

# Association of IL-13, RANTES, and leukotriene C4 synthase gene promoter polymorphisms with asthma and/or atopy in African Americans

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**Purpose:** IL-13, RANTES (Regulated on Activation, Normal T cells Expressed and Secreted), and cysteinyl leukotrienes are asthma and atopy mediators. Two RANTES –403(G to A) and –28(C to G), an –1055 IL-13(C to T), and a –444(A to C) leukotriene C4 synthase (LTC4S) single nucleotide polymorphisms (SNPs) have been shown in Caucasians and Asians as asthma and atopy risk factors. We studied these SNPs in African Americans with asthma and/or atopy. **Methods:** We studied 61 patients with asthma and/or atopy and 129 to 157 newborn controls for the –403 RANTES, –28 RANTES, and –1055 IL-13 SNPs, as well as 47 patients and 60 newborn controls for the –444 LTC4S SNP. **Results:** The two groups did not significantly differ at the genotypes of the –403 and –28 RANTES SNP. On the other hand, the mutant TT genotype for the –1055 IL-13 SNP was detected in 19.7% of patients versus 12.7% in controls ( $P < 0.04$ , OR 2.9, 95% CI 1.0–8.0), and the mutant T allele in 58.3% versus 36.6% in controls ( $P < 0.02$ , OR 2.4, 95% CI 1.1–5.2). In a similar fashion, for the –444 LTC4S SNP, the mutant AC genotype was detected in 19.1% versus 10.0% in controls ( $P > 0.28$ ); mutant C allele had an OR of 2.1 (95% CI 0.7–6.3). **Conclusion:** African American asthmatics/atopics had higher frequency of the TT mutant gene for the –1055 IL-13 SNP and of its mutant T allele. Regarding the –444 LTC4S SNP, there was a definite difference, although not statistically significant, with an OR of 2.1 for the mutant AC genotype in patients. If these findings become reproduced by larger studies, it may suggest that IL-13 and LTC4S SNPs can be used as predictive markers for asthma/atopy in African Americans. *Genet Med* 2005;7(6):406–410.

**Key Words:** asthma, atopy, IL-13, RANTES, LTC4S

Asthma (a chronic complex inflammatory airway disease) and atopy (a condition of hyper-responsiveness of IgE to allergens) are both caused by an interaction between genetic and environmental factors.<sup>1</sup> Genetic predisposition is probably caused by a characteristic pattern of polymorphism in multiple genes involved in the regulation of the allergic reaction.<sup>2</sup> Several candidate genes for atopy and asthma have been described<sup>3–5</sup>; three of them are IL-13, RANTES (Regulated upon Activation, Normal T cells Expressed and Secreted), and LTC4S (leukotriene C4 synthase) genes.

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The IL-13 gene lies on chromosome 5q31-33 regions, which contain a number of genes involved in the etiology of allergic asthma.<sup>5–8</sup> IL-13 promotes the differentiation and survival of eosinophils and mast cells. It also induces the IgE isotype switch and VCAM-1 expression. However, it inhibits monocyte-mediated antibody-dependent cellular cytotoxicity, proinflammatory cytokine/chemokine production, and nitric oxide secretion. Administration of IL-13 induces eosinophilic infiltration of the airways.<sup>9</sup> IL-13–overexpressing mice exhibit eosinophilic bronchial inflammation, mucus hypersecretion, fibrosis, eotaxin production, and nonspecific airway hyperactivity.<sup>10</sup> The –1055(C to T) SNP in the promoter region of IL-13 is associated with increased risk of allergic asthma in Dutch Caucasians.<sup>11</sup>

The RANTES gene is located on chromosome 17q11.2-q12.<sup>28</sup> RANTES is a CCL5 chemokine that can induce chemotaxis and activation of several cells (mast cells, eosinophils, basophils, and T-cells). Higher RANTES levels have been demonstrated in bronchoalveolar lavage fluid of patients with active asthma.<sup>12</sup> Furthermore, significant rise in plasma RANTES level has been found in asthmatics during acute attacks.<sup>13</sup> It has been reported that two functional polymorphisms in the proximal promoter region of the RANTES gene

