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ORIGINAL ARTICLE

An 18-year Follow-up of Allergy Development Related to Nasal Metachromatic Cell Findings During Infancy

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ABSTRACT

Background: The ability to predict the development of allergic diseases in infants is important. Predictive biomarkers are wanted to improve the risk evaluation in addition to known heredity of allergy. Biomarkers taken during infancy need to be evaluated through longitudinal studies into adulthood. *The objective of this study was to analyse the occurrence of metachromatic cells in the nasal mucosa during infancy ($MC_{infancy}$) and evaluate the cells as predictive biomarkers of allergy development*.

Methods: Previously, $MC_{infancy}$ occurrences were analysed in 64 infants with and without allergy heredity, and related to allergy development at 18 months and 6 years of age. In this third follow-up at 18 years of age, current allergy symptoms were analysed. $MC_{infancy}$ findings were related to the cumulative number of allergic subjects. The predictive values of $MC_{infancy}$ and known heredity were compared.

Results: The cumulative number of subjects with allergy was 46, probable allergy 5, and no allergy 13. Detected $MC_{infancy}$ predicted allergy with high accuracy (31/33), but negative $MC_{infancy}$ findings did not exclude the risk (15/31). In the group of allergic subjects positive $MC_{infancy}$ were found in 31/46 (67%), positive heredity in 37/46 (80%) and one/both factors positive in 43/46 (93%). Detection of $MC_{infancy}$ could precede the debut of allergy symptoms by many years.

Conclusions: Detected $MC_{infancy}$ predicted allergy development, but absence of $MC_{infancy}$ did not exclude the risk, and therefore this biomarker was not found to be adequate. There is a further need to find biomarkers with high ability to both predict and exclude the risk.

KEY WORDS

allergy, cohort study, metachromatic cells, nasal epithelium, prediction

INTRODUCTION

Atopic disease is one of the most common chronic disorders worldwide both among children and adults.[1,2] As the impact from these disorders on affected individuals, their families and on the community as a whole is considerable, measurements aiming at prevention or reduction of allergy morbidity are continuously regarded to be of great importance.[3-5] In order to optimize all efforts, individuals at risk who may best benefit from good clinical management have to be identified early in life.[6] Although a family history of asthma and atopy is well known to be one of the most important predictive factors,[7-9] there is a need to improve risk evaluation. Thus, much research has been done to identify additional risk factors and predictive biomarkers. Sensitization before the age of two years is found to be associated with asthma at school age in children with parental asthma or atopy.[10] Environmental factors, such as exposure to tobacco smoke and furry pets, are also regarded as risk factors for respiratory symptoms.[11]

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Authors' Contributions: KI and MPB made the study design of this paper. KI performed the examination of the participants and evaluation of the results. Both authors wrote the manuscript.
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Understanding the immunological mechanisms underlying inflammation has identified a number of biomarkers, but few of them have proven useful as predictors.[12] The recent advances in technology in studies of functional genomics[13] may be a method for ensuring the best reliable predictive biomarkers, but as findings analysed during infancy need to be evaluated through longitudinal studies into adulthood, the results using this relatively new method will be delayed for another one or two decades.

Our ambition to find biomarkers during infancy for allergy development by means of a simple test procedure started with a prospective study, including 67 infants between 1985 and 1988. Most of the invited infants had a history of allergy in immediate family members, ensuring development of atopy in early life. A smaller group with no family history of allergy was included to ensure healthy controls.

