Respiratory Medicine (2005) **99**, 216–219

respiratoryMEDICINE

RANTES G-401A polymorphism is associated with allergen sensitization and FEV_1 in Chinese children

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Received 21 July 2003; accepted 2 June 2004

KEYWORDS
Asthma;
Atopy;
Polymorphism;
Regulated on activation, normal T cell expressed and secreted;
Spirometry

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_1; $P=0.044$). *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_1 in Chinese children.

Introduction

Regulated on activation, normal T cell expressed and secreted (*RANTES*) is one of the most extensively studied C–C chemokines in allergic inflammation.[1] Elevated *RANTES* level was detected in bronchoalveolar lavage fluid of patients with acute asthma and following allergen challenge.[2–4] Genome-wide screen in African Americans also linked asthma to the C–C chemokine gene cluster on chromosome 17p12-17q11.2.[5] Two mutations at -401 and -28 in *RANTES* resulted in increased transcriptional activity of the mutant promoter.[6–9] In Caucasian adults, *RANTES* G-401A was associated with asthma, skin test positivity and forced expiratory volume in 1-s (FEV_1).[10] This polymorphism was also associated with atopic dermatitis in German children.[9] This study aims at investigating

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doi:10.1016/j.rmed.2004.06.004

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.[11] Briefly, these patients had ⩾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 microparticle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL, USA), and specific IgE to locally relevant aeroallergens [12] (*D. pteronyssinus*, cat, dog, cockroaches and mixed molds) by fluorescent enzyme immunoassay (AutoCAP system, Pharmacia Diagnostics AB, Uppsala, Sweden). Atopy is defined by ⩾ one aeroallergen-specific IgE. All asthmatics also underwent simple spirometry, and results were compared with local references.[13]

Genotyping *RANTES* polymorphisms

RANTES G-401A and C-28G were genotyped by restriction fragment length polymorphism as described.[6,7] 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 *Mnl*1 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, χ^2 or Fisher exact test as appropriate. The distribution of *RANTES* genotypes or alleles between patients and controls were analyzed by χ^2 or Fisher exact test. Plasma IgE concentrations and FEV_1 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; $P < 0.0001$).

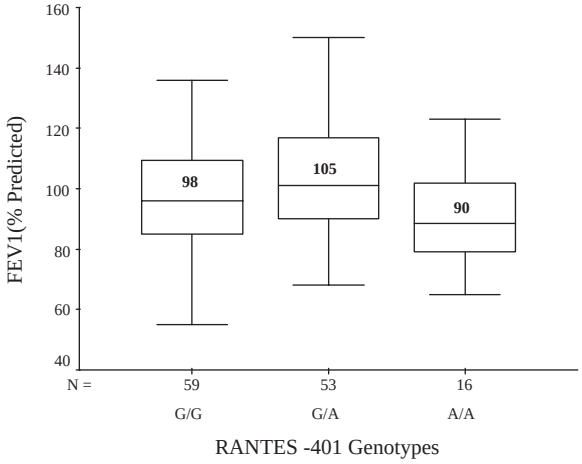

Figure 1 Boxplots of percent FEV_1 versus *RANTES* G-401A genotypes. The median percent FEV_1 was provided for each of the three genotypes ($P = 0.044$ by Kruskal–Wallis test).