(-28 C to G and -403 G to A) increase transcriptional activity and subsequent RANTES expression in human cell lines.<sup>14,15</sup> The -403 allele was found to be associated with atopic dermatitis (but not with asthma) in German children,<sup>15</sup> as well as with increased susceptibility to both asthma and atopy and increased severity of airway obstruction in British population.<sup>16</sup> The -28 C to G RANTES SNP represented a genetic risk factor for life-threatening asthma attacks in Chinese children.<sup>17</sup>

Cysteinyl leukotrienes (Cys-LTs) are critical mediators of bronchial smooth muscle contraction and eosinophilia in asthma and atopy. Arachidonic acid is the precursor fatty acid that is transformed into the Cys-LTs by the 5-lipoxygenase (5-LO) pathway.<sup>18</sup> LTC<sub>4</sub>S, a 5-LO enzyme, catalyzes the production of the unstable LTC<sub>4</sub>, which then in the extracellular environment is converted to its bioactive metabolites LTD<sub>4</sub> and LTE<sub>4</sub>. An -444(A to C) LTC<sub>4</sub>S SNP was identified in the gene promoter region of the chromosome 5q35<sup>19</sup> and was predicted to create an extra recognition site for the AP-2 transcription factor and lead to increased gene transcription (transcriptional upregulation). Hence, the -444C mutant allele is a risk factor for aspirin-induced asthma (AIA).<sup>20</sup> The role of LTC<sub>4</sub>S promoter polymorphism in AIA is unclear, with several studies indicating a functional role,<sup>21</sup> whereas others question its significance.<sup>22</sup> The -444C allele is present in 20% to 30% of the general Caucasian and Asian population.<sup>23</sup>

There is no data on African Americans on the association of the RANTES -403(G to A) and -28(C to G), IL-13 -1055(C to T), or LTC<sub>4</sub>S -444(A to C) SNP with asthma and/or atopy. Hence, we investigated the possibility of such an association.

## MATERIALS AND METHODS

### Subjects

Our subjects were African Americans from Northwestern Louisiana who have been followed at the Allergy/Immunology Clinics of the Louisiana State University Health Sciences Center with already established diagnoses of allergic rhinitis, bronchial asthma, atopic dermatitis, or combinations. The diagnosis was established according to the classic findings in the medical history, physical examination, and relevant laboratory tests. Empirically the total group was referred to as "asthma/atopy" and composed of 61 patients 2 to 74 years of age.

Atopy was determined by a positive allergy skin test or serum specific IgE to common environmental allergens. Skin testing was done by the prick method (SPT) and the reaction was considered positive if it was  $\geq 2+$ , compared to histamine (1:1000) and a negative control. More specifically scoring of the SPT was considered  $\geq 2+$  if the response was larger than the negative control by 50% to 75% (wheal diameter at least 3 mm), and as  $\geq 4+$ , the response larger than the positive histamine control by 25% to 50% (wheal with pseudopodes).<sup>26</sup>

Serum specific IgE antibody level was measured by the ImmunoCAP method (Pharmacia Diagnostics, Kalamazoo, MI) and was considered positive if  $\geq 0.35$  kU/L. According to the laboratory's criteria, the test results was considered 2+ for spe-

cific IgE levels of 0.71 to 3.50 kU/L, 3+ for levels of 3.51 to 17.51 kU/L, and 4+ for levels of 17.51 to 50.00 kU/L.<sup>27</sup>

Patients' DNA was compared with that of healthy African American newborn controls: 129 to 157 for IL-13 and RANTES and 60 for LTC<sub>4</sub>S. The polymorphic assays on controls were performed on newborns' DNA stored specimens that were obtained for previous studies.

### Genotyping

PCR-restriction fragment length polymorphism was used.<sup>25</sup> Aliquots of PCR products were subjected to 2% agarose gel electrophoresis. The IL-13, RANTES, and LTC<sub>4</sub>S genotyping and PCR conditions are summarized in Table 1. Genotyping assays for LTC<sub>4</sub>S SNP (47 patients) and for IL-13 and RANTES (61 patients) were originally done for two separate investigations that were presented as abstracts at two different meetings. In this article, we combined the two pieces of work, hence the discrepancy in numbers.