The main focus of the study was on the occurrence of metachromatic cells (MC, mast cells, basophils) in the nasal mucosa, as new information on human MC heterogeneity had recently become available at the time of the study start.[14] Specimens for cell analysis were taken repeatedly from the nasal mucosa during infancy and could be evaluated in 64 babies. At the first follow-up at 18 months of age, findings of demonstrable MC during infancy ($MC_{infancy}$) were significantly associated with allergy, both for respiratory tract symptoms and for atopic dermatitis.[15] Nasal eosiophils were commonly found in allergic and also in healthy children and therefore considered to be of less diagnostic and predictive value.[16] The association between $MC_{infancy}$ and allergy development still persisted at the second follow-up at 6 years of age.[17]

The aim of this third follow-up was to analyse, after getting detailed information and examinations of current allergy symptoms, if the association between $MC_{infancy}$ and allergy development still persisted at the age of 18 years. Based on this result the value of $MC_{infancy}$ findings as predictive biomarker for allergy development could be evaluated and compared to the predictive value of allergy heredity.

METHODS

SUBJECTS

The original study group comprised 67 children. Specimens for analysis of MC occurrence in the nasal mucosa had been taken at 3, 6, 9 and 18 months of age ($MC_{infancy}$) in 64 infants, while results from three babies could not be evaluated due to insufficient compliance with the repeated examinations during infancy.[15] $MC_{infancy}$ were detected in 33 and not detected in 31 infants.

The family history of allergy was previously established in detail.[15] Briefly, the definition of a positive allergy heredity was based on a history in immediate family members of allergy symptoms in the upper/lower airways verified by a positive skin prick test to a relevant air-born allergen and/or a history of atopic eczema; possible allergy heredity was defined as a history of mild airway allergy not verified by the skin prick test, or no allergy history but a positive skin prick test, or a history of atypical eczema; negative heredity was defined as a history of healthy immediate family members verified by negative skin prick tests. Allergy heredity in the 64 infants with assessed findings of $MC_{infancy}$ was found to be positive in 44, possible in 11 and negative in only 9 subjects.

In this 18-year follow-up, information on allergy development was obtained from all of the individuals in the original cohort by questionnaires, personal interviews and telephone calls, and the invitation to participate in various examinations was accepted by 49 of them. Test results from subjects, who reported current treatment with pharmacological agents with possible effects on the results, were excluded in the statistical evaluations. The numbers of included values in the separate tests are shown in the 'Results' section.

This follow-up was performed during wintertime out of the pollen season. The participants had to be free from airway infections for at least 10 days prior to the examinations.

CLINICAL EXAMINATIONS

All of the participants were examined by anterior rhinoscopy, inspection of the throat, epipharynx, hypopharynx and larynx, and by otomicroscopy and tympanometry.

QUESTIONNAIRE

The questions about upper and lower airways and the skin were based on questionnaire modules for teenagers in the study protocol for ISAAC studies.[18] Additional questions about time relations between exposure to offending allergens and airway symptoms and the current use of pharmaceutical agents were added.

SKIN PRICK TEST AND PHADIATOP® ANALYSIS

A skin prick test (SPT) was performed using ALK extracts (ALK, Denmark) including pollen allergens (birch, timothy, mugwort) and perennial allergens (horse, cat, dog, D pteronyssinus, D. farinae, Alternaria, Cladosporium). The SPT was defined as positive if the mean wheal diameter (half of the sum of the largest diameter and its perpendicular) was at least 3 mm.[15] Histamine hydrochloride 10 mg/ml served as a positive control and normal serum albumin was used as a negative control. ImmunoCAP® (Phadia AB, Uppsala, Sweden) with the corresponding allergens as in SPT was used in one male with allergy including severe eczema. Sensitization to inhalant allergens was also examined in all of the subjects by Phadiatop® analysis (Phadia AB, Uppsala, Sweden). Positive results in the serological analyses were

values above the detection limit of 0.35 kU/L, according to the manufacturer.

