

Adenosine deaminase polymorphism and the relationship of total immunoglobulin E with skin prick test: A study on school children

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ABSTRACT

Recent studies suggest that genetic polymorphism modulates immunoglobulin E (IgE)-mediated atopic reactions. Adenosine is an important local hormone influencing immune function and tissue reactivity; because adenosine concentration is regulated by adenosine deaminase (ADA), we have investigated the possible effect of ADA polymorphism on the relationship between IgE and positive prick test. A random sample of 160 schoolchildren from the population of Viterbo (Italy) were studied. Prick testing was performed with a panel of local allergens. Total IgE assay was performed according to standard clinical procedure. ADA phenotype was determined according to Spencer et al. A highly significant correlation between prick test and IgE was observed. However, the strength of correlation was moderate ($\eta^2 = 0.16$), indicating that positive prick testing depends on other variables besides IgE. The relationship between IgE and positive prick testing is stronger in carriers of ADA*2 allele than in ADA*1/*1 subjects. Also, sensitivity and predictive values are higher in ADA*2 carriers than in homozygous ADA*1/*1 children. The data suggest that the effect of IgE level on local reactivity is influenced by ADA polymorphism; at low level of IgE, the presence of the ADA*2 allele seems to protect from positive prick testing. (Allergy Asthma Proc 27:115–118, 2006)

Skin-prick testing and/or serum level of specific immunoglobulin E (IgE) currently are considered the gold standard for sensitivity to air allergens.¹ However, although statistically significant, the strength of the relationship between the two tests is moderate, suggesting an important role of other factors, probably also genetic, in the relationship between IgE and cutaneous reactivity.² A recent study, in fact, has shown that IL1A genetic polymorphism is involved in the regulation of IgE-mediated atopic reactions.³ Moreover, IL1A receptor α -chain and ACP1 genetic polymorphisms are associated with serum IgE levels.^{4,5}

Adenosine, a purine nucleoside present in plasma and other extracellular fluids, is an important local hormone (such as prostaglandins) regulating blood flow, neurotransmission, physiology of smooth muscles, and platelet aggregation. Current interest is focused on a wide variety of effects produced by adenosine *via* activation of cell surface adenosine receptors (ARs).^{6,7} Through its effects on the AR, the concentration of this substance may influence immune function and tissue reactivity. Local concentration of adenosine depends on the activity of adenosine deaminase

(ADA), a polymorphic enzyme showing different enzymic activity among genotypes.^{8,9} An association between ADA polymorphism and bronchial asthma in children has been described.¹⁰ Such association and the regulatory role of adenosine concentration makes this enzyme a candidate gene for IgE regulation (see also Discussion). In this study we have investigated the effect of ADA genetic variability on the relationship between total IgE level and prick test positivity.

SUBJECTS AND METHODS

As a part of a Project on Preventive Pediatrics, we have studied a random sample of 160 schoolchildren from the white population of Viterbo, Italy. The survey was approved by the Sanitary Authority and informed consent was obtained by the parents to include their children in the study. The population living in this area of central Italy is homogeneous and relatively free from migratory phenomena.

Skin-prick testing was performed with a panel of the following local allergens (produced by Lofarma Allergeni S.P.A., Milan, Italy): *Dermatophagoides pteronyssinus* and *farinae*, *Alternaria tenuis*, *Aspergillus fumigatus* (mold), *Poa pratensis* (grass), *Artemisia vulgaris* (composita), *Parietaria officinalis* (Urticaceae), *Olea europea* (olive), milk albumin, egg white, and cat dander.

Histamine chloride, 1 mg/mL, and control solution were tested also at the distal end of the forearm. Allergens were applied to the volar side of both forearms and tested in the aforementioned order with a distance of ~2 cm between each application. Ten to 15 minutes

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Table 1 Demographic Characteristics of the Sample Study

	Mean	Proportion (%)	Total n°
Age (yr)	12.9 (SE = 0.02)		
Boys		51.2	160
ADA*2 carriers		18.1	125
Positive skin-prick		24.7	160
Asthmatic children		13.5	160

after skin prick, the mean allergen wheal diameter (minus the diameter of the control solution wheal, when this differed from zero) was recorded. Each allergen test also was evaluated, comparing its wheal with the size of the histamine wheal (1 mg/mL): allergen wheal one-half the size of the positive reference or bigger were considered positive. Subjects in whom at least one of the tests of allergens gave a significant wheal were considered skin-prick positive.

Total IgE assay was performed according to standard clinical procedure. History of asthmatic episodes was reported by children and confirmed by the pediatrician in charge of the school. ADA phenotype was determined in 125 children according to Spencer *et al.*⁸

Three-way contingency table analysis was performed according to Sokal and Rohlf.¹¹ Other statistical analyses were performed by the SPSS program (SPSS, Inc., Chicago, IL).¹² The η^2 is a measure of the strength of association.

RESULTS

Table 1 shows some demographic characteristics of the subject sample. The proportion of ADA*2 carriers in Viterbo, Italy, is similar to that of other Italian populations (mean value, 16.7%).¹⁰ Given the low number of expected homozygous ADA*2/*2 genotype, Hardy-Weinberg equilibrium can not be adequately tested.