### Statistical analysis

The association between the asthma and/or atopy and the polymorphic alleles was analyzed in our data using the Chi-square test. The association was considered significant if the *P* value was  $< 0.05$ .

## RESULTS

### IL-13, RANTES, and LTC<sub>4</sub>S genotypes of asthma and/or atopy patients in comparison to healthy controls

Table 2 shows the comparison of the three IL-13, RANTES, and LTC<sub>4</sub>S SNPs, in asthma/atopy patients versus healthy controls. The *P* value was statistically significant ( $P < 0.04$ , odds ratio 2.9, 95% CI 1.0-8.0) only for the IL-13 TT mutant allele. Regarding the LTC<sub>4</sub>S SNP, the *P* value was not significant ( $P > 0.28$ ), but the odds ratio of the C allele was 2.1 (95% CI = 0.7-6.3).

### IL-13 -1055TT, RANTES -403AA, and LTC<sub>4</sub>S -444AC mutant genotypes according to atopic disease

We analyzed the data according to the three major atopic diagnoses, i.e., allergic rhinitis (AR), bronchial asthma (BA), and atopic dermatitis (AD). Compared to controls, no statistically significant differences were noted for any of the three SNPs studied (Table 3).

### Relationship of allergen sensitization and IL-13, RANTES, and LTC<sub>4</sub>S SNPs

We investigated the possible relationship of the SNPs to the degree of positivity (i.e., sensitization) to the aeroallergen skin prick tests (SPT) or the specific IgE (Table 4). Because normal subjects can have a positive skin test or serum specific IgE to one or a few aeroallergens without clinical relevance, we classified our patients into 2 groups ( $\geq 2+$  and  $\geq 4+$ ) according

**Table 1**  
PCR primers, assay conditions, and restriction enzymes for genotyping

Polymorphism	Primers (5', 3')	MgCl <sub>2</sub>	Annealing Temp. °C	Restriction enzyme	Alleles	Fragment sizes (bp)
IL-13 C-1055T	5'-ACTTCTGGGAGTCAGAGCCA-3' 5'-TACAGCCATGTCGCCTTTTCCTGCTCTTCCGTC-3'	3.25 mM	62	Hpy99 I	CC	339 33
					TT	372
					CT	339 33 372
RANTES G-403A	5'-CACAAAGAGGACTCATTCCAACCTCA-3' 5'-GTTCTGCTTATTCATTACAGATCGTA-3'	2.6 mM	55	Rsa I	GG	180 26
					GA	206 180 26
					AA	206
RANTES C-28G	5'-ACTCCCCTTAGGGGATGCCCGT-3' 5'-GCGCAGAGGGCAGTAGCAAT-3'	2.6 mM	55	Hinc II	CC	152 23
					CG	175 152 23
					GG	175
LTC <sub>4</sub> S A-444C	5'-CAACGACTAAGGCTGGCAGG-3' 5'-CACTTTCTCCAGGGCCTTGC-3'	2.5 mM	62	Msp I	AA	199
					AC	167 199
					CC	167

**Table 2**  
IL-13, RANTES, and LTC<sub>4</sub>S genotype of asthma and/or atopy patients compared to controls

		Asthma/atopy	Controls	p value	Odds ratio
IL-13 -1055	CC	13 (21.3%)	62 (39.5%)	0.04	2.9 95% CI = 1.0-8.0
	CT	36 (59.0%)	75 (47.8%)		
	TT	12 (19.7%)	20 (12.7%)		
	Total patients	61	157		
RANTES -403	GG	16 (26.2%)	35 (26.7%)	NS*	
	GA	34 (55.7%)	72 (55.0%)		
	AA	11 (18.0%)	24 (18.3%)		
	Total patients	61	131		
RANTES -28	CC	59 (96.7%)	129 (100%)	NS*	
	CG	2 (3.3%)	0 (0%)		
	GG	0 (0%)	0 (0%)		
	Total patients	61	129		
LTC <sub>4</sub> S -444	AA	38 (80.6%)	54 (90.0%)	0.303 (NS)	2.01 95% CI = 0.624-6.656
	AC	9 (19.1%)	6 (10.0%)		
	Total patients	47	60		

NS, Not significant.

to the degree of positivity to at least 50% of the allergens tested in each individual patient. We found statistically significant difference in the category of sensitization  $\geq 2+$  of the IL-13 T mutant allele (*P* value 0.019, odds ratio 2.42, 95% CI = 1.143-5.165) in comparison to newborn controls.