DEFINITIONS OF ALLERGY DIAGNOSES

The definitions of allergic rhino-conjuctivitis and allergic bronchial symptoms were based on a history of relevant symptoms (itching in eyes or nose, tearing, eye redness, runny nose, sneezing, nasal obstruction, shortness of breath, chest wheezing/whistling, increased mucus production, coughing) during the last two years at least twice in relation to exposure to an airborne allergen. The symptoms had to be supported by at least one positive SPT reaction and a positive Phadiatop® test. The definition of asthma was according to the changes in the spirometric results in the exercise provocation test (see below). Atopic eczema was evaluated according to the SCORAD index.[19] Symptoms not fulfilling all of the criteria were defined as 'probable allergy'. A symptom-free period during the last two years in relation to previously offending allergen exposures was regarded as recovery from disease.

EXERCISE PROVOCATION TEST AND ASTHMA DEFINITION

A standardized method was used to evaluate the function in the lower airways. The individuals had to run on a treadmill for 6 minutes to achieve a pulse rate of $\geq$160 beats per minute. Spirometry (Microlab) was performed before exercise, immediately after exercise and repeated after another 15 minutes; a reversal dose of a β-agonist was then given followed by a fourth measurement 15 minutes later.

Asthma definition was based on the values of the forced expiratory volume in 1 second (FEV_1): an initial FEV_1 value $\leq$80% of the expected value, a reduction of FEV_1 $\geq$15% after the physical exercise in combination with an increase of FEV_1 $\geq$15% after the bronchodilatation. Individuals reporting relevant symptoms from the chest related to allergen exposures but not fulfilling the asthma criteria were regarded to suffer from allergic bronchitis.

CYTOSPIN PREPARATIONS OF NASAL MUCOSAL CELLS

Nasal mucosal cells were harvested by a gentle nasal brushing using a 5.5 mm diameter nylon brush (Doft AB, Östhammar, Sweden). The brush was immediately placed in a tube containing physiological saline and twirled for several seconds. After cytocentrifugation onto glass slides, the materials were air-dried and fixated in 95% ethanol for later staining with toluidine blue for analysis of MC, according to the method used in our previous follow-ups.[15,17] Analysis of the numbers of MC was performed blindly by light microscopy (magnification ×250) with slides coded. Occurrence of one or more MC was regarded as a positive result, and absence of demonstrable MC as a negative result, provided the density of epithelial cells was >25 cells per visual field.

STATISTICAL ANALYSIS

Fisher's exact test was used when comparing two qualitative data results between two groups. A probability level of less than 5% was considered to be significant.

ETHICS

The study was approved by the Ethical Committee at the University Hospital in Linköping, Sweden (03694). A written consent was obtained from each of the participants after full information of the study.

RESULTS

CLINICAL MANIFESTATIONS OF ALLERGY AND TEST RESULTS AT THE AGE OF 18 YEARS

Diagnosis of Allergy by History of Symptoms and Findings at the Clinical Examinations

The history of allergy symptoms from the 67 participants revealed current allergy symptoms in 39 individuals, probable allergy in four and no allergy in 24 individuals.

The development of allergy from all follow-ups is shown in Figure 1. The details from the first two follow-ups are presented previously.[15,17] Since the previous 6-year follow-up, ten individuals reported debut of symptoms, two of the six individuals with previous recovery had regained allergy symptoms, and only one subject had recovered at this follow-up.

The most common problems at the age of 18 years were symptoms in the upper airways, which was a shift among allergy symptoms, as skin and bronchial symptoms were previously dominating during infancy and preschool age (Table 1). Thus, nasal symptoms were reported by all individuals with airway symptoms and in 11 of the 17 subjects with skin symptoms.

The clinical findings were normal in most individuals, probably due to the examinations being performed during winter time with a symptom free interval in the pollen sensitive subjects and weak influences from allergen exposures in subjects with perennial allergy.

There was a trend toward a higher number of individuals reporting allergy symptoms among those attending the clinic for tests (31/49) compared to those who refrained from the visit (8/18).

