

A NOS1 Gene Polymorphism Associated with Asthma and Specific Immunoglobulin E Response to Mite Allergens in a Colombian Population

B. Martínez^a K. Barrios^a C. Vergara^a D. Mercado^a S. Jiménez^a L. Gusmão^b
L. Caraballo^a

^aInstitute for Immunological Research, University of Cartagena, Cartagena, Colombia;

^bIPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal

Key Words

Asthma • Immunoglobulin E • House dust mite •
Nitric oxide synthase • Population study • Polymorphism

Abstract

Background: Nitric oxide (NO) is involved in asthma pathogenesis and is synthesized by three isoforms of NO synthase, one of them encoded by *NOS1* gene. The CA-repeat and the C5266T SNP in *NOS1* exon 29 have been associated with asthma and IgE levels. We thought to test the association of asthma and asthma-related phenotypes with the exon 29 CA-repeat and the C5266T SNP in a Colombian population sample. **Methods:** The CA-repeat and the C5266T SNP were genotyped in 167 asthmatics and 166 controls using PCR-based fragment length polymorphism and TaqMan assay. We also determined total and mite-specific IgE against *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. **Results:** Three new CA-repeat alleles, 14, 23 and 24 repeats were detected. Allele comprising 16 repeats was associated with asthma (OR: 1.90 (CI 1.22–2.97, $p_c = 0.028$) and low total ($p_c = 0.02$) and specific IgE to *B. tropicalis* ($p_c < 0.0001$) and *D. pteronyssinus* ($p_c < 0.0001$). We found no association of the C5266T SNP and asthma or IgE levels. **Conclusion:** *NOS1* exon 29 CA-repeat may be a risk factor for asthma susceptibility and mite specific IgE response in a Colombian population.

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Introduction

Asthma is a chronic inflammatory disease highly associated with atopy, characterized by reversible airflow obstruction and bronchial hyperresponsiveness. It affects millions of people worldwide, having a considerable socioeconomic impact [1–3]. It is considered a multifactorial disorder involving complex interactions between genetic and environmental factors [4]. Several reasons make *NOS1* an important candidate gene for asthma susceptibility, including its location on 12q14–24.2, a chromosomal region consistently reported to be linked to asthma and atopy in several studies [5–8] and the relationship of NO with asthma pathogenesis.

NOS1 codifies for neuronal NOS, one of the three isoforms of NOS, that convert L-arginine to L-citrulline and NO. Some studies have detected associations between the genes of the other two isoforms, NOS2 (inducible NOS) and NOS3 (endothelial NOS), and asthma [9–13]; however, regarding this disease, most of the positive findings have been with *NOS1*. NO produced by *NOS1* is a neurotransmitter for ‘nonadrenergic noncholinergic’ bronchodilator nerves [14]. NO concentration in exhaled air of asthmatics is higher than that of normal subjects [15, 16] and decreases after treatment with corticosteroids [17]. Furthermore, *NOS1* knock-out mice showed lower exhaled NO than wild-type mice and were less responsive

Table 1. Associations of *NOS1* gene with asthma and asthma-related phenotypes

Author, year of publication	Population	Phenotype and p value	<i>NOS1</i> Polymorphism	Reference
Gao P, et al., 2000	Caucasian	asthma, p = 0.03	intron 2 CA repeat	9
Grasemann H, et al., 2000	Caucasian	asthma, p = 0.01	exon 29 CA repeat	27
Shao C, et al., 2004	Japanese	asthma, p = 0.04	intron 2 CA repeat	28
Immervol T, et al., 2001	Caucasian	total IgE, p = 0.02	C5266T SNP	29
Ali M, et al., 2003	Australian	atopy, p = 0.03	intron 20 AAT repeat	31
Hollá L, et al., 2004	Caucasian	total IgE, p = 0.05	C5266T SNP	30
Leung T, et al., 2005	Chinese	total IgE, p = 0.01; IgE to Dp, p = 0.04	C5266T SNP	13
Wechsler M, et al., 2000	White, Hispanic	FE _{NO}	intron 20 AAT repeat	32

to methacoline [18, 19]. In addition to being a potent effector molecule in the innate immune response [20], NO also influences the adaptative immune response in both mouse and humans by exerting a regulatory role in Th1/Th2 generation [21–24] and it has been suggested that could contribute to asthma pathogenesis by selective downregulation of the Th1 response or promoting the Th2 response [25, 26].

