A genetic basis for heterogeneity of asthma syndrome in pediatric ages: Adenosine deaminase phenotypes

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Wheezing in children is characterized by at least two major syndromes: "wheezing associated with respiratory infections" and "atopic asthma." The distinction between these two forms is not easy as similarities exist between them. The purpose of this study was to try to differentiate between them. We determined the phenotype of adenosine deaminase (ADA) in 291 children ages 1 mo to 15 yr who had been affected by attacks of wheezing recently requiring clinical attention. The results demonstrated that the frequency of 2-1 ADA phenotype was significantly reduced in wheezers compared to control subjects. Among wheezers, the 2-1 ADA phenotype was normally represented under 5 yr of age but was particularly rare among asthmatic children 5 to 15 yr old. Thus ADA phenotypes may represent a genetic basis for some of the heterogeneity of the asthma syndromes in children. (J ALLERGY CLIN IMMUNOL 74:81, 1984.)

Wheezing is a symptom observed very frequently in pediatric practice, and it is considered currently as a prerequisite to a number of different diagnostic categories. Even if the terminology is still somewhat controversial, one could reasonably accept the existence of at least two syndromes categorizing children with wheeze: WARI and atopic asthma. Regardless of definitions, childhood syndromes present similarities and differences, which may be age-related. In several cases it is possible to observe that the same subject wheezes in different circumstances for different causes. For example, 30% to 50% of young children (usually under age 2 yr) who had viral bronchiolitis may subsequently develop asthma.[1-3] Some authors[4] have been unable to detect differences between children with "asthma" and those with "wheezy bronchitis."

Abbreviations used

A:	Adenosine
ADA:	Adenosine deaminase
WARI:	Wheezing associated respiratory injections

In general, children who wheeze during the first year of life may do so especially during viral infections and may have more atopy and a familial history of atopy compared to the general population.[2, 3, 5] Asthma attacks may be precipitated by infections, by other agents such as allergens, or both. Furthermore, asthmatic children have a greater frequency of viral infections than their nonasthmatic siblings.[6] Thus wheezing attacks may be due to atopy, or viral infections, or both throughout childhood. However, higher frequency of atopy is demonstrable in those who wheeze over the age of 6 to 8 yr, whereas viral "WARI" is more a typical condition of children less than 5 yr. There is a rapid decline of WARI with age, and these patients cease to wheeze.[1] Children with WARI under age 5 yr may later become susceptible to noninfectious-induced wheeze. These differences are important enough to maintain a separation between

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Received for publication Aug. 23, 1982.
Accepted for publication Jan. 30, 1984.

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TABLE I. Age and sex distributions of the study groups

Study groups	Ages	Male	Female	Total
Cases				
Rome	<15	159	76	235
Northern Italy	<15	43	13	56
				291
Controls				
Rome	<1	305	267	572
	<21	—	—	320
Northern Italy	<21	248	277	525
				1417

asthma and WARI and to examine characteristics that may differentiate these two groups. Laboratory data had not previously substantiated the difference between "WARI" and atopic asthma.

It is proposed that wheezing may be a common hallmark of certain genetically predisposed individuals. It has been suggested previously[7] that atopy and predisposition to asthma are inherited independently and that atopy enhances the liability of asthma. It is our hypothesis that certain phenotypes of certain genetically determined characteristics could be differentiating factors.

This article attempts to demonstrate that phenotypes of ADA may be one of these genetic markers. We attempt to demonstrate that the distribution of phenotypes of ADA is significantly different in a pediatric population of wheezers compared to normal subjects. In the wheezers, there are differences also according to atopic status and age.

MATERIAL AND METHODS

We examined 291 children (202 males and 89 females), ages 1 mo to 15 yr. Of these, 235 were patients observed in the outpatient pediatric pulmonary clinic of the University of Rome or were admitted in the wards of the same clinic for acute respiratory episodes. The only criterion for admission of patients to the study was the occurrence of a history of at least two episodes of wheezing in the last 6 mo, irrespective of the possible etiology/pathogenesis of the attacks. In addition, 24 children less than 1 yr of age were included in the study with only one certified episode of wheezing, clinically classified as "bronchiolitis" according to a score in use in our clinic; all 24 were admitted to the hospital during that episode. We enrolled an additional 56 children who were residents in a House for Asthmatic Children ("Pio XII") in Misurina (Belluno), northern Italy.