Table 2 shows the relationship between skin-prick test and IgE levels. A significant correlation is observed between the two variables. However, the strength of correlation evaluated by η^2 is moderate (0.16), indicating that positive skin-prick test depends on other vari-

ables besides IgE. The receiver operator characteristic curve has shown that IgE discriminates skin-prick test classes, the optimal cutoff being 100 IU/mL (data not shown).

Table 3 shows the effect of ADA phenotype on the relationship between skin-prick test and IgE level. There is a significant interaction among ADA and IgE concerning their effect on the skin-prick test; the association between IgE and skin-prick test is much stronger in carriers of ADA*2 alleles than in ADA*1/*1 children (0.32 versus 0.12 for η^2). At a low level of IgE, ADA*2 allele seems to protect from a positive skin-prick test. Sensitivity and predictive value are also higher in ADA*2 carriers than in homozygous ADA*1/*1 children.

We also have studied the association between IgE and asthma and the effect of ADA on such association. The association of asthma with IgE is marginally significant and the strength of the relationship is very low ($\eta^2 = 0.06$; data not shown).

DISCUSSION

The data suggest that ADA polymorphism may influence the effect of IgE level on local reactivity: at a low level of IgE, the presence of ADA*2 allele corresponding to a low enzymic activity and to higher local concentrations of adenosine seems to protect from positive skin-prick test as compared with ADA*1/*1 genotypes. Our data have been obtained through a study in an ethnically homogeneous area of central Italy. However, the sample study is relatively small. Thus, our observations need to be confirmed in other populations before drawing definite conclusions.

ADA is a polymorphic enzyme controlled by a locus with two codominant alleles ADA*1 and ADA*2. The corresponding three common ADA genotypes have different enzymic activities: ADA*1/*1 is 15% more active than ADA*1/*2 and 30% more active than ADA*2/*2.⁹

ADA is an enzyme of the purine metabolism, which catalyzes the irreversible deamination of adenosine to inosine. It is ubiquitous in human tissues and its classic function is considered to be the regulation of intra- and extracellular levels of adenosine. Through the regula-

Table 2 The Relationship between Skin-Prick Test and IgE Level

IgE level (IU/mL)	≤ 20	$>20, \leq 40$	$>40, \leq 100$	$>100, \leq 230$	>230
Proportion of positive skin-prick test	17.4%	6.4%	21.9%	31.2%	40.6%
Total n°	33	31	32	32	32
Test of association of skin-prick test to IgE (p)					
Independence				<0.001	
Linear association				<0.001	
η				0.40	
η^2				0.16	

Table 3 The Effect of ADA Phenotype on the Relationship between Skin-Prick Tests and IgE Level

ADA1-1	IgE level	≤100	>100
	Proportion of positive skin-prick	18.0%	41.5%
	Total number	61	41
Carriers of ADA*2	Proportion of positive skin-prick	0.0%	50.0%
	Total number	11	12
Tests for interaction skin-prick-IgE-ADA (three-way contingency table analysis by a log linear model) x = prick test; y = IgE; z = ADA			
xyz Interaction		$p < 0.05$	
Tests of association of skin prick to IgE			
Significance of association (χ^2 test of independence)	ADA1-1 subjects P < 0.01	Carriers of ADA*2 allele P < 0.01	
Strength of association (η^2)	0.12	0.32	
Sensitivity and Specificity			
	Sensitivity	Specificity	Positive Predictive Value
ADA1-1 children	61%	68%	41%
Carriers of ADA*2 allele	100%	65%	50%
Cutoff point for IgE		100 IU/mL	Negative Predictive Value 82% 100%

tion of extracellular levels of adenosine, ADA could modulate the action of its important autacoid mediator on the AR.

ADA was supposed to be an exclusively cytosolic enzyme until the recent demonstration that is present on the surface of erythrocytes and platelets.¹³ Subsequently, it has been shown that ADA is present on the surface of many cell types including lymphocytes, where it can act as an ectoenzyme.¹⁴ The functional role of ecto-ADA is related not only to degrade extracellular adenosine¹⁴ but also to transduce signals through its interaction with CD26 or A1R, two ADA anchoring proteins. In this way it acts as a costimulatory molecule that facilitates a variety of specific signaling events in different cell types.¹⁵ The most interesting aspect is the possible participation of the ectoenzyme in cell-to-cell contacts during ontogenesis and maturation of the immune system.¹⁶ Quantitatively, the strength of the signal would depend on the concentration of ecto-ADA available. ADA biochemical polymorphism could contribute to modulate ecto-ADA function and, consequently, the development and functionality of lymphoid tissue.

The values of the strength of correlation between the skin-prick test and IgE found in this study are within the range reported in a recent study.² Such low levels are remarkable and indicate that IgE variance accounts for <20% of skin-prick variance. Our observation suggests a role of ADA probably through the regulation of adenosine level. However, the genetic variability of this enzyme accounts for only a small portion of skin-prick variance. Thus, it is likely that other unknown

factors besides IgE level contribute to the positivity of this important clinical test.

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