NOS1 polymorphisms have been associated with asthma [9, 27, 28], IgE levels [13, 29–31] and exhaled NO concentration [32]. The three studies showing association with asthma were performed in Caucasian and Japanese populations, two of them with the *NOS1* intron 2 CA repeat and the other with the exon 29 CA repeat. Regarding to IgE levels and atopy, the studies were done in Caucasians and Chinese, three of them with the C5266T SNP and one with the intron 20 AAT repeat (table 1). Replicating them in different populations with a different genetic background and living in other geographic conditions, is very important in order to explore the relevance of these association studies. *Blomia tropicalis* and *Dermatophagoides pteronyssinus* are the main source of sensitization in tropical environments [33–35]; remarkably, the prevalence of IgE sensitization to other aeroallergens is very low in asthmatics and general population of Cartagena [36]. Since the SNP C5266T seems to be mainly associated with IgE levels and the exon 29 CA repeat has been associated with asthma phenotype, we hypothesized that the effects of these polymorphisms upon IgE responses could be better detected among individuals living in a tropical environment highly exposed to mite allergens. Therefore, we sought to test associations of two polymorphisms located in exon 29 of *NOS1*, the CA repeat and the C5266T SNP, with asthma and IgE response to mites in a Colombian population and compare their allele distributions with those described for other populations.

Materials and Methods

Subjects

The study was approved by the Bioethics Committee of the School of Medicine of The University of Cartagena. A full verbal explanation of the investigation was given and all subjects gave a written consent for their inclusion in the present study. One hundred and sixty-seven unrelated asthmatics were randomly selected from patients who visited three public health centers in Cartagena (Colombia). Asthma was defined and classified according to the Global Initiative for Asthma Criteria, GINA [37], using a standardized questionnaire previously tested [3, 38] in patients with a history of physician-diagnosed asthma. The diagnosis was confirmed by a physician belonging to the research staff, sustained on a clear clinical history with clinical symptoms and the presence of reversible airways disease on pulmonary function testing. Reversibility of lung function was confirmed by an increase of 15% or more in FEV₁ after inhaling 400 µg of salbutamol. Subjects meeting the following criteria were recruited: age of 8 years or greater having at least two years of asthma evolution, three or more episodes of asthma symptoms (wheezing, chest tightness and dyspnea) in the last 12 months or absence of symptoms because of using anti-asthmatic medications. Children under 8 years of age were excluded to avoid asthma misdiagnosis due to the high prevalence of transitory wheezing at this age range. As a control group, 166 nonrelated individuals without clinical history of asthma or other chronic diseases were recruited randomly from the same neighborhoods of patients. Both patients and controls lived in the same urban area and belonged to the prevalent ethnic group in Cartagena, mulattos, an admixture originating mainly from Spaniards and Africans [39, 40]. Subjects with specific IgE to at least one of the mites tested were considered atopic.

Total and Specific IgE

Total serum levels of IgE were quantified by duplicate using a commercial enzyme-linked immunosorbent assay (ELISA) kit (RIDASCREEN® Total IgE; R-Biopharm, Darmstadt, Germany). Specific IgE against *B. tropicalis* and *D. pteronyssinus* was detected by ELISA. Mite extracts were obtained as described previously [41]. ELISA was performed as described previously [42], but with modifications. Briefly, extracts were diluted 1:50 in 0.2 M sodium carbonate/bicarbonate buffer (pH 9.2) and 100 µl were added to

Table 2. Demographic and laboratory characteristics of the subjects

Characteristics	Asthmatics (n = 167)	Controls (n = 166)	p value
Mean (SD) age, years	33.15 (17.40)	29.02 (16.03)	0.060
Male gender, %	67 (40.2)	79 (47.6)	0.16
Smokers, %	9.7	0.7	<0.0001
Median of total IgE, IU/ml (interquartile range)	900.6 (395.6–1,163.5)	83.4 (38.7–192.1)	<0.0001
Median OD Bt-specific IgE (interquartile range)	1.05 (0.15–2.4)	0.09 (0.08–0.1)	<0.0001
Median OD Dp-specific IgE (interquartile range)	0.43 (0.15–1.3)	0.09 (0.08–0.1)	<0.0001

each well of microtiter plates (Immulon-4 Dynatech, Alexandria, Va., USA), incubated overnight at room temperature and washed five times (PBS pH 7.4, 0.1% Tween 20). The plates were blocked with 100 μ l 3% bovine serum albumin in PBS. One hundred microliters of each serum sample diluted 1:5 in blocking buffer were added to the wells and placed overnight in a humid chamber. Plates were washed and incubated with 100 μ l of anti-human IgE alkaline phosphatase conjugate, diluted 1:500 (Sigma, St Louis, Mo., USA) and developed with *p*-nitrophenylphosphate substrate. Absorbance was measured at 405 nm using a Spectrophotometer (Spectra MAX 250 Molecular Device Sunnyvale, Calif., USA) and expressed as Optical Density (OD) units. Samples were assayed by duplicated and levels of specific IgE above 0.110 OD (mean OD of 5 nonallergic controls + 3 SD) were considered positive.