A sample of normal adults from a population sample in Rome, previously studied by another group,[8] and a consecutive series of newborn infants, studied by our group in Rome,[9] were considered normal controls for the study population in Rome. A sample of 487 random adults from the Po Delta, studied by the Center for Evolutionary Genetics in Rome,[10] were considered normal controls for the study population in northern Italy.

The age and sex distributions of the study groups are shown in Table I.

Several examinations have been performed in most of the patients included in this study, related to other clinical protocols or for clinical use. Only data relevant to the ADA results are quoted in the present paper. In all cases, a detailed questionnaire was administered to derive family and personal history. All children were prick skin tested with common environmental antigens. Questionnaires and tests were administered by trained physicians; there were no interobserver differences in administration or interpretation.

ADA phenotype was determined in all subjects according to the method of Spencer et al.[11] Blood samples were maintained at less than 4° C up to the moment of determination, which was always initiated within 12 hr from the time of sampling. The determinations were performed in the Genetics Laboratory, Camerino Institute, or in the Genetics Institute of Rome, by use of the same technique. There were no interlaboratory differences in percentages of ADA phenotypes, either in patients or control subjects. Data were analyzed by standard statistical techniques on an H-P programmable calculator.

RESULTS

There were no demonstrable differences in ADA phenotypes by sex in any of the groups, so gender was not considered in further analyses. Table II demonstrates the distribution of ADA phenotypes on the three different control samples; no significant differences were observed between the different control groups. Table II also demonstrates the distribution of ADA phenotypes in the case samples ("wheezers"). ADA distributions were the same in the two different case groups of children. The prevalence of phenotype 2-1 is significantly reduced in cases compared to controls ($p < 0.025$). The relative risk ratio (the odds ratio in a case-control study like this) for cases (i.e., wheezers) to be ADA phenotype 1 is 1.63 ($p < 0.05$). The attributable risk (the probability of ADA type 1 is a case/wheezes) due to ADA type 1 is 34% (see Table II).

Within cases, by age (less than 5 yr and more than 5 yr), we examined the proportions with a positive family history for atopy, with positive immediate prick skin tests for common allergens, and the prevalence of ADA phenotype 2-1, as seen in Table III. It can be observed that the differences in prevalences of skin test positivity and of ADA phenotype 2-1 are correlated significantly with the age of children. ADA 2-1 is significantly less common ($p < 0.002$) in wheezy children over the age of 5 yr, although it is somewhat more common in those age 5 yr or less (who are more likely to have WARI). Patients with

TABLE II. Relative frequency distribution of ADA phenotypes*

Groups	ADA phenotype 1	2-1	2	N
Controls				
Rome newborn infants	0.836	0.159	0.005	572
Rome adults	0.825	0.172	0.003	320
Northern Italy adults	0.834	0.162	0.004	525
All controls	0.833	0.163	0.004	1417
Cases (children)				
Rome	0.881	0.115	0.004	235
Northern Italy	0.911	0.071	0.018	56
All cases	0.887	0.107†	0.007	291

*Two samples of "wheezy" children in patients and three samples in controls.

†Statistically significant difference ($p < 0.025$ by chi^2) between ADA 2-1 vs. 1; all controls vs. all cases. The odds ratio, or relative risk ratio, of being ADA 1, given that one is a case, is 1.63 ($p < 0.02$). The attributable risk = the probability that an ADA-1 is a case (i.e., has wheeze) caused by ADA-1 is 34%.[19]

episodes of wheeze over the age of 5 yr have an increased relative risk ratio (3.04) of being ADA 1 when compared to patients under the age of 5 yr. Over age 5 yr, the proportion of those with positive prick skin tests is significantly greater ($p < 0.001$). Thus atopic children over age 5 yr with episodic wheeze (i.e., asthma and thus atopic asthma) have a much greater likelihood of having ADA phenotype 1.

Children whose symptoms started after age 2 yr had a lower proportion of ADA phenotype 2-1 than the total case group (significant for males and total) (Table IV), although the relative risk ratio for ADA-1 is only 2.4.

