

BRIEF COMMUNICATION

Constitutive nitric oxide synthase gene polymorphisms and house dust mite respiratory allergy in an Algerian patient group

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Abstract

Genetic polymorphisms in neuronal nitric oxide synthase (NOS1) and calmodulin-dependent endothelial NOS (NOS3) genes are known to influence the course of allergic respiratory disorders. We investigated the role of NOS1 -84 G→A and NOS3 -786 T→C, 894 G→T and 27 base pair (bp) repeat polymorphisms in 125 patients suffering from asthma and/or rhinitis and monosensitized against *Dermatophagoides pteronyssinus* (*Dpter*) and 111 controls from Algeria. We found a higher frequency of the -786 C NOS3 allele in patients than in controls [corrected *P* value (*P_c*) = 0.04], especially in female cases (*P_c* = 0.02) and that the 'ab' genotype of the 27-bp polymorphism was significantly associated with specific immunoglobulin E production against *Dpter* (*P* = 0.006). This study brings further support for the participation of NOS3 gene polymorphism in the pathogenesis of respiratory allergic disorders.

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Allergic affections including asthma and rhinitis are common disorders, highly heterogeneous and at constant increase in their prevalence during the last decades (1, 2). The development of such affections is known to be the result of interactions between genetic background of the affected subject and environmental factors, which include common allergens such as house dust mite and pollens (3). A large number of studies have reported associations between various polymorphic genomic markers and atopy/asthma in different population groups (4–6). Among the studied loci, those encoding nitric oxide synthase (NOS) enzymes have attracted particular attention because of their catalytic role in NO synthesis, a molecule playing a prominent role in the regulation of airway function (7). The NOS family

consists of three main members divided into two functional groups: (i) the constitutively expressed Ca²⁺/calmodulin-dependent endothelial NOS (NOS3) and neuronal NOS (NOS1) allow a rapid but small increase in NO production and (ii) the inducible isoform (NOS2) promotes sustained and enhanced synthesis of NO by inflammatory cells induced by cytokines and other proinflammatory molecules (8). In the airway, NO, a key physiological regulator of functions such as bronchodilatation and neurotransmission can become deleterious under certain circumstances (9). The nocive effects of NO include plasma exsudation and intensification of pre-existing inflammation (10, 11) as evidenced by the high-exhaled NO levels in asthmatic patients (12). Interestingly, a correlation exists between

exhaled NO levels and the number of blood eosinophils (13). It is also believed that the NOS system may influence the T helper 2 (Th2)/Th1 disbalance known to characterize allergic diseases (14). Given the previous description of functionally relevant polymorphisms in *NOS1* and *NOS3* genes, several studies have focused on the potential impact of some of their genetic variations on susceptibility/resistance to allergic manifestations. In *NOS1* gene, these polymorphisms are mainly represented by a variable number of simple sequence repeats (CA repeat in intron 2 and exon 29, and AAT in intron 20), and a punctual C5266T substitution (15). The most studied variations of *NOS3* gene include a polymorphic 27 base pair (bp) tandem repeat in intron 4, a common Glu298Asp change in exon 7 (894 G→T) and a T to C change at position -786 in the promoter region (16). While the *NOS1* variants had consistently been associated with high incidence of asthma, elevated levels of immunoglobulin E (IgE) and exhaled NO in asthmatic patients (17, 18), the data are less clear concerning association of *NOS3* polymorphic markers with allergic disorders. An association between *NOS3* 27-bp tandem repeat polymorphism with bronchial asthma was reported (19–21), while the Glu298Asp variant in exon 7 was not found to influence the course of asthma and/or related atopic phenotypes (19) but was associated with the level of exhaled NO in asthmatic patients (22). Finally, a very recent study implicated the -786 polymorphic position in allergy pathogenesis but only in affected male patients (23).