Genotyping

Genomic DNA was extracted using conventional 'salting out' technique [43]. The CA polymorphism was genotyped by PCR and GeneScan analysis; PCR reactions were performed in a total volume of 25 μ l containing 100 ng of genomic DNA, 200 μ M dNTPs, 1.5 U of *Taq* Gold Polymerase (Applied Biosystems, USA), *Taq* gold GeneAmp® PCR buffer 1 $\times$ and 10 pmol of each forward (6-FAM labeled) and reverse primers. Primers used were: forward 5'-TGC AGG AAC TAG GCA CAA GC-3' and reverse 5'-GAT CGA CAC ACT TGT GCA GG-3' [27]. PCR conditions were as follows: an initial denaturation step at 95°C for 6 min, followed by 35 cycles at 95°C for 1 min, 59°C for 1 min, and 72°C for 1 min. A final extension step was carried out at 72°C for 5 min. To determine the number of CA repeats in different alleles, PCR products were separated by electrophoresis in an ABI PRISM 310™ sequencer and GeneScan software (Applied Biosystems, Foster City, Calif., USA) was used to assign genotypes by fragment size analysis.

The TaqMan assay was designed and manufactured by Applied Biosystems. Probes for genotyping the C5266T (rs2682826) were: 5'-CATGTTCCAGTGGTGTGTCATGCCCCATGAGTTGCCCTTGTCGGCAAGAGAG-3' (VIC probe) and 5'-CATGTTCCAGTGGTGTGTCATGCACCCGTGAGTTGCCCTTGTCGGCAAGAGAG-3' (FAM probe). The PCR reaction was done in a final volume of 25 μ l containing 50 ng of DNA, 12.5 μ l of 2 $\times$ Universal PCR Master Mix, 1.25 μ l of assays on demand 20 $\times$ and 6.25 μ l of bidistilled water. Thermal cycling conditions were: AmpErase® UNG activation at 50°C for 2 min followed by AmpliTaq® Gold DNA pre-incubation for 10 min at 95°C; 50 cycles of 15 s at 92°C and 60°C for 1 min. Automatic calling was done

with a quality value above 99% using the 7300 system SDS software in a 7300 Real-Time PCR System (Applied Biosystems, Foster City, Calif., USA).

Sequencing

All the samples suspected of having new alleles were sequenced. The number of repeats in each allele was confirmed by sequencing by the dideoxy chain termination method using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, Calif., USA) according to the manufacturer's instructions. The sequencing reactions were done in a volume of 20 μ l using the same reverse primer as for the PCR reaction. Sequencing products were separated and detected in an ABI PRISM 310 Genetic Analyzer.

Statistical Methods

Hardy-Weinberg equilibrium (HWE) was tested in asthmatics and controls by the Markov Chain method using Arlequin software [44]. Allele and genotype frequencies of the studied groups were compared by Fisher's exact test in 2 $\times$ 2 tables of the counts of a given allele or genotypes versus all others. Patients and controls characteristics were compared using Student's *t* test as needed. Normal distribution of IgE levels was evaluated by Kolmogorov-Smirnov test and the relationship between total and specific IgE levels and genotypes was assessed by non parametric tests. Statistical analysis was performed with the SPSS software (SPSS 13 for Windows, SPSS Inc., Chicago, Ill., USA). When analyzing associations with the CA repeat polymorphism, *p* values were corrected for multiple tests (10 alleles) as Bonferroni (*p*_c); values <0.05 were considered statistically significant.

Results

Some demographic and laboratory characteristics of patients and controls are described in table 2. The groups were matched by age and sex; total and specific IgE levels were significantly higher in the asthmatic subjects compared with the non asthmatic controls. Neither total nor mite-specific IgE levels were significantly different between smokers and nonsmokers, both in patients and controls (data not shown). 91.6% of patients were atopic

Table 3. Allele distribution of CA repeats in exon 29 of *NOS1* in Colombian subjects and comparison with published data for other ethnic groups [45]

Allele repeats	Colombian (n = 166)	p ¹	African-American (n = 210)	p ²	Caucasian (n = 538)	p ³	Japanese (n = 250)
14 (170 bp)	6 (1.8)	–	–	–	–	–	–
15 (172 bp)	19 (5.7)	0.65	14 (6.7)	<0.001	1 (0.2)	<0.001	57 (22.8)
16 (174 bp)	40 (12.0)	0.002	9 (4.3)	<0.001	3 (0.5)	<0.001	4 (1.6)
17 (176 bp)	135 (40.7)	0.96	85 (40.5)	<0.001	414 (77.0)	<0.001	155 (62.0)
18 (178 bp)	33 (9.9)	<0.001	62 (29.5)	0.29	66 (12.3)	0.27	32 (12.8)
19 (180 bp)	21 (6.3)	0.005	28 (13.3)	<0.001	8 (1.5)	<0.001	2 (0.8)
20 (182 bp)	17 (5.12)	0.026	3 (1.4)	<0.001	7 (1.3)	–	–
21 (184 bp)	27 (8.13)	0.05	8 (3.8)	0.56	38 (7.1)	–	–
22 (186 bp)	14 (4.22)	0.009	1 (0.5)	<0.001	1 (0.2)	–	–
23 (188 bp)	19 (5.72)	–	–	–	–	–	–
24 (190 bp)	1 (0.3)	–	–	–	–	–	–

p¹ = Comparing Colombian subjects with African-American subjects; p² = comparing Colombian subjects with Caucasian subjects; p³ = comparing Colombian subjects with Japanese subjects.

