |                  |

A, mutant -401 allele; CI, confidence interval; G, wild-type -401 allele, OR, odds ratio.

\*Analyzed between two groups by Pearson  $\chi^2$  or Fisher exact test.

†Results for *D. pteronyssinus*, dog and cockroach sensitization were insignificant and thus not included.

‡Compared with age-matched upper limits of reference ranges (5–6 years: 120 kIU/l; 6–7 years: 160 kIU/l; ≥ 8 years: 180 kIU/l) for local Chinese children.

Asthma was also significantly associated with sensitization to *D. pteronyssinus* (OR 5.40, 95% CI 2.60–11.29;  $P < 0.0001$ ) and cat (OR 5.24, 95% CI 1.11–33.80;  $P = 0.017$ ).

The distributions of *RANTES* -401 and -28 alleles followed Hardy–Weinberg equilibrium. Table 2 summarizes genotype and allele frequencies of *RANTES* G-401A in various asthma or atopy phenotypes. *RANTES*-401A was associated with the presence of cat-specific IgE (OR 2.35; 95% CI 1.15–

4.77;  $P = 0.010$ ). Plasma cat-specific IgE concentrations were higher in subjects with -401A/A as compared to G/A and G/G ( $P = 0.034$  for trend). Sensitization to mixed molds was also more common in subjects carrying -401A (OR 3.82; 95% CI 1.24–12.14;  $P = 0.007$ ). Spirometrically, 20 (16%) asthmatics had FEV<sub>1</sub> ≤ 80%. Fig. 1 shows that FEV<sub>1</sub> was significantly lower in patients with -401A/A. *RANTES* -28G was present in 10% patients and 9% controls. None of them was homozygous for this

mutant, and C-28G was not associated with any asthma or atopy phenotype (results not shown).

## Discussion

This study shows for the first time that *RANTES* polymorphism is linked to allergen sensitization and asthma severity as assessed by FEV<sub>1</sub>. *RANTES* G-401A is associated with sensitization to cat and mold allergens. Asthmatic children with -401A/A also have lower FEV<sub>1</sub>. *RANTES* C-28G is not associated with any outcome variable in our Chinese children. Fryer and colleagues showed similarly a significant association between *RANTES* G-401A and skin test positivity to aeroallergens and asthma diagnosis in Caucasians.<sup>10</sup> In contrast, Yao and colleagues found that *RANTES* -28G but not -401A was significantly associated with near-fatal asthma in Chinese children. *RANTES* -28G was also associated with peripheral eosinophilia and bronchial hyperresponsiveness.<sup>16</sup> These discrepant effects might be explained by tight linkage disequilibrium between G-401A and C-28G.<sup>17</sup> The importance of *RANTES* on asthma and atopy was further supported by the finding that its blood level was increased in patients with severe asthmatic exacerbation.<sup>3,18</sup>

*RANTES* -401A was found in 9–16% of Caucasians,<sup>6,9,10</sup> but its frequency was much higher in Chinese (25%) and Japanese (33%).<sup>16,17</sup> *RANTES* -28G, another gain-of-function mutation, was present in 4% of Caucasians, 12% of Chinese and 12–17% of Japanese.<sup>7,8,16,17</sup> In the present study, -401A was present in 28% of controls whereas -28G was detected in 9% of them, and these figures were similar to those of Chinese and Japanese but much higher than in healthy Caucasians. Further studies are needed to delineate the allele frequencies for *RANTES* -401 and -28 in different ethnic groups.

The main weakness of this study relates to the sample size. There has not been any report on allele frequencies of *RANTES* G-401A and C-28G in Chinese at the time of this study, and this made sample size estimation difficult. Retrospective analysis showed that our sample size was able to detect an odds ratio of 2.6 for G-401A with asthma with 80% power and 95% confidence. This study may thus be underpowered in detecting any association between G-401A and rare outcomes. Despite this, this study detects an association between this polymorphism and FEV<sub>1</sub> and aeroallergen sensitization.

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