TABLE III. Proportions of patients with family histories of atopy with positive prick skin tests and with ADA phenotype 2-1 by age

Age group	N	Family history of atopy	Positive skin tests	ADA 2-1
≤5	95	0.52	0.37	0.189
>5 (−15)	196	0.57	0.85	0.071
Total	291	0.55	0.69	0.110
Difference between age groups		NS	$p < 0.001$	$p < 0.002$

TABLE IV. Proportion of ADA phenotype 2-1 in patients according to age at first symptoms of bronchial obstruction

	≤2	>2 (−15)	Significance* (P)
Males			
Proportion ADA 2-1	0.154	0.055†	<0.05
N	104.0	91.0	
Females			
Proportion ADA 2-1	0.136	0.114	>0.05
N	44.0	44.0	
Total			
Proportion ADA 2-1	0.149	0.074†	<0.05
N	148.0	135.0	

*By chi.^{2}

†Significantly lower than all cases proportion of ADA 2-1 ($p < 0.01$) by chi.^{2}

DISCUSSION

Our results have demonstrated that recurrent wheezing is associated with a significant lack of the ADA phenotype 2-1. This occurs at an age when atopic features are more likely present and are likely to influence the episode of wheeze. Children who wheeze over the age of 5 yr have a highly reduced frequency of phenotype 2-1 compared to the younger "wheezeers." As the relative risk ratio of children to be ADA 1 type is 1:63 ($p < 0.05$) and the attributable risk of ADA 1 as a marker of wheezers is 34%, the heterogeneity of the asthmatic disorder in childhood appears to have, at least in part, a genetic basis. Children under 5 yr or whose symptoms start at age 2 yr or earlier appear quite different from the older group in which ADA-1 is associated with asthma. Nevertheless, ADA 1 itself is not a powerful marker, as a high proportion of all nonwheezers (controls) had ADA phenotype 1 also (83%), even if the atopic asthmatic over age 5 yr does have a very high relative risk ratio (3.04) associated with a relative frequency of 93% with ADA phenotype 1.

The concordance of results obtained from two different, independent populations of asthmatic children and produced by two different laboratories appears to be a reasonable guarantee against biases caused by stratification or artifacts of the procedure. It was noted also in controls that the proportion of ADA phenotypes does not differ by age, likely indicating little difference in penetrance with age. Nevertheless, the results would have been more powerful if we could have matched cases and controls by age.

The rationale for searching for such an association between late childhood atopic asthma and ADA phenotype is on the basis of several factors. A can be transported into or released by several types of

cells.[12, 13] Its extracellular activity helps regulate both basophiles and leukocytes through specific membrane receptors, which can control the release of histamine on challenge.[14] Intracellular adenosine is considered to have a central role in the biochemical pathway of purine nucleotides, which helps control the effector function of lymphocytes and their capability for differentiation.[15] The intracellular concentration of A greatly depends on the competitive mechanism between the ways of intracellular nucleotide utilization.

In 50% of cases of severe combined immunodeficiency in which there is a complete lack of ADA activity (Null ADA phenotype), there is an increased level of A and its derivative compounds (including cAMP) in plasma, urine, lymphocytes, and erythrocytes.[16] In certain congenital hemolytic anemias, an increased level of ADA activity has been found in erythrocytes (but not in other cell populations), which then results in a lowered intraerythrocyte level of ATP and other adenine nucleotides.[17]

Thus it appears that the activity level of ADA is one of the main regulatory systems for the utilization of A to form ATP and cAMP.

It has been demonstrated that the three phenotypes of ADA recognizable in a normal population (1-1, 2-1, 2-2) have different enzymatic activity. The phenotype 1-1 is more active than the other two phenotypes: +15% compared to 2-1 and +30% compared to 2-2.[18] Thus one of the possible conclusions from these data is that the intracellular concentration of A and of other nucleotides could be different in individuals bearing ADA phenotypes 2-1 and 2-2 because of their lower enzymatic activity.

It has been demonstrated that A, at concentrations reported to occur in vivo, inhibits the antigen or IgE-induced release of histamine from human basophils, an inhibition caused by an increase of intracellular cAMP.[14] Similar observations have occurred with studies of leukocytes.[15] This may be a mechanism for the protection afforded by ADA phenotype 2-1 in atopic asthma. If so, the pathogenetics of "WARI under the age of 5 yr," that is not prevented by the 2-1 ADA phenotype, is triggered by a viral infection, is not clearly associated with a family history of atopy or personal atopy (related to IgE) is in some way different from pathogenetic pathways of later childhood "atopic asthma." If so, these two broad types of pediatric respiratory illnesses are widely different.

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