Table 4. *NOS1* allele frequencies in asthmatic and control subjects

Allele repeats	Basepairs	Asthmatics (%) n = 167	Controls (%) n = 166	OR	CI	p value
14	170	8 (2.4)	6 (1.8)	1.33	0.42–4.38	0.59
15	172	22 (6.6)	20 (6.02)	1.10	0.56–2.15	0.76
16	174	69 (20.7)	40 (12.0)	1.90	1.22–2.97	0.0027*
17	176	110 (32.9)	135 (40.7)	0.72	0.52–1.00	0.038
18	178	37 (11.1)	33 (9.9)	1.13	0.67–1.91	0.63
19	180	22 (6.6)	21 (6.3)	1.04	0.54–2.02	0.89
20	182	11 (3.3)	16 (4.8)	0.67	0.29–1.56	0.31
21	184	27 (8.1)	27 (8.13)	0.99	0.55–1.79	0.98
22	186	14 (4.2)	14 (4.22)	0.99	0.44–2.25	0.98
23	188	11 (3.3)	19 (5.72)	0.56	0.25–1.26	0.13
24	190	3 (0.9)	1 (0.3)	3.00	0.28–75.19	0.31
Total		334	332			
* p _c = 0.028.						

and 78.4% had both asthma and rhinitis. Classification of asthma severity showed that 65.2% (109/167) of patients had intermittent asthma, 18% (30/167) mild persistent, 12% (20/167) moderate persistent and 4.8% (8/167) severe persistent. 91.6% of patients were atopic.

CA Repeat Alleles in Colombian and Other Populations

We found eleven alleles of the CA dinucleotide repeat, ranging from 170 to 190 bp; three of them had not been

previously described, corresponding to 14 (170 bp), 23 (188 bp) and 24 (190 bp) CA repeats (GenBank Accession numbers DQ011899, DQ011900, DQ011901, respectively) (table 3). Genotype distribution did not deviate from Hardy-Weinberg expectancy among controls. The overall distribution of alleles in our control group differed significantly from that previously described in African Americans, Caucasians and Japanese ($p < 0.001$ for each comparison) [45], however, the commonest allele in those populations (17 repeats), was also the most frequent in

Fig. 1. Effect of allele 16 (174 bp) on the specific IgE response to domestic mites: *Dermatophagoides pteronyssinus* (Dp) (a) and *Blomia tropicalis* (Bt) (b). p_c corrected value (p_c) for both analyses is <0.0001 .

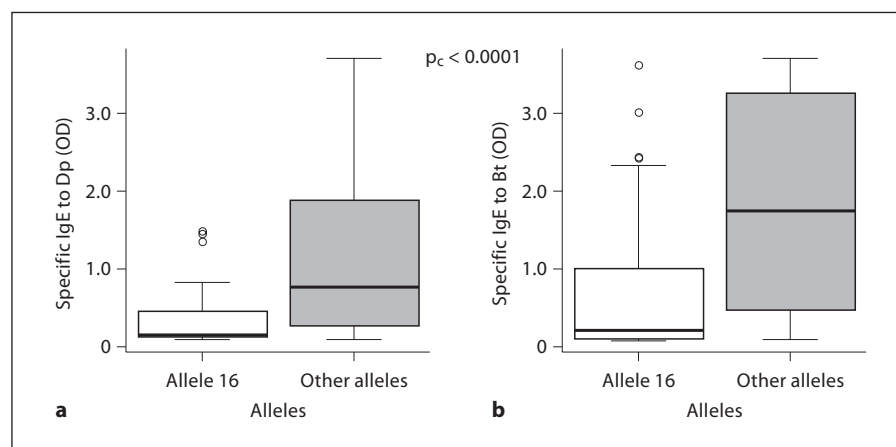
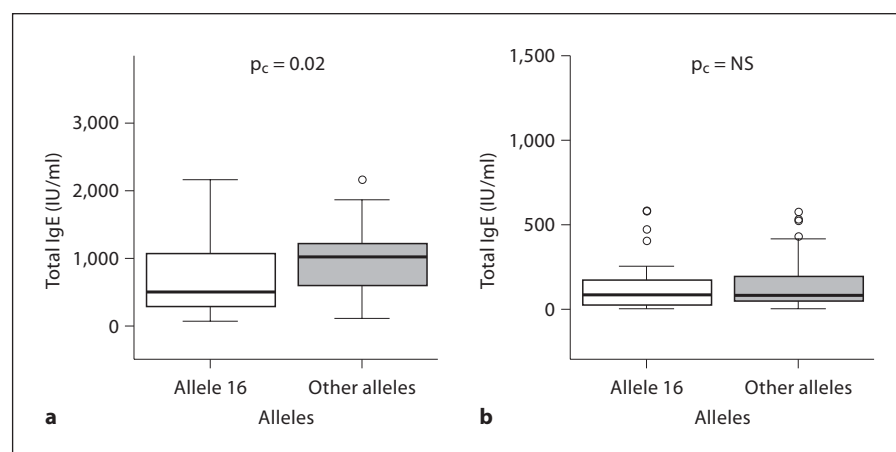


Fig. 2. Effect of allele 16 (174 bp) on the serum levels of total IgE in asthmatic patients (a) and healthy controls (b).



our control group. Table 3 shows the comparison of allele distribution of the CA-repeat in our healthy control group and other three populations described by Grasemann [45].