In this study, we evaluated the potential impact of *NOS1* and *NOS3* polymorphisms on the level of IgE production and incidence of asthma and/or rhinitis in a patient group as compared with healthy controls from Algeria, a population group that had not been previously studied. DNA samples were obtained from 125 patients attending the pulmonary and allergy speciality departments of the Beni-Messous and Ben-Aknoun hospitals in Algiers, Algeria along with 111 ethnically matched healthy controls (all individuals were Arab or Berber of ethnic origins). The patients were diagnosed according to the American Thoracic Society criteria (24). At the time of inclusion, the patient group fell into three phenotypic groups: 76% of patients with both allergic rhinitis and asthma, 26.5% with allergic rhinitis alone and 3.2% with asthma alone. Informed consents were obtained for all patients and controls. Skin prick test (SPT) was performed using commercially available sets (SPT-Stallergènes®, Stallergènes, Antony, France) for house dust mites [*Dermatophagoides pteronyssinus* (*Dpter*) and *Dermatophagoides farinae*], common grass, five pollen mixtures, moulds (*Alternaria alternata*), cat, dog, olive tree (*Olivier*) and cockroach (*Blatella germanica*). The total serum IgE level was measured by a home-designed (Pasteur Institute of Algeria, Algiers, Algeria) enzyme-linked immunosorbent assays and specific IgE against *Dpter* was detected by Immulite® (DPC, Gwynedd, UK). Diagnosis of atopy was

based on combined criteria, which include clinical signs of atopic status, positive SPT and raised total and specific serum IgE levels greater than 150 IU/ml and 35 kU/l, respectively. Genotyping was performed by a TaqMan® 5'-nuclease assay (Applied Biosystems, Foster City, CA) with allele-specific fluorogenic oligonucleotide probes for *NOS1* -84 G→A and for *NOS3* -786 T→C variations as described previously (25, 26). The *NOS3* 894G→T was analysed by a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP), whereas alleles of *NOS3* 27-bp repeat polymorphism (a, b and c alleles) were discriminated by agarose gel electrophoresis of amplified products (27, 28). Alleles and genotype frequencies were determined and compared using a standard chi-squared test. *P* values were corrected (*P_c*) and considered statistically significant at values less than 0.05. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Student's *t*-test (SPSS 8.0 software package) was used to compare IgE means accounting patient's genotypes. *P* < 0.05 were considered significant.

Phenotype characteristics of patient and control groups are summarized in Table 1. All patients were found to be monosensitized reacting only to *Dpter*, whereas controls provided negative reactions. Serum total IgE levels were significantly higher in patients than in controls [median IgE level: 350 IU/ml in patients vs 46.5 IU/ml in controls, *P* < 0.00001 (quartile range: 195–654 IU/ml vs 35.5–58.5 IU/ml and mean: 420.32 ± 273.23 vs 51.20 ± 22.39)]. Similarly, serum-specific IgE levels produced against *Dpter* were also significantly higher in patients than in control individuals [median IgE level: 80 kU/l vs 0.35 kU/l, *P* < 0.00001 (quartile range: 16–100 kU/l vs 0.35–0.35 kU/l and mean: 59.70 ± 40.37 vs 0.44 ± 0.44, respectively)]. Both total and specific IgE levels correlated with clinical respiratory manifestations and skin test results (*P* < 0.00001 and *P* < 0.0001, respectively).

In terms of genotyping, we found that *NOS3* -786 C allele was more frequent in the patient group than in healthy controls (48.4% vs 37.8%; *P_c* = 0.04, OR = 1.54, 95%

Table 1 Phenotype characteristics of patients with respiratory allergy and controls

	Control group (<i>n</i> = 111)	Allergic patients (<i>n</i> = 125)
Age, years (mean ± SD)	25.46 ± 10.48	21.92 ± 11.27
Male, <i>n</i> (%)	33 (29.7)	53 (42.4)
Female, <i>n</i> (%)	78 (70.2)	72 (57.6)
Sex ratio	2.36	1.35
Allergy symptoms (%)	0	100
Skin prick test (%)	0	100
Total IgE, IU/ml (mean ± SD)	51.20 ± 22.39	420.32 ± 273.23
Specific IgE <i>Dpter</i> (mean ± SD)	0.44 ± 0.44	59.70 ± 40.37

Dpter, *Dermatophagoides pteronyssinus*; IgE, immunoglobulin E.

CI = 1.05–2.46) (Table 2). Interestingly, stratification of patients and controls based on gender showed that frequency of –786 C allele is significantly higher in female patients than in female controls (52.08% vs 37.82%; $P_c = 0.02$, OR = 1.79, 95% CI = 1.10–2.91), whereas it was at comparable frequency in male subgroups (Table 3). Otherwise, we noted also a trend towards a higher prevalence of this allele in female patients as compared with male patients (52.08% vs 43.39%) but statistically non-significant likely because of the small number of male as compared with female patients (53 and 72 patients, respectively). The overall frequency of the 27-bp repeat and 894 G→T polymorphisms of NOS3 gene and that of NOS1 locus did not show any difference between patients and controls. However, analysis of the distribution of the 27-bp repeat genotypes among patients showed that in those bearing the compound heterozygous 'ab' ($n = 34$), *Dpter*-specific IgE levels were higher than those bearing the other genotypes, i.e. bb, aa, ac and bc ($n = 84, 4, 2$ and 1 , respectively) (75.9 ± 6.3 kU/l vs 53.6 ± 4.4 kU/l, $P = 0.006$). In this context, both NOS3 –786 T→C, 894 G→T and NOS1 –84 G→A polymorphisms were not found to be associated with any of the disease characteristics (serum total IgE, *Dpter*-specific IgE and other atopy-related clinical phenotypes).

