

## Short communication

# Lack of association between atopic eczema/dermatitis syndrome and polymorphisms in the promoter region of RANTES and regulatory region of MCP-1

**Background:** Chemokines play an important role in the pathophysiology of atopic eczema/dermatitis syndrome (AEDS) and allergy. Recently polymorphisms in the promoter region of RANTES (regulated on activation normal T cell expressed and secreted) and in the gene regulatory region of MCP-1 (monocyte chemoattractant protein-1) have been found, which increase the expression of these chemokines. The –403A allele of the RANTES promoter region was found associated with AEDS in German children. We investigated whether the presence of these polymorphisms was associated with AEDS or allergy in Hungarian children.

**Methods:** One hundred and twenty-eight children with AEDS, 102 allergic children without AEDS and 303 children of comparable ages without allergic disorders were screened for genotype with a PCR-based assay.

**Results:** There were no significant differences in the frequency of these polymorphisms, or in the distribution of genotypes between the groups. The total IgE concentration, the white blood cell count and the blood eosinophil cell count did not differ between the genotypes.

**Conclusion:** In this cohort of Hungarian children there was no association between –28G, and –403A alleles in the RANTES promoter, –2518G polymorphism in the distal regulatory region of the MCP-1 and AEDS, or allergy.

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In this manuscript according to the proposal of the EAACI (1) we will use the revised allergy nomenclature and the term atopic eczema/dermatitis syndrome (AEDS) to describe what is currently referred to as atopic dermatitis. AEDS is a chronically relapsing inflammatory skin disease in which both inherited and environmental factors play a role in maintaining and exacerbating the condition. Tissue eosinophilia is one of the characteristic findings after allergen exposure and in allergic inflammation seen in AEDS (2). In recent years numerous studies suggest a major involvement of T lymphocytes in the pathogenesis of the disease, with increased numbers of Th lymphocytes expressing type 2 cytokines at the initial phase of skin inflammation, whereas both Th2 and Th1 cytokines are up-regulated in chronic lesions (3).

Chemotactic cytokines, or chemokines, are small signalling proteins which are deeply involved in the physiology and pathophysiology of acute and chronic inflammatory processes by attracting and stimulating

specific subsets of leukocytes (4). Several studies have suggested an important role of C-C chemokines in the pathogenesis of allergic inflammation (5,6).

RANTES (regulated on activation normal T cell expressed and secreted) is one of the most extensively studied chemokines in allergic diseases and has been found to be associated with AEDS (7). RANTES protein was detected at high levels in skin scales of AEDS patients, and dermal fibroblast were identified as a major source of this chemokine (8). Furthermore, cutaneous challenge of human subjects with RANTES induced eosinophil recruitment in the skin (9).

Two polymorphisms in the RANTES promoter region (–28C/G and –403G/A) have been found affecting the transcription of the RANTES gene (10,11). In human cell lines the –28G and the –403A were shown to increase promoter activity of RANTES compared to the more frequent –28C and the –403G, respectively, suggesting that these polymorphisms

increase RANTES expression in the human body (10,11). In a recent study the -403A allele was found associated with AD in German children (10).

Monocyte chemoattractant protein-1 (MCP-1) may play a significant role in the allergic responses because of its ability to drive undifferentiated T lymphocyte populations toward an IL-4 producing Th2 type cell (12). IL-4 can up-regulate eotaxin, which is the most potent chemoattractant for the eosinophils (13).

Recently a bi-allelic A/G polymorphism in the MCP-1 distal gene regulatory region at position -2518 (numbers indicate nucleotide positions relative to the major transcription start sites) has been found that affects the level of MCP-1 expression in response to an inflammatory stimulus (14). Monocytes from individuals carrying a G allele at -2518 produce more MCP-1 after treatment with IL-1 $\beta$  than monocytes from A/A homozygous subjects. The effect of the G allele appears to be dose-dependent as cells from individuals homozygous for G at -2518 produced more MCP-1 than cells from G/A heterozygotes (14).

Given the importance of RANTES and MCP in allergic inflammation, we hypothesized that polymorphisms which influence the level of these chemokines might play a role in the susceptibility to AEDS or allergy. Therefore we determined the prevalence of the RANTES -28G and -403A, and MCP-1 -2518G polymorphisms in three groups of Hungarian children. The first group included children with AEDS, the second children had clinical signs of severe allergy but without AEDS, and the third group included children with no allergic disorders. We investigated whether the presence of these polymorphisms was associated with AEDS or allergy in these children.

## Material and methods

### Subjects

One hundred and twenty-eight children with AEDS, 102 allergic children without AEDS and 303 children of comparable ages without allergic disorders were enrolled in the study. All patients were Hungarian and lived in Budapest. Some characteristics of the allergic patients can be found in Table 1.