Distributions of the CA Repeat Polymorphism in Asthmatics and Controls

There was a significant difference of the frequency of allele 16 (174 bp) in asthmatic and control subjects (OR: 1.9; CI 1.22–2.97, $p_c = 0.028$) (table 4). In addition, association of this allele with the mild intermittent and moderate forms of asthma was found, however, after correction, only the association with moderate asthma remained significant ($p_c = 0.04$). There were no significant differences in the distribution of CA repeat alleles between patients with intermittent-to-mild persistent asthma and those with moderate persistent-to-severe asthma ($p_c = 0.5$) although allele 16 was more frequent in the latter

group. Allele 17 was associated as a protecting factor, but the significance ($p = 0.038$) disappeared after applying a correction for multiple tests. No significant differences were found on genotype distribution between patients, including asthma severity groups, and controls. Since 91.6% of patients were atopic, further analysis of atopy as such was not performed.

Influence of CA Repeat Alleles and Genotypes on IgE Levels among Patients

The distribution of total serum IgE levels and their logarithmic transformed values did not show a normal distribution. In patients, but not in controls, Mann-Whitney analysis showed that significantly lower IgE levels to *B. tropicalis* ($p_c < 0.0001$) was found in the group ($n = 63$) with allele 16 (median 0.20 OD) in comparison to patients ($n = 104$) without this allele (median 1.76 OD). Also, significantly lower IgE to *D. pteronyssinus* ($p_c <$

0.0001) was found in patients with allele 16 (median 0.15 OD) in comparison to patients without this allele (median 0.76 OD) (fig. 1). Furthermore, significantly lower total IgE levels ($p_c = 0.02$) were found in patients ($n = 63$) with allele 16 (median 480.2 IU/ml) in comparison to patients ($n = 104$) without this allele (median 985.0 IU/ml). This result was not observed in the subgroup of controls (fig. 2). The genotype 16/15 was also associated with low IgE levels to *B. tropicalis* ($p_c = 0.01$). In contrast, the genotype 17/17 (176/176 bp) was associated with high IgE response to *B. tropicalis* ($p_c = 0.01$) and high total IgE levels ($p_c = 0.02$). Genotype 16/16 was associated with low IgE levels to *D. pteronyssinus* ($p = 0.01$) but the significance disappeared after correction.

Total IgE was also analyzed as a qualitative trait using a cut-off level at 343 IU/ml (mean total IgE in the control group + 1 SD). Allele 16 was associated with low IgE levels ($p = 0.018$) and allele 17 with high IgE levels ($p = 0.025$). However, these two p values were not significant after correction for multiple tests.

The C5266T SNP and Asthma and IgE Levels

No association was found between alleles or genotypes of this SNP and asthma or IgE levels. Allele's distribution in our control population (minor allele T = 20%) was not significantly different to those reported for several populations in the SNP database of GenBank, with the exception of Japanese, where the distribution was significantly different ($p < 0.0001$). Also, the distribution was different to that reported for healthy Chinese ($p < 0.0001$) in reference [13].

Discussion

During the last 6 years several variants of *NOS1* have been associated with asthma and IgE phenotypes. Although those associations involve different polymorphisms and alleles inside the same gene, a common feature described for association studies in asthma [46], they consistently show that probably *NOS1* plays an important role in asthma pathogenesis. In this study, we found an association between both asthma and IgE levels and the CA repeat polymorphism in exon 29 of the *NOS1* gene in a Colombian population. Allele 16 (174 bp) was associated with asthma and, in patients, with low IgE response to mites and low total IgE levels. This allele was also weakly associated with mild intermittent asthma and significantly with moderate persistent asthma, suggesting that the association observed with asthma in general is

determined by the more severe forms of the disease; however, due to the small number of patients in the group of moderate persistent asthma, this result should be taken cautiously and further evaluated in a larger population. The 17/17 genotype was significantly associated with high total and specific IgE levels but allele 17 was inversely associated with asthma, although this association was not statistically significant. In general, these results support a relationship of the CA repeat polymorphism with asthma and asthma-associated phenotypes; however, there are differences regarding the associated alleles in other reports. Grasemann et al. [27] found an association of this polymorphism with asthma in Caucasians, but with allele 17. They also found that allele 18 had a protector effect against asthma, but we found no association with this allele; moreover, although nonstatistically significant, the slightly protective effect we found was with allele 17. These differing results could be due to genetic background differences between the two populations and more specifically in the allele distribution of this polymorphism. However, these results on the exon 29 CA repeat, together with two other reports associating asthma with the Intron 2 CA repeat (table 1) suggest an actual role of some *NOS1* alleles as risk factors for asthma susceptibility.

