

ORIGINAL ARTICLE

## Atopic and Nonatopic Asthma in Children

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In 155 asthmatic children we have studied the relationship between prick test positivity and a set of genetic factors previously found to be associated with bronchial asthma. Among these factors, MN system ( $p = 0.009$ ) and age at onset of symptoms ( $p = 0.05$ ) are the most important variables separating prick test negative from prick test positive children. MN and age at onset influence independently prick test positivity pointing to an additive effect of the two variables. M phenotype appears correlated positively with an increased susceptibility to nonallergic asthma in all age groups, whereas N phenotype appears correlated positively with age at onset but in allergic asthma only. The MN system codifies for glycoprotein A, a sialoglycoprotein that represents a major ligand for several bacteria and viruses that recognize the N-acetylneuraminic acid present in this protein. The present data suggest that genetic variability in this system might influence bacterial and viral competition and mucosal damage influencing susceptibility to asthmatic reactions in absence of IgE hyperproduction.

**Keywords** atopy, asthma, genetics, MN system, onset age

### INTRODUCTION

The recent observations that early contact with stables exerts a protective effect not only on allergic but also on nonallergic asthma cannot be easily explained within the framework of Th1/Th2 paradigm and calls for alternative or concurrent mechanisms to understand the origin of asthmatic disorders (1–3). Besides environmental and lifestyle, genetic factors influencing immune response or adhesion of infectious agents to cell membrane of the respiratory tract could have important and different roles in the pathogenesis of this heterogeneous group of disorders. The current surge of interest in these problems prompted us to analyze in asthmatic children the relationship between prick test positivity and a set of genetic factors previously found to be associated with bronchial asthma.

### METHODS

The sample study is from the population of Rome and has been previously described (4). It is composed of 155 children, 97 males and 58 females aged from 1 month to 12 years. The criterion for inclusion in the study was a history of two or more episodes of wheezing in the last 6 months, irrespective of the etiology/pathogenesis of the attack, 78 children were less than (or equal to) 24 months old (55 males and 23 females), and 77 were more than 24 months old (48 males and 29 females).

Atopic status was assessed with a panel of the following local allergens (produced by Lofarma Allergeni S.P.A.,

Milan): *Dermatophagoides pteronissinus* and *farinae*, *Alternaria tenuis*, *Aspergillus fumigatus* (mold), *Poa pratensis* (grass), *Artemisia vulgaris* (composita), *Parietaria officinalis* (urticacea), *Olea Europea* (olive), milk albumin, egg white, and cat dander.

Histamine chloride (1 mg/mL) and control solution were also tested at the distal end of the forearm. Allergens were applied to the volar side of both forearms and tested in the order above with a distance of about 2 cm between each application. Ten to fifteen minutes 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 was also evaluated by comparing its wheal with the size of the histamine wheals (1 mg/mL): allergen wheals half the size of the positive reference or bigger were considered positive. This would allow for differences due to age (5). Subjects in whom at least one of the test of allergens gave a significant wheal were considered prick positive. The study was approved by the institutional review board. Parents gave consent to include their children in the study.

Methods for phenotype determination of the genetic systems examined have been previously reported [MN (4), ADA (6), ACP1 (7), ABO and Secretor (8)].

Logistic regression analysis and chi-square test of independence have been carried out according to SPSS programs (9). Three-way contingency table analysis by a log linear model has been carried out according to standard methods (10).

### THE GENETIC SYSTEMS STUDIED

#### *Adenosine Deaminase (ADA)*

ADA is a polymorphic enzyme controlled by a locus with two codominant alleles, ADA\*1 and ADA\*2. The

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TABLE 1.—Logistic regression analysis.

|                             |              |        |            |
|-----------------------------|--------------|--------|------------|
| Initial chi-square (step 1) | 18.882       | df = 7 | p = 0.0086 |
| Final chi-square            | 13.386       | df = 2 | p = 0.0012 |
| Variable in the equation    | Significance |        |            |
| MN                          | 0.0095       |        |            |
| Age at onset                | 0.0544       |        |            |
| Residual chi-square         | 4.999        | df = 5 | p = 0.4160 |

Dependent prick test positivity. Independent: age at onset of symptoms, ABO blood group system, Secretor, MN blood group system, ADA (adenosine deaminase), ACP1 (acid phosphatase locus 1) and sex. Method: backward stepwise procedure.

corresponding three common ADA genotypes have different enzymatic activity: ADA\*1/\*1 is 15% more active than ADA\*1/\*2 and 30% more active than ADA\*2/\*2 (11).

ADA occurs in a small molecular form (MW 33,000) called red blood cell (RBC) ADA and in a large molecular form (MW 200,000) called tissue-specific ADA. The various ADA tissue enzymes consist of one or more molecules of RBC ADA and one molecule of adenosine deaminase complexing protein (ADPC) (12).