The allergic children attended the Allergic Outpatient Consultations of the Heim Pál Pediatric Hospital, the 1st Department of Pediatrics of Semmelweis University Budapest and the Budai Children's Hospital. All the allergic children had specialist physician-diagnosed allergy with the following criteria (a): clinical signs of severe allergy (including allergic rhinitis, AEDS, food allergy and urticaria); (b) positive skin prick test and/or IgE sensitized (see below).

AEDS was defined as described elsewhere. The diagnosis of AEDS was based on (a): physician's diagnosis; (b) manifestation of "dry skin"; and (c) three or more symptoms of AEDS at three or more areas. All patients had a history of AEDS within the last two years.

The control children were randomly selected from patients in Heim Pál Pediatric Hospital who met the following criteria: (a) no symptoms or history of allergic diseases, and (b) total serum IgE levels below the general population mean for their ages.

Table 1. Some clinical and biological characteristic of AEDS patients and allergic patients without AEDS

| Clinical and biological characteristics      | AEDS patients | Allergic patients without AEDS |
|----------------------------------------------|---------------|--------------------------------|
| Number                                       | 128           | 102                            |
| Age (range: 3–18 years)                      | 6.3 $\pm$ 4.4 | 7.5 $\pm$ 4.0                  |
| Gender (male/female)                         | 68/60         | 56/46                          |
| Total IgE kU/l (range: 9–>5000)              | 474 $\pm$ 465 | 572 $\pm$ 621                  |
| White blood cell count (10 <sup>6</sup> /ml) | 7.1 $\pm$ 1.9 | 6.9 $\pm$ 1.6                  |
| Eosinophil percentage                        | 4.0 $\pm$ 1.9 | 3.6 $\pm$ 1.5                  |

Informed parental consent was obtained for each patient and the study was approved by an institutional review committee.

### Laboratory analysis

Total genomic DNA was extracted from white blood cells using the method of Miller (15).

The MCP-1 -2518A/G, RANTES -28C/G and -403G/A polymorphisms were determined as described previously (16).

Total serum IgE levels and specific IgE levels to more than 100 allergens were determined by the Pharmacia (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) CAP System. IgE levels were defined as high in subjects whose total serum IgE exceeded 100 kU/L, and specific IgE was considered positive in those having detectable allergen-specific IgE (>0.35 kU/L) (IgE sensitized patients). Patients with positive skin prick test to at least one allergen (wheal diameter 3 mm greater than saline control) were defined as positive.

### Statistical methods

Allele frequencies were calculated by allele counting. Data were analyzed using MEDCALC and ARLEQUIN programs. "Hardy-Weinberg" equilibrium was tested by using a  $\chi^2$  goodness-of-fit test. Fisher's exact test was used to test for differences in allele distribution between the groups. Exact test of population differentiation was used to test if the genotypic composition of the groups was significantly heterogeneous.

## Results

The Table 2 shows the prevalence of polymorphisms in the three groups of children. Overall the results showed Hardy-Weinberg equilibrium. There were no significant differences in the frequency of these polymorphisms, or in the distribution of genotypes between the groups. In contrast to the results of Nickel et al. (10), the RANTES -403A allele was not significantly more frequent in AEDS patients than in control patients, or in patients with allergic disorders without AEDS. In our study a total of 533 Hungarian children were investigated. The frequency of the RANTES -403A in our patients was 16.8%, which is in good correlation with that found previously in two groups of Caucasian children (German: 20%; American: 15%), but significantly lower ( $P < 0.001$ ) than in African-American (43%), Afro-Caribbean (44%) and Colombian (30%) children (10). The frequency of the RANTES -28G allele in our patients is 3.7%, which is comparable with that of a Hungarian adult population (3.1%) (16), but significantly lower ( $P < 0.001$ ) than in a Japanese population

Table 2. Genotype and allele frequencies for RANTES -28 and -403, and MCP-1-2518 in three groups of children

| Polymorphism 1/2* | Population            | Genotype |     |     | Total | Allele 2 frequencies |
|-------------------|-----------------------|----------|-----|-----|-------|----------------------|
|                   |                       | 11*      | 12* | 22* |       |                      |
| RANTES -28 C/G    |                       |          |     |     |       |                      |
|                   | AEDS                  | 120      | 8   | 0   | 128   | 3.1                  |
|                   | Allergic without AEDS | 90       | 12  | 0   | 102   | 5.9                  |
|                   | Controls              | 284      | 19  | 0   | 303   | 3.1                  |
| RANTES -403 G/A   |                       |          |     |     |       |                      |
|                   | AEDS                  | 86       | 38  | 4   | 128   | 18.0                 |
|                   | Allergic without AEDS | 71       | 29  | 2   | 102   | 16.2                 |
|                   | Controls              | 211      | 84  | 8   | 303   | 16.5                 |
| MCP-1 -2518 A/G   |                       |          |     |     |       |                      |
|                   | AEDS                  | 83       | 35  | 10  | 128   | 21.5                 |
|                   | Allergic without AEDS | 64       | 32  | 8   | 102   | 23.5                 |
|                   | Controls              | 194      | 90  | 19  | 303   | 21.1                 |

