

Letter to the Editor

The interaction of *IL1A* and endothelial nitric oxide synthase polymorphisms is associated with the degree of atopy

To the Editor:

There is increasing evidence that nitric oxide (NO) may play an important role in allergic inflammation. Endothelial NO synthase (eNOS) is one of the enzymes catalyzing NO synthesis. It is induced in a variety of cell types by proinflammatory cytokines, such as IL-1, TNF- α , IFN- γ , and by microbial products such as LPSs.¹ Previous studies on the role of the *eNOS* gene in the pathogenesis of atopy have produced conflicting results.^{2,3}

IL-1 α is a proinflammatory cytokine that mediates the inflammatory reaction in the skin.⁴ IL1 α has also been found to accelerate cutaneous inflammation.⁵ We have earlier shown that *IL1A* (G/T base change at +4845) polymorphism is associated with atopy; the risk for atopy was higher in subjects having genotype GG of *IL1A* gene.⁶ We have expanded the study by analyzing *eNOS* (T/G base change at +894) polymorphism from the same cohort and investigated whether these genes show an epistatic effect on the degree of atopy.

Altogether 254 female and 151 male (mean age, 60 years; age range, 31-89 years) participated in the study. The subjects were controls without asthma of a Finnish population-based case-control study intended to investigate the risk factors of adult asthma. Detailed information about the whole material has been presented earlier in the Journal.⁶ *IL1A* (+4845G>T, rs17561) polymorphism was detected as previously described.⁶ The *eNOS* (+894T>G, rs1799983) polymorphism was analyzed by allele-specific hybridization method (TaqMan; Applied Biosystems, Foster City, Calif). The nucleotide sequences of the primers and probes used in the PCR were deduced from published sequences deposited in the GenBank database and chosen and synthesized in conjunction with Applied Biosystems (Foster City, Calif). The genotype distributions followed Hardy-Weinberg equilibrium. Sensitization to allergens was determined by skin prick test (SPT) performed as previously described.⁶ The degree of atopy was defined as the number of positive SPTs. Thus, the SPT-positive patients were divided into those with 1 or 2 positive SPTs (SPT+[1]) and 3 or more positive SPTs (SPT+[2]).

As seen in Table I, *IL1A* genotype GG increased the risk for sensitization to allergen; the number of patients in SPT+(2) group was especially increased (odds ratio [OR], 2.09; 95% CI, 1.23-3.57; $P = .006$, χ^2 test; $df = 1$). *eNOS* polymorphism did not have an independent effect on sensitization to allergen but interacted with *IL1A* gene. The interaction of these genes was calculated by using multinomial logistic regression analysis. The model showed that there is a slight interaction between these genes on the degree of sensitization ($P = .08$; Table II). There was a significant interaction between the SPT-negative and SPT+(2) groups ($P = .026$), but not between the SPT-negative and SPT+(1) groups ($P = .785$, bilogistic regression analysis; Table II). If the age and sex were included in the model, the results did not change (data not shown).

Earlier studies have shown that exhaled NO levels in adults correlate with atopy (with a number of positive SPTs and total IgE) rather than bronchial hyperreactivity and lung function.^{7,8} Here the *eNOS* gene did not have an independent role in the pathogenesis of atopy. Its effect was dependent on *IL1A* gene. In addition, *eNOS* polymorphism itself or together with *IL1A* polymorphism did not have an effect on asthma susceptibility (Pessi et al, Unpublished data, November 2005). It seems that the effect of *eNOS* gene on atopy is dependent on another proinflammatory gene, like *IL1A*. This interaction between *eNOS* and *IL1A* genes may also explain some earlier conflicting results^{2,3} on the effect of *eNOS* polymorphisms on atopy.

We cannot exclude the possibility that the effect of the *eNOS* gene is on the diagnostic phase—that is, the high producer allele T of *eNOS* gene⁹ makes the SPT more sensitive by inducing stronger endothelial enlargement on the site of allergen application. However, we did not observe any effect of the *eNOS* genotype on the response induced by the histamine control (data not shown), thus speaking against this alternative.

In our study, we show for the first time that *IL1A* and *eNOS* genes have an interactive effect on the degree of atopy (ie, the epistatic effect of 2 genes). Both genes are involved in the initiation of the immune response and probably affect the T_H1/T_H2 balance.¹ At the inductive phase of T_H1 cells, NO has shown an enhancing effect

TABLE I. Independent effect of *IL1A* (single nucleotide polymorphism +4845) and *eNOS* (single nucleotide polymorphism +894) on sensitization on allergen: the degree of atopy*

	SPT–		SPT+(1)		SPT+(2)		SPT– vs SPT+(1)	SPT– vs SPT+(2)
	N	%	N	%	N	%	P	P
<i>IL1A</i> GG	99	40.1	43	55.1	42	58.3	.02	.006
<i>IL1A</i> GT/ TT	148	59.9	35	44.9	30	41.7		
<i>eNOS</i> TT/ TG	112	45.3	41	52.6	31	43.1	.226	.73
<i>eNOS</i> GG	135	54.7	37	47.4	41	56.9		

*P value calculated in 2 $\times$ 2 table by χ^2 test.

TABLE II. Epistatic effect of *IL1A* (single nucleotide polymorphism +4845) and *eNOS* (single nucleotide polymorphism +894) on sensitization on allergen: the degree of atopy

							Interaction analyses		
	SPT–		SPT+(1)		SPT+(2)		All groups	SPT– vs SPT+(1)	SPT– vs SPT+(2)
	N	%	N	%	N	%	P*	P†	P†
<i>eNOS</i> TT/TG& <i>IL1A</i> GG	44	39.3	23	56.1	23	74.2	.08	.785	.026
<i>eNOS</i> TT/TG& <i>IL1A</i> GT/TT	68	60.7	18	43.9	8	25.8			
<i>eNOS</i> GG& <i>IL1A</i> GG	55	40.7	20	54.1	19	46.3			
<i>eNOS</i> GG& <i>IL1A</i> GT/TT	80	59.3	17	45.9	22	53.7			

*Multinomial logistic regression analysis.

†Bilogistic regression analysis.

on the induction and differentiation of T_H1 cells, whereas it has little or no effect on fully committed T cells. On the other hand, NO at noncytotoxic concentrations has been demonstrated to downregulate the T_H1-type cytokines and/or increase the T_H2-associated molecules.¹ The high NO synthesis is linked to atopy⁷ and to the genotypes of TT and GT of *eNOS* gene.⁹ Previously, we showed that subjects having genotype GG of *IL1A* had a significantly increased risk for SPT positivity.⁶ Probably the allele G is associated with low capacity to produce IL-1 α , a weak inflammatory response induced is known to favor the differentiation of the T_H2 cells.¹⁰ Thus, it can be concluded that if a subject has 2 atopy risk genes, such as a high NO-producing allele of *eNOS* gene together with *IL1A* atopy risk gene, T_H2 responses like sensitization to allergens are even further increased.

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