Allergy Tests

Signs of sensitization, according to positive findings in the allergy tests, were evaluated in all of the 49 individuals accepting the invitation for examination. The SPT results were in agreement with the Phadiatop® analyses in all except one subject, who had recovered from previous allergy and was SPT negative but Phadiatop positive. The allergy tests were posi-

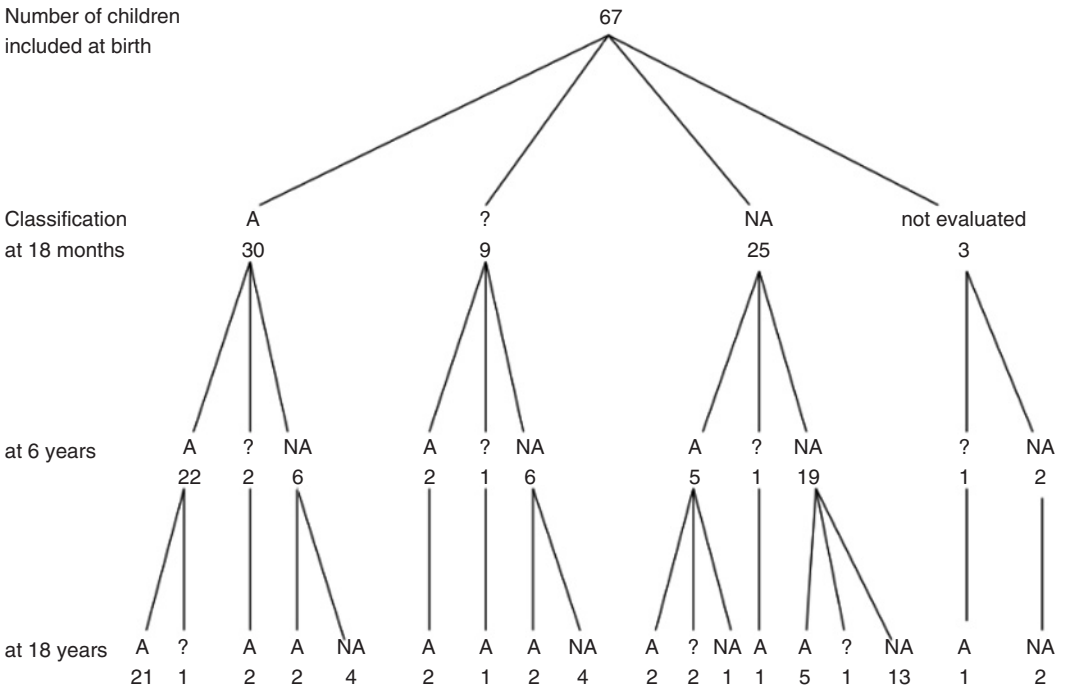

Fig. 1 Number of subjects reporting allergy (A), probable allergy (?) and no allergy symptoms (NA) at the follow-ups at 18 months, 6 years and 18 years of age.

Table 1 Number of individuals and the percentage (in parentheses) of different allergy symptoms at the follow-ups

History of allergy symptoms	18 months† (*n* = 64)	6 years‡ (*n* = 67)	18 years (*n* = 67)
Allergy symptoms	30	29	39
- single/multiple	18/12	16/13	17/22
- symptoms in the:			
skin	21 (70%)	19 (66%)	17 (44%)
lower airways	16 (53%)	11 (38%)	15 (38%)§
upper airways	2 (7%)	16 (55%)	33 (85%)
Probable allergy	9	5	4
No allergy	25	33	24

†data from reference 15; ‡data from reference 17; §asthma verified in 2/15 individuals reporting bronchial allergy symptoms.

tive to at least one allergen in 26 out of 27 individuals with airway allergy; one man with a convincing history of birch pollen allergy was negative in both of the tests. According to the profile of sensitization the subjects with airway allergy were grouped into a subgroup sensitized only to pollens (n = 8) and a subgroup sensitized to perennial allergens regardless of concomitant positive pollen test (n = 18). The tests were negative in all of the four individuals with symptoms limited to the skin, in three of the four individuals with probable airway allergy and in all of the 14 non-allergic individuals.