NOS1 gene, by means of the C5266T SNP, has also been associated with total IgE levels in Caucasian and Chinese populations [13, 29, 30]. Furthermore, in Chinese subjects this polymorphism was also associated with specific IgE to *D. pteronyssinus*, as detected by skin test [13]. Our results support these and other data [31] suggesting that *NOS1*, or a close linked gene, could be influencing the IgE response. However, contrarily to the expected, we found no association of IgE levels with the C5266T SNP but with the CA repeat of exon 29, being the third polymorphism of *NOS1* gene associated with this phenotype.

As mentioned, differences in allele distributions could partially explain the diversity of associations reported within this gene; however the role of environmental factors should also be considered. Both studies from China [13] and Colombia, two countries located in subtropical and tropical zones and consequently exposed to mite allergens [47, 48], have shown association with specific IgE to domestic mites. In our particular case, under this environmental conditions and the genetic background of the population, a frequent and strong IgE response to mite allergens in asthmatics is induced [33, 36], making easier to identify 'high' and 'low' responders phenotypes and their relationship with genotypes; accordingly, fur-

ther studies evaluating the level of mite-allergen exposure among patients and controls should be done to analyze more accurately this relationship.

Interestingly, the associations of allele variants of *NOS1* with asthma and IgE are consistently present even in populations with different genetic backgrounds and significant differences in *NOS1* allele distribution. Leung et al. [13] found significant inter-ethnic differences in allele frequencies of the AAT repeat when compared their results in Chinese subjects with those from Caucasians and African Americans. However they were able to identify an association between this polymorphism and total IgE levels. In addition, associations of the *NOS1* intron 2 GT repeat and asthma have been found in both British and Japanese population [9, 28]. The present study also show associations with the CA repeat in exon 29, even though the distribution of most *NOS1* alleles in our population is significantly different to those reported for other ethnic groups. Given the frequency of CA repeat allele 16 and the size of the population included, our study has 88% power to detect a minimum odds ratio of 1.9. All these are additional evidences showing that *NOS1* is a susceptibility gene for asthma in diverse populations, which is compatible with the hypothesis that genetic effects are usually consistent across human populations [49].

NOS1 is a structurally complex gene with an intricate genetic regulation and diversity in the transcript generation. The exon 29 CA repeat polymorphism is located in the 3' UTR and can affect the size of *NOS1* mRNA [50, 51] which in turn could influence the production of the enzyme and, in consequence, the NO synthesis. NOS is not known to be directly involved in IgE synthesis; therefore, associations of variants of *NOS1* gene with IgE levels are more likely the result of linkage disequilibrium with other genes in this chromosomal region, more functionally related with IgE production. However, the growing evidence of the immunoregulatory effects of NOS2-induced NO [25, 26, 52] opens the pos-

sibility for a role of NOS isoforms in the Th1/Th2 polarization of the adaptative immune response and IgE production, particularly because it has been shown that NOS1 derived NO downregulates NF- κ B-dependent NOS2 expression [53].

Several authors have speculated about the physiological basis for associations between *NOS1* gene and asthma or atopy [27, 31]. Our finding that allele 16 is associated with asthma and also with low total and specific IgE levels is unexpected and intriguing. Since there is not experimental evidence of a direct effect of this enzyme on the regulation of IgE response, we can only speculate that the variant of *NOS1* associated with allele 16 is associated with inhibited nonadrenergic noncholinergic bronchodilation as well as with changes on its capacity to regulate NOS2, altering the influence of this enzyme on the basal *NOS1*-derived anti-inflammatory effect and the polarization of immune response. Alternatively, allele 16 could be causative for part of the asthmatic phenotype but being close linked to an IgE-downregulating variant of other gene in the chromosomal region. Functional analyses have to further clarify the physiological mechanisms conferred by *NOS1* polymorphisms.

In summary, our results add evidence about the relationship of *NOS1* gene and asthma. They also support previous reports showing that this gene is associated with the IgE immune response, although a possible cause-effect relationship between these two findings is not clear and the physiological mechanisms supporting them remain unknown.

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References

- 1 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al: International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-491.
- 2 Beasley R: The burden of asthma with specific reference to the United States. *J Allergy Clin Immunol* 2002;109(5 suppl):S482-S489.
- 3 Dennis R, Caraballo L, Garcia E, Caballero A, Aristizabal G, Cordoba H, et al: Asthma and other allergic conditions in Colombia: a study in 6 cities. *Ann Allergy Asthma Immunol* 2004;93:568-574.
- 4 Cookson W: The alliance of genes and environment in asthma and allergy. *Nature* 1999;402(6760 suppl):B5-B11.