Endogenous NO production is a tightly controlled process involving distinct NO synthases, either constitutive (eNOS, NOS1 and NOS3) or inducible (NOS2), the expression of which had been incriminated in susceptibil-

ity/resistance to allergic diseases (29). In terms of NOS3 gene diversity, the herein NOS3 –786 C allele association with atopic phenotype (overall respiratory allergy) is consistent with the recent finding in a Czech asthmatic male patients (23). Of interest is that the allele C had previously been associated with deficient (50% reduction) NOS3 transcription (30) and with similar reduction in NOS3 expression (31) as well as with diminished serum nitrite/nitrate levels (32). Interestingly, this polymorphism had also been identified as a gender-specific genetic modulator of acute chest syndrome in sickle cell disease (26). In our study, a similar contribution to the pathogenic effect was noted, likely reflecting a hormonal effect, in particular oestrogen (33). In this context, it is interesting to remind that this nucleotide substitution is in close proximity with a predicted oestrogen-responsive element and a DNA-binding protein, replication protein A1 that had been shown to reduce the –786 C promoter activity (34). Concerning NOS3 27-bp repeat polymorphism, it has been shown that the level of plasmatic NO in homozygous subject for the rare 'a' allele was nearly 20% lower than that of subjects carrying the common 'b' allele (29). However, the potential implication of this variation in the genetic control of NO production provided inconsistent data in association studies involving different ethnic groups. Among our respiratory allergic patients, the reported association between the compound heterozygote 'ab' genotype and high specific serum IgE levels possibly reflected a linkage disequilibrium of a yet to be defined susceptibility locus. This situation could also evoke a potential dominant effect of the 'a' allele, but such effect could not be proven in our work because of the low number of homozygous cases of this variant. In addition and consistent with previous findings, the common NOS3 894G→T variation in exon 7 was not associated with disease phenotype in our study. Finally, the –84 G/A NOS1 gene polymorphism, thought to decrease promoter activity by about 30% (25) but the relevance of which in allergic disorders is unknown, was not found to influence clinical phenotypes in our work.

Altogether, our data from a population group from North Africa, albeit requiring confirmation by a much larger study, suggest a genetic contribution of endogenous NO in allergic diseases. This is further highlighted by studies

Table 2 NOS3 –786 T→C allele and genotype frequencies among patients and controls

	Patients ($n = 125$)	Healthy controls ($n = 111$)	χ^2	P	P_c
Alleles, n (%)					
–786 T	129 (51.6)	138 (62.1)	—	—	—
–786 C	121 (48.4)	84 (37.8)	5.33	0.02	0.04
Genotypes, n (%)					
–786 T/T	36 (28.8)	44 (39.6)	—	—	—
–786 T/C	57 (45.6)	50 (45)	—	—	—
–786 C/C	32 (25.6)	17 (15.3)	3.78	0.05	NS

NOS3, endothelial NOS; NS, not significant.

Table 3 Endothelial NOS –786 allele frequencies after gender stratification

Alleles	Females		χ^2	P	P_c	Males		χ^2	P	P_c
	Patients ($n = 72$)	Controls ($n = 78$)				Patients ($n = 53$)	Controls ($n = 33$)			
–786 T, n (%)	69 (47.91)	97 (62.17)	—	—	—	60 (56.60)	41 (62.12)	—	—	—
–786 C, n (%)	75 (52.08)	59 (37.82)	6.1	0.01	0.02	46 (43.39)	25 (37.87)	—	NS	—

NOS, nitric oxide synthase; NS, not significant.

in NOS3 transgenic mice, which show that overexpression of NOS3 could prevent/attenuate airway hyperresponsiveness and inflammation (35). Of interest also is the recently proposed hypothesis involving a crosstalk between constitutive and inducible forms of NOS; a decrease in NOS3-related NO production below a certain threshold may enhance the expression of NOS2 gene (36, 37), thus contributing to sustained synthesis of NO as in asthmatic patients.

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