\*1 = frequent allele; 2 = rare allele

(16.6%) (11). In the present study the frequency of MCP-1 -2518G is 21.7%, which is lower, although not significantly ( $P=0.07$ ), than in an American Caucasian population (29%), but significantly lower ( $P<0.001$ ) than in Asian (47%), and Mexican (47%) populations (14).

The total IgE concentration, the white blood cell count and the relative eosinophil cell count did not differ between the genotypes (data not shown).

## Discussion

In a recent report Nickel et al. found an association between AEDS and the RANTES -403A allele in a cohort of German children (10). Our investigation, however, could not reproduce this finding in Hungarian children. The RANTES -403A allele was not significantly more frequent in AEDS patients than in control patients, or in patients with allergic disorders without AEDS.

Chronic allergic diseases such as AEDS are characterized histologically by the preferential infiltration of eosinophils (17). This can be seen in experimental antigen challenges of the skin of allergic subjects, in whom eosinophils can account for as much as 60% of the total infiltrating cells, despite the relative paucity of eosinophils in the circulation (usually <4% of the total cells) (18). Eosinophils, by virtue of their release of pro-inflammatory mediators, are thought to be responsible for some of the histopathologic and physiologic changes observed in these diseases. Several reports suggested that RANTES has a major role in recruiting eosinophils to the site of the inflammation (9,19). RANTES was demonstrated as one of the most potent *in vitro* eosinophil chemoattractants, which induced eosinophil transendothelial migration (20,21) and could be detected at high levels in skin scales of AEDS patients (8). Therefore, it was reasonable to hypothesize that polymorphisms that increased the expression of

RANTES might increase the susceptibility to AEDS. Our study, however, failed to detect an association between the -28G and -403A alleles of the RANTES promoter and AEDS.

The study of genetic associations is difficult, and is confounded by small sample sizes, sample selection, genetic admixture, and regional differences in gene frequencies and gene-environmental interactions. Although both German and Hungarian populations are Caucasian the German genetic admixture was especially strong in Budapest from where our samples were collected. Furthermore, the frequencies of the -403A allele were comparable in the two cohorts of children. On the other hand, there are wide variations in prevalence of AEDS both within and between countries inhabited by similar ethnic groups, suggesting that environmental factors are critical in determining disease expression. Investigation of higher numbers of AEDS patients in different populations, measurement of the level of RANTES protein in the skin, with or without these polymorphisms, or experiments with transgenic animal models and cell cultures may explore the exact biological role of these alleles in the disease.

Although MCP-1 does not directly induce the chemotaxis and release of eosinophils, the finding that neutralization of MCP-1 drastically reduces eosinophil recruitment to the lungs suggests a role of MCP-1 in eosinophil function (22). In addition, recently the -2518G at the distal gene regulatory region of the MCP-1 has been found to be associated with increased blood eosinophil levels in asthmatic patients (23). Furthermore, MCP-1 can drive undifferentiated T lymphocyte populations towards Th2 type cells (12). Since in acute lesions of AEDS preferential accumulation of Th2 type cytokines can be detected, the MCP-1 -2518G allele, which was found to increase the MCP-1 expression, was a possible candidate for disease susceptibility polymorphism. Our results, however, did not support this assumption. In this cohort of children, there is no detectable association between the MCP-1 -2518G allele and AEDS, or allergy in general.

It is noteworthy that there are considerable differences in the frequencies of these polymorphisms among the different races. Because these polymorphisms are functional, i.e., they alter the expression of important biological active compounds, these inter-race differences must have an impact on disease processes, or responses to infections or drugs. It must be noted, however, that susceptibility to common disorders or responses to drugs usually result from the cumulative effect of many independent genetic loci and environmental influences. As a consequence, large-scale single nucleotide polymorphism (SNP) screenings linked with personal medical and environmental information are necessary to elucidate the real

significance of these polymorphisms in different races and to predict susceptibility to diseases, and tailor therapy to the individual.

In conclusion, in this cohort of Hungarian children there was no association between  $-28\text{G}$ , and  $-403\text{A}$  alleles in the RANTES promoter,  $-2518\text{G}$  polymorphism in the distal regulatory region of the MCP-1 and AEDS, or allergy in general.

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