- 5 Barnes KC, Neely JD, Duffy DL, Freidhoff LR, Breazeale DR, Schou C, et al: Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. *Genomics* 1996;37:41–50.
- 6 A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). *Nat Genet* 1997;15:389–392.
- 7 Wjst M, Fischer G, Immervoll T, Jung M, Saar K, Rueschendorf F, et al: A genome-wide search for linkage to asthma. German Asthma Genetics Group. *Genomics* 1999;58:1–8.
- 8 Dizier MH, Besse-Schmittler C, Guilleud-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;162:1812–1818.
- 9 Gao PS, Kawada H, Kasamatsu T, Mao XQ, Roberts MH, Miyamoto Y, et al: Variants of NOS1, NOS2, and NOS3 genes in asthmatics. *Biochem Biophys Res Commun* 2000;267:761–763.
- 10 Konno S, Hizawa N, Yamaguchi E, Jinushi E, Nishimura M: (CCTTT)_n repeat polymorphism in the NOS2 gene promoter is associated with atopy. *J Allergy Clin Immunol* 2001;108:810–814.
- 11 Lee YC, Cheon KT, Lee HB, Kim W, Rhee YK, Kim DS: Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with asthma. *Allergy* 2000;55:959–963.
- 12 Holla LI, Buckova D, Kuhrova V, Stejskalova A, Francova H, Znojil V, et al: Prevalence of endothelial nitric oxide synthase gene polymorphisms in patients with atopic asthma. *Clin Exp Allergy* 2002;32:1193–1198.
- 13 Leung TF, Liu EK, Tang NL, Ko FW, Li CY, Lam CW, et al: Nitric oxide synthase polymorphisms and asthma phenotypes in Chinese children. *Clin Exp Allergy* 2005;35:1288–1294.
- 14 Belvisi MG, Ward JK, Mitchell JA, Barnes PJ: Nitric oxide as a neurotransmitter in human airways. *Arch Int Pharmacodyn Ther* 1995;329:97–110.
- 15 Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ: Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133–135.
- 16 Massaro AF, Mehta S, Lilly CM, Kobzik L, Reilly JJ, Drazen JM: Elevated nitric oxide concentrations in isolated lower airway gas of asthmatic subjects. *Am J Respir Crit Care Med* 1996;153:1510–1514.
- 17 Kharitonov SA, Yates DH, Barnes PJ: Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996;153:454–457.
- 18 De Sanctis GT, MacLean JA, Hamada K, Mehta S, Scott JA, Jiao A, et al: Contribution of nitric oxide synthases 1, 2, and 3 to airway hyperresponsiveness and inflammation in a murine model of asthma. *J Exp Med* 1999;189:1621–1630.
- 19 De Sanctis GT, Mehta S, Kobzik L, Yandava C, Jiao A, Huang PL, et al: Contribution of type I NOS to expired gas NO and bronchial responsiveness in mice. *Am J Physiol* 1997;273:L883–L888.
- 20 Bogdan C: Nitric oxide and the immune response. *Nat Immunol* 2001;2:907–916.
- 21 Taylor-Robinson AW: Inhibition of IL-2 production by nitric oxide: a novel self-regulatory mechanism for Th1 cell proliferation. *Immunol Cell Biol* 1997;75:167–175.
- 22 Bauer H, Jung T, Tsikas D, Stichtenoth DO, Frolich JC, Neumann C: Nitric oxide inhibits the secretion of T-helper 1- and T-helper 2-associated cytokines in activated human T cells. *Immunology* 1997;90:205–211.
- 23 Chang RH, Feng MH, Liu WH, Lai MZ: Nitric oxide increased interleukin-4 expression in T lymphocytes. *Immunology* 1997;90:364–369.
- 24 Huang FP, Niedbala W, Wei XQ, Xu D, Feng GJ, Robinson JH, et al: Nitric oxide regulates Th1 cell development through the inhibition of IL-12 synthesis by macrophages. *Eur J Immunol* 1998;28:4062–4070.
- 25 Morita R, Uchiyama T, Hori T: Nitric oxide inhibits IFN- α production of human plasmacytoid dendritic cells partly via a guanosine 3',5'-cyclic monophosphate-dependent pathway. *J Immunol* 2005;175:806–812.
- 26 Eriksson U, Egermann U, Bihl MP, Gambazzi F, Tamm M, Holt PG, et al: Human bronchial epithelium controls TH2 responses by TH1-induced, nitric oxide-mediated STAT5 dephosphorylation: implications for the pathogenesis of asthma. *J Immunol* 2005;175:2715–2720.
- 27 Grasmann H, Yandava CN, Storm van's Gravesande K, Deykin A, Pillari A, Ma J, et al: A neuronal NO synthase (NOS1) gene polymorphism is associated with asthma. *Biochem Biophys Res Commun* 2000;272:391–394.
- 28 Shao C, Suzuki Y, Kamada F, Kanno K, Tamari M, Hasegawa K, et al: Linkage and association of childhood asthma with the chromosome 12 genes. *J Hum Genet* 2004;49:115–122.
- 29 Immervoll T, Loesgen S, Dutsch G, Gohlke H, Herbon N, Klugbauer S, et al: Fine mapping and single nucleotide polymorphism association results of candidate genes for asthma and related phenotypes. *Hum Mutat* 2001;18:327–336.
- 30 Holla LI, Schuller M, Buckova D, Vacha J: Neuronal nitric oxide synthase gene polymorphism and IgE-mediated allergy in the Central European population. *Allergy* 2004;59:548–552.
- 31 Ali M, Khoo SK, Turner S, Stick S, Le Souef P, Franklin P: NOS1 polymorphism is associated with atopy but not exhaled nitric oxide levels in healthy children. *Pediatr Allergy Immunol* 2003;14:261–265.
- 32 Wechsler ME, Grasmann H, Deykin A, Silverman EK, Yandava CN, Israel E, et al: Exhaled nitric oxide in patients with asthma: association with NOS1 genotype. *Am J Respir Crit Care Med* 2000;162:2043–2047.
- 33 Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR: Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. *J Invest Allergol Clin Immunol* 1993;3:198–204.
- 34 Chew FT, Lim SH, Goh DY, Lee BW: Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999;54:1150–1159.
- 35 Ferrandiz R, Casas R, Dreborg S: Sensitization to *Dermatophagoides siboney*, *Blomia tropicalis*, and other domestic mites in asthmatic patients. *Allergy* 1996;51:501–505.
- 36 Caraballo L, Puerta L, Fernandez-Caldas E, Lockey RF, Martinez B: Sensitization to mite allergens and acute asthma in a tropical environment. *J Invest Allergol Clin Immunol* 1998;8:281–284.
- 37 Global Strategy for Asthma Management and Prevention: Global Initiative for Asthma. NIH Publication No 01-3659, NHLBI/WHO, 2002.
- 38 Caraballo L, Cadavid A, Mendoza J: Prevalence of asthma in a tropical city of Colombia. *Ann Allergy* 1992;68:525–529.
- 39 Caraballo LR, Marrugo J, Erlich H, Pastorizo M: HLA alleles in the population of Cartagena (Colombia). *Tissue Antigens* 1992;39:128–133.
- 40 Builes JJ, Martinez B, Gomez A, Caraballo L, Espinal C, Aguirre D, et al: Y chromosome STR haplotypes in the Caribbean city of Cartagena (Colombia). *Forensic Sci Int* 2007;167:62–69.
- 41 Puerta Llerena L, Fernandez-Caldas E, Caraballo Gracia LR, Lockey RF: Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp-allergic individuals. *J Allergy Clin Immunol* 1991;88:943–950.
- 42 Labrada M, Uyema K, Sewer M, Labrada A, Gonzalez M, Caraballo L, et al: Monoclonal antibodies against Blo t 13, a recombinant allergen from *Blomia tropicalis*. *Int Arch Allergy Immunol* 2002;129:212–218.
- 43 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- 44 Schneider S, Roessli D, Excoffier L: Arlequin ver 2.000: A Software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland, 2000.