ADPC is identical with CD26 (a T-cell activator molecule). It has been suggested that ADA expression may be connected with the process of T cell activation.

ADA catalyzes the irreversible deamination of adenosine to inosine pointing to an important role in regulating adenosine concentration. Adenosine is an important local hormone (like prostaglandins) regulating blood flow, neurotransmission, physiology of smooth muscle, and platelet aggregation. Current interest is focused on the modulatory effect of this substance on the immune system (13). ADA was supposed to be an exclusively cytosolic enzyme until the recent demonstration that is present on the surface of erythrocyte and platelets. 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 adenosine 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, which facilitates a variety of specific signaling events in different cell types. The most interesting aspect is the possible participation of the ectoenzyme in cell to cell contacts during ontogenesis and maturation of immune system (14). 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.

Previous studies by our group have shown a reduced frequency of ADA\*2 carriers among children with bronchial asthma, suggesting a protective action of this allele against the disease (6).

#### *Acid Phosphatase Locus 1 (ACP1)*

The ACP1 or cLMPTP (cytosolic low molecular weight protein tyrosine phosphatase) is an enzyme involved in

T-cell receptor signal transduction (15). It has also been shown to modulate insulin as well PDGF receptor signal transduction (16). ACP1 is a polymorphic enzyme showing a strong quantitative variation of total enzymatic activity. In Caucasian populations, six genotypes are observed designed: \*A/\*A, \*A/\*B, \*B/\*B, \*A/\*C, \*B/\*C, and \*C/\*C attributed to the presence of three codominant alleles, ACP\*A, ACP\*B, and ACP\*C, at the autosomal locus. Spencer et al. (17), found the following activities ( $\mu\text{mol}$  p-nitrophenol produced in 30' per g of Hb at 37°C): ACP1\*A/\*A = 122.4; ACP1\*A/\*B = 153.9; ACP1\*B/\*B = 188.3; ACP1\*A/\*C = 183.8, and ACP1\*B/\*C = 212.3.

Intracellular signal transduction is mediated by a balance between kinases and phosphatases, and both have a crucial role in immunologic and metabolic responses (18, 19). Genetic variability of kinases and phosphatases involved in these signaling pathways could influence lymphocyte activation threshold as well as cellular responses to cytokines and growth factors.

Previous studies by our group have shown an increased frequency of carriers of ACP1\*A allele in atopic dermatitis (7) and a lower level of IgE in carriers of ACP\*C allele (20).

#### *MNSs System*

MNSs is a polymorphic system controlled by two closely linked genes on chromosome 4q28-q31 encoding MN and Ss antigens. The two major glycoproteins of the human red cell membrane (glycophorin A and B) carry the antigen specificity of the system. Glycophorin A (MN sialoglycoprotein) is the most abundant in the red cell membrane (about 1 million copies per cell). Less is known about glycophorin B (Ss sialoglycoprotein). It is similar in structure to the glycophorin A (21–23). Glycophorin A is one of the major ligand of the Plasmodium Falciparum. It is also an important ligand for some bacteria and for viruses (ortho and paramixovirus, reovirus, picornavirus) that recognize the N-acetylneuroaminic acid abundant in the glycophorin (23).

Previous studies by our group have shown a strong association of MN genotype with age at onset of bronchial asthma, suggesting that, in atopic children with N genotype,

TABLE 2.—Analysis of interaction between MN blood group system and age at onset concerning the effects on prick test positivity.

| Age at onset    | M    | MN   | N    |
|-----------------|------|------|------|
| ≤ 24 months (%) | 57.7 | 72.1 | 77.7 |
| Total no.       | 26   | 43   | 9    |
| >24 months (%)  | 70.6 | 91.9 | 95.7 |
| Total no.       | 17   | 37   | 23   |

Three-way contingency table analysis by a log linear model

|                           | G      | df | p     |
|---------------------------|--------|----|-------|
| xyz interaction           | 1.280  | 2  | NS    |
| influence of x and z on y | 17.561 | 5  | 0.005 |

Age at onset into two categories: below 24 months and above 24 months. The table shows the proportion of children with a positive prick test in relation to MN genotype and age at onset of symptoms.

x: MN; y: prick test; z: age at onset.

asthma becomes evident at a more advanced age of development compared with those of M genotype (4).

### ABO and Secretor

The secretor gene (FUT2) that encodes for a 2- $\alpha$ -L-fucosyltransferase and the ABO blood group system that encodes for glycosyltransferases, act in concert to build up oligosaccharide structures in exocrine secretion systems, including the respiratory tract (24–26).

