

relationship to specific causes, and with reactions to insect stings and their characteristics.

A population of industrial workers in a factory in Milan was investigated. All subjects had to answer to the questionnaire and those responding with positive answers were requested to undergo allergy skin tests consistent with the symptoms identified by the questionnaire.

Skin tests were performed by a standard panel of aeroallergens and food extracts, and by purified *Hymenoptera* venom. In total 304 subjects (203 males and 101 females, age 22–61 years, mean 40.2 years) were investigated. Of these, 207 answered negatively to all questions while 98 (32.2%) answered positively to one or more questions. Specifically, 87 (28.6%) had symptoms of respiratory allergy, five (1.6%) of food allergy, four (1.3%) of insect allergy, and two (0.6%) of drug allergy. The mean age was 39.6 years in symptomatic and 40.5 years in asymptomatic subjects.

Fifty-two subjects agreed to undergo the tests, while 46 refused. Table 1 reports the results according to different allergies.

In the present study the prevalence of hypersensitivity symptoms as assessed by the questionnaire was 32.2%, but according to the results of skin tests it was 21.7%, a figure that agrees with recent reports for western developed countries. Most symptoms were respiratory and they correlated very well with skin tests when seasonal, less when perennial. Non IgE-mediated disorders, such as idiopathic rhinitis, NARES, nasal polyposis, and nonallergic asthma, may account for this discrepancy. In accordance with known epidemiological data (3,4), the prevalence of

hypersensitivity to foods and insect stings was low. However, regarding foods, the possible correlation assessed by the questionnaire was only for skin symptoms, which overlooked anaphylaxis, causing an underestimation of reactions to foods, and to drugs. Moreover, according to results of the skin tests, food allergy was virtually absent, but the well-known low diagnostic accuracy of food extracts must be considered partly responsible. It is notable that the questionnaire completely prevented the reported phenomenon of exaggerated self-perception of food allergy (5), by excluding all symptoms unrelated to IgE-mediated reactions.

Our results suggest that a simple questionnaire consisting of a few questions can detect subjects with any kind of allergy in the general population. Its value may be improved by the addition of more questions dealing with anaphylaxis.

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The ecNOS gene in allergic Czech children

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Key words: IgE-mediated allergy; nitric oxide synthase; NOS; polymorphism; steroid.

● ALLERGIC DISEASES are considered to be multifactorial, involving complex interactions between genetic and environmental influences. There is increasing evidence that nitric oxide (NO) plays a key role in regulation of airway function and in the pathophysiology of inflammation in IgE-mediated allergic diseases (1). NO is produced in increased amounts in asthma and allergic rhinitis. NO in exhaled air correlates with asthma severity, sputum eosinophils, and with peak flow variability, probably related to airway inflammation (2). That is why the gene for endothelial NO synthase (ecNOS; endothelial constitutive nitric oxide synthase), one of three NO synthases, seems to be a candidate gene. The gene encoding ecNOS is located on chromosome 7p35–36 and a number of polymorphisms have been identified in this gene. A 27-bp repeat in intron 4 has been associated with atopic asthma in Koreans (3).

Our study was carried out (1) to determine whether the allele and

Table 1. Positive skin tests according to the kind of symptoms

Kind of symptoms	Positive skin tests	(%)
Seasonal respiratory symptoms	29/31	(94%)
Perennial respiratory symptoms	5/17	(29%)
Reactions to foods	0/3	
Reactions to insect stings	1/2	(50%)
Overall symptoms	36/53*	(68%)

*. One subject was tested with both foods and insect venoms.

genotype distributions in childhood patients with IgE-mediated allergic diseases in our Czech population differ from an earlier study; and (2) to see if this polymorphism is associated with the severity or intermediary phenotype markers of allergic diseases.

There is an association with need for topical steroids.

This was a cross-sectional study of 66 pediatric patients with IgE-mediated asthma and rhinitis and their combinations (45 boys, 21 girls; 14 ± 6 years). Children were investigated and treated at the Faculty Children Hospital Brno, according to standard protocols (4,5), using adequate controls. IgE-mediated allergy was recognized by specific IgE or positive skin prick test to any one or more allergens as described previously (6).

Project approval was granted by the local ethics committee at Medical Faculty MU Brno and written informed consent for genetic analysis was obtained from parents of all subjects.

We used peripheral blood leukocytes for DNA extraction. Then the 27-bp repeat polymorphism in intron 4 of the ecNOS gene was detected using our modified PCR method according to Lee et al. (3). An individual's genotype was evaluated as homozygote (bb), 602 bp (five repeats of the 27-bp); heterozygote (ab), 602 and 575 bp; or homozygote (aa), 575 bp (four repeats of the 27-bp).

We detected 6.1% ($n=4$) subjects homozygous for the deletion (ecNOS 4a/a), 31.8% ($n=21$) heterozygotes (ecNOS 4a/b) and 62.1% ($n=41$) homozygotes for the common insertion allele (ecNOS 4b/b). There was a statistically significant difference in the frequencies of individual alleles ($P=0.0018$) and the genotypes ($P=0.0108$) of this polymorphism between patients whose clinical condition required compensation using topical corticoids (inhaled or nasal) (allele: $a/b=0.316/0.684$; genotype: $aa=4$ (10.5%), $ab=16$ (42.1%), $bb=18$ (47.4%)) and children whose compensation was achieved without corticoid therapy (allele: $a/b=0.089/0.911$, genotype: $aa=0$ (0%), $ab=5$ (17.9%) and $bb=23$ (82.1%)). We failed

to find any association of genotype with intermediary phenotype.

Genetic, as well as environmental, factors may contribute not only to the manifestation of the disease, but also to its severity. The polymorphism studied here is responsible for part of the genetic variability in the control of the plasma NO levels (lower by about 20% in the aa homozygotes as compared to bb homozygotes) (7).

Our study ascertained the bb genotype in 62% of allergic patients, a much lower occurrence than in the Korean extrinsic asthmatics (86%), and even less than the Korean controls (75%) (3). More detailed analysis of our allergic children revealed statistically significant differences in the frequencies of alleles and the genotype combinations between the group of patients requiring inhaled or nasal steroid treatment (the frequency of the rarer a allele exceeding 30%, the frequency of the bb genotype being only 47%) and the group not requiring this therapy (allele a only 9%, genotype bb 82%). Thus we can speculate that although the manifestation of IgE-mediated allergic disease can be associated with the bb genotype (3), the a variant (or genotype aa) in the manifest allergic disease relates to a persisting airway inflammation where the clinical condition must be compensated by applying inhaled corticosteroids, i.e. a higher degree of therapy.

The discrepancy between the 'risk' allele associated by the Korean authors and our study is interesting; it could be related to genetic heterogeneity of allergic asthma and rhinitis which could, in addition, be population dependent. Reproduction of our findings in other populations could suggest whether association between the risk factors (genes in this case) and manifestation of IgE-mediated allergic disease (or its severity) can be generalized.

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