- 45 Grasemann H, Drazen JM, Deykin A, Israel E, De Sanctis GT, Pillari A, et al: Simple tandem repeat polymorphisms in the neuronal nitric oxide synthase gene in different ethnic populations. *Hum Hered* 1999;49:139–141.
- 46 Ober C: Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol* 2005;116:274–278.
- 47 Fernandez-Caldas E, Puerta L, Caraballo L, Mercado D, Lockey RF: Sequential determinations of *Dermatophagoides* spp. allergens in a tropical city. *J Invest Allergol Clin Immunol* 1996;6:98–102.
- 48 Puerta L, Fernandez-Caldas E, Mercado D, Lockey RF, Caraballo LR: Sequential determinations of *Blomia tropicalis* allergens in mattress and floor dust samples in a tropical city. *J Allergy Clin Immunol* 1996;97:689–691.
- 49 Ioannidis JP, Ntzani EE, Trikalinos TA: ‘Racial’ differences in genetic effects for complex diseases. *Nat Genet* 2004;36:1312–1318.
- 50 Hall AV, Antoniou H, Wang Y, Cheung AH, Arbus AM, Olson SL, et al: Structural organization of the human neuronal nitric oxide synthase gene (NOS1). *J Biol Chem* 1994;269:33082–33090.
- 51 Wang Y, Newton DC, Marsden PA: Neuronal NOS: gene structure, mRNA diversity, and functional relevance. *Crit Rev Neurobiol* 1999;13:21–43.
- 52 Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J: Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol* 2005;175:342–349.
- 53 Togashi H, Sasaki M, Frohman E, Taira E, Ratan RR, Dawson TM, et al: Neuronal (type I) nitric oxide synthase regulates nuclear factor kappaB activity and immunologic (type II) nitric oxide synthase expression. *Proc Natl Acad Sci USA* 1997;94:2676–2680.