Specific oligosaccharide epitopes are necessary for recognition of microorganisms (27). The product of ABO and Secretor genes seems to influence the adhesion of infectious agents, thus having a modulatory effect on viral and bacterial respiratory infections (26, 28).

In a previous article (8), our group showed that the proportion of O/non-Secretor in asthmatic children is higher than in controls, thus confirming a similar association found in adults.

## RESULTS

Table 1 shows a logistic regression analysis with dependent prick test positivity and independent age at onset, sex, and a set of variables previously found to be associated with asthmatic disorders [MN (4), ADA (5), ACP1 (6), and ABO and Se (8)]. The set of variables shows a strong discriminant power on prick test positivity. MN system and age at onset of symptoms are the most important variables separating prick test-negative from prick test-positive children.

Table 2 shows an analysis of interaction between MN system and age at onset of symptoms concerning their effects on prick test positivity. The lack of three-way interaction suggests that MN and age at onset influence independently prick test positivity: their effects are additive as suggested by the significance of the model testing the independence of prick positivity on MN and age at onset. The minimum proportion of positivity is observed in M subjects with an age at onset below 24 months (57.7%),

whereas the maximum (95.7%) is observed in N subjects with an age at onset above 24 months.

Table 3 shows the distribution of MN phenotypes according to age at first symptoms of asthma and to prick test positivity. The relationship previously described (5) between age at onset and MN is present only among prick-positive children. In prick-negative children, chi-square analysis indicates an increase of M phenotype in both age groups ( $p < 0.01$ ). In prick-positive children, there is a significant increase of N phenotype in those more than 24 months of age at the onset of asthmatic symptoms ( $p < 0.02$ ). It appears that the M phenotype is correlated positively with an increased susceptibility to nonallergic asthma in all age groups (prick negative;  $p < 0.01$ ; OR = 2.5), whereas the N phenotype seems to be correlated positively with age at onset but in allergic asthma only (prick positive;  $p < 0.02$ , OR = 2.07 in age >24; OR = 0.56 for all other categories).

## DISCUSSION

Among the genetic systems previously found to be associated with bronchial asthma, the MN system seems to bear a direct relationship with prick test positivity. In the context of the recent observation (1–3) that early contact with an animal stable exerts a protective effect on non-allergic asthma, the fact that M phenotype is correlated positively with an increased susceptibility to nonallergic asthma seems of special interest.

Because the MN system codifies for glycophorin A, a sialoglycoprotein that represents a major ligand for several bacteria and viruses that recognize the N-acetylneuraminic acid present in this protein (23), genetic variability in this system might influence mucosal damage by infectious agents enhancing in some genotypes susceptibility to asthmatic reactions in the absence of IgE hyperproduction.

Age of children appears also very important because N phenotype is correlated positively with age at onset, but in allergic asthma only.

It is likely that development of allergy is dependent on genetic background, developmental parameters, and environmental exposure especially to bacterial endotoxins. However, a separate analysis of single factors is a reductionist approach that cannot solve the problem of allergy and asthma. The simultaneous study of genetic, developmental, and environmental factors and of their interactions may be more productive to unravel pathogenetic mechanisms.

## ABBREVIATIONS

|      |                        |
|------|------------------------|
| Se   | secretor               |
| ADA  | adenosindeaminase      |
| ACP1 | acid phosphatase locus |
| MN   | blood group system     |
| ABO  | blood group system     |

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TABLE 3.—Distribution of MN blood group system phenotype according to age at first symptoms of asthma and to prick test positivity.

|                                  | MN phenotype |        |        | Total no. |
|----------------------------------|--------------|--------|--------|-----------|
|                                  | M (%)        | MN (%) | N (%)  |           |
| Prick negative                   |              |        |        |           |
| Age $\leq$ 24 months (a)         | 44.0         | 48.0   | 8.0    | 25        |
| Age >24 months (b)               | 55.6         | 33.3   | 11.1   | 9         |
| Prick positive                   |              |        |        |           |
| Age $\leq$ 24 months (c)         | 28.3         | 58.5   | 13.2   | 53        |
| Age >24 months (d)               | 17.6         | 50.0   | 32.5   | 68        |
| General population (e)           | 26.2         | 55.0   | 18.8   | 309       |
| Chi-square test of independence  |              |        |        |           |
|                                  | Chi-square   | df     | p      |           |
| All classes/all MN phenotypes    | 18.09        | 8      | 0.02   |           |
| (a + b) vs. (c)/(M) vs. (N + MN) | 6.99         | 1      | < 0.01 |           |
| (c) vs. (e)/(N) vs. (M + MN)     | 0.33         | 1      | NS     |           |
| (d) vs. (e)/(N) vs. (M + MN)     | 6.19         | 1      | < 0.02 |           |

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