## DISCUSSION

Different polymorphisms have been previously identified in candidate genes associated with asthma and/or atopy in Caucasians. In the present study on African Americans, we inves-

**Table 3**IL-13 –1055 TT, RANTES –403 AA, and LTC<sub>4</sub>S –444AC mutant SNPs in allergic rhinitis (AR), bronchial asthma (BA), and atopic dermatitis (AD)

DIAGNOSIS	n	IL-13 –1055				P Value	RANTES –403					P Value	LTC <sub>4</sub> S –444			
		CC Wild	TT Mutant	CT	CT		n	GG Wild	AA Mutant	GA	GA		n	AA Wild	AC Mutant	AC
AR	46	8	6	32	NS	46	11	6	29	NS	37	30	7	NS		
BA	38	6	9	23	NS	38	13	4	21	NS	30	26	4	NS		
AD	16	3	4	9	NS	16	4	2	10	NS	13	10	3	NS		

NS, Not significant compared with African American newborn controls.

**Table 4**Number of patients genotyped among those positive by allergy skin prick test/specific IgE in IL-13, RANTES –403, and LTC<sub>4</sub>S SNPs, according to degree of positivity to at least 50% of allergens tested

SPT/sIgE Score	IL-13 C –1055T					RANTES G –403A					LTC <sub>4</sub> S A –444C			
	n	CC	TT	CT	P value	n	GG	AA	GA	P value	n	AA	AC	P value
≥ 2+	18	1	4	13	0.019	18	2	1	15	NS	15	13	2	NS
	(100%)	(5.5%)	(22.2%)	(71.5%)		(100%)	(11.1%)	(5.5%)	(83.3%)		(100%)	(86.6%)	(13.3%)	
≥ 4+	5	1	1	3	NS	5	1	1	3	NS	3	3	0	NS
	(100%)	(20%)	(20%)	(60%)		(100%)	(20%)	(20%)	(60%)		(100%)	(100%)	(0%)	

NS, Not significant compared with African American newborn controls.

tigated the association of three critical SNPs (IL-13, RANTES, and LTC<sub>4</sub>S) that control the expression of important asthma and/or atopy molecules.

We found statistically significant difference in the IL-13 –1055 TT mutant genotype in our patients, compared to African American newborn controls. This is consistent with the findings of a similar study on this specific SNP in a Dutch Caucasian population.<sup>11</sup>

There was no significant difference between the patients and controls regarding RANTES –403 and –28 SNP. An association has been previously reported of the –403 RANTES SNP with atopic dermatitis, but not with asthma, in German children<sup>15</sup> as well as an increased susceptibility to asthma/atopy, and increased severity of airway obstruction in a British population.<sup>16</sup>

The –444 LTC<sub>4</sub>S SNP has been studied before in Caucasian and Japanese patients with aspirin-induced asthma (AIA) and aspirin tolerant asthma (ATA). The presence of the C allele was identified as a risk factor for AIA Caucasian patients compared with ATA or healthy subjects (odds ratio 3.89).<sup>20</sup> The role of the LTC<sub>4</sub>S promoter SNP in AIA is unclear. One study supports its functional role,<sup>21</sup> whereas three others question its significance.<sup>22–24</sup> Our study is the first to suggest a role for LTC<sub>4</sub>S SNP in the development of asthma/atopy in African Americans, with an odds ratio of 2.01 (95% CI 0.624–6.656), although the association was not statistically significant, possibly due to the small sample size. Nevertheless, our findings are probably valid by their similarity to those in previous studies on Caucasian<sup>20</sup> or Asian<sup>23</sup> populations.

Larger studies are needed to validate our findings, which would suggest that IL-13 and LTC<sub>4</sub>S SNPs can be used as predictive markers for asthma/atopy in African Americans.

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