Thus, there was a high agreement between signs of sensitization in individuals reporting airway symptoms (96%), and no signs of sensitization in healthy individuals (100%) and in subjects with symptoms limited to the skin (100%).

Exercise Test

The exercise test was performed in 47 individuals, as two subjects with upper and lower airway symptoms refrained from performing the test due to personal reasons. The test was normal in all subjects with no symptoms in the lower airways. Out of 11 individuals reporting allergic symptoms in the lower airways, the criteria of asthma diagnosis were fulfilled in only two subjects, both sensitized to perennial and pollen allergens, and nine were regarded to suffer from allergic bronchitis.

MC Findings

Occurrence of nasal MC ($MC_{18\ years}$) was evaluated in 45 individuals, as two subjects were using pharmacological agents, which might have had effects on the cell findings (non-allergic systemic diseases treated with methotrexate and oral steroids, respectively) and another two subjects refrained from the nasal brushing due to personal reasons.

The number of individuals with demonstrable $MC_{18\ years}$ were significantly higher (p = 0.01) in the group with allergy symptoms (16/30) compared to the group of individuals with no allergy (1/12). However, no statistical difference was found in the number of subjects with demonstrable $MC_{18\ years}$ when

Table 2 Number of subjects with findings of metachromatic cells ($MC_{18\ years}$) related to current allergy symptoms and allergen sensitization at the 18-year follow-up

History of allergy symptoms in:	$MC_{18\ years}$ present	not present	Totals
Skin only	1	3	4
Airways			
allergen sensitization			
- pollens only	4	4	8
- perennials	10	7	17
not sensitized	1	0	1
Probable airways	2	1	3
No allergy	1	11	12
Totals	19	26	45

A small subset of subjects with airway allergy had also mild eczema. A subset of subjects sensitized to perennials was also sensitized to pollens.

comparing the allergy subgroups sensitized to pollens only and to perennial allergens (4/8 and 10/17, respectively, Table 2).

DEVELOPMENT OF ALLERGY SYMPTOMS FROM INFANCY TO THE AGE OF 18 YEARS IN RELATION TO THE METACHROMATIC CELL FINDINGS DURING INFANCY AND TO THE FAMILY HISTORY OF ALLERGY

Reports of allergy symptoms could be obtained in this third follow-up from all of the 64 individuals with assessed findings of $MC_{infancy}$ and known allergy heredity. During the period of 18 years, altogether 46 of the 64 individuals were suffering (n = 38) or had been suffering previously (n = 8) from allergy symptoms, five had reported probable symptoms during the period, while 13 subjects never had experienced any allergy symptoms (Fig. 1).

Predictive Value of $MC_{infancy}$

In the group of individuals with demonstrable $MC_{infancy}$, allergy developed in 31/33 (94%), probable allergy in 2/33 and none remained healthy. The intervals until debut of allergy symptoms with clinical manifestations in airways and/or skin in relation to findings of $MC_{infancy}$ are shown in Table 3. In most of the $MC_{infancy}$ positive individuals symptoms developed during infancy, but in one third of the subjects the debut of allergic symptoms was delayed until preschool age or adolescence. Fourteen of 19 subjects with positive $MC_{18\text{-}years}$ findings were $MC_{infancy}$ positive and five subjects were $MC_{infancy}$ negative. $MC_{infancy}$ were demonstrated in the respiratory mucosa in four of the five subjects developing atopic eczema without airway symptoms.

In the group of individuals with no detectable $MC_{infancy}$, 13/31 (42%) remained healthy, 3/31 (10%) suffered from probable allergy and 15/31 (48%) developed allergy symptoms.

Predictive Value of Allergy Heredity

In the group of subjects with a positive family history of allergy, 37/44 (84%) developed allergy, 3/44 (7%) reported probable allergy and 4/44 (9%) remained free from allergy symptoms. Among the subjects with no heredity, 3/9 developed allergy problems and 6/9 remained healthy.

Predictive Value of Combined Information from $MC_{infancy}$ Findings and Allergy Heredity

Positive findings of $MC_{infancy}$ and/or positive allergy heredity were found in 51 subjects, and 43/51 (84%) developed allergy, 4/51 (8%) probable allergy and 4/51 (8%) remained healthy. Among individuals with no demonstrable $MC_{infancy}$ and negative heredity, 2/8 developed allergy problems and 6/8 remained healthy.

Thus, among the subjects developing allergy 31/46 (67%) had positive $MC_{infancy}$ findings, 37/46 (80%) had a positive heredity of allergy, and in 43/46 (93%) of the subjects either or both of predictive factors were positive.

DISCUSSION

This study demonstrated that detection of MC in nasal epithelium during infancy is associated with development of allergic symptoms up to the age of 18 years, as all of the individuals with demonstrable $MC_{infancy}$ developed allergy or, in a few individuals, suspected allergy symptoms and none of them remained completely free from allergy problems. Detection of the $MC_{infancy}$ often preceded the debut of allergy symptoms during infancy[15] and childhood[17] and this follow-up showed an interval of many years with debut of allergy symptoms not before adolescence in some subjects. It is also interesting to note that $MC_{infancy}$ in nasal mucosa were found, not only in individuals developing airway allergy, but also in individuals developing atopic eczema without airway symptoms. These findings are in agreement with the concept of atopy as a systemic disease.

Our findings of demonstrable $MC_{infancy}$ have lead us to some speculations, although we have no ambition to give an adequate explanation for the development of allergy diseases. One of our speculations is, that subjects with a propensity for allergy development might have an innate constitution with a higher number of MC, as reflected by the $MC_{infancy}$ findings, regardless of future symptoms in lower airways, skin as well as in the upper respiratory tract, described as the atopic march. The presence of MC early in life might contribute to sensitization, as mast cells have been proposed to be promotors of local IgE synthesis via B cell activation.[20,21] We also have speculated that an $MC_{infancy}$, provided that the infiltration in the nasal mucosa has a corresponding $MC_{infancy}$ infiltration in

Table 3 Number of subjects with/without nasal metachromatic cells during infancy ($MC_{infancy}$) in relation to debut of allergy symptoms during the entire period up to 18 years of age

$MC_{infancy}$	First report of allergy symptoms at the age of: 18 months/6 years/18 years			Symptoms in		Probable allergy	Never allergy
				airways ± skin	skin only		
Present (*n = 33*)	20	5	6	27	4	2	0
Not present (*n = 31*)	10	2	3	14	1	3	13
Totals	30	7	9	41	5	5	13

Subgroups with symptoms in airways (with/without skin symptoms) and the skin only are also shown.

the bronchial wall, might contribute to the development of asthma, as mast cells are found in increased in numbers within the airway smooth muscle bundles of asthmatic patients[22] and this infiltration has been related to airway dysfunction and asthma severity.[23]

In contrast to the possibility of future allergy prediction based on detectable $MC_{infancy}$, absence of $MC_{infancy}$ findings did not exclude the risk of allergy development. In all of the three follow-ups absence of MC were found in a proportion of individuals expected to have demonstrable MC due current allergy symptoms.

One explanation for not finding expected occurrence of MC in this 18-year follow-up might be the time of the year of the examinations, which were performed out of the pollen season to ensure the same environmental conditions to all of the participants. Provided the same redistribution of mast cells from the lamina propria to the epithelium, as described after exposure in pollen allergic patients,[24,25] also takes place due to exposure to perennial allergens, reduced numbers of subjects with detectable $MC_{18\ years}$ could be expected in the subgroup sensitized to only pollens with no allergen exposure for more than three months compared to the subgroup sensitized to perennial allergens with persistent allergen exposure. However, no statistical difference in the number of subjects with/without detectable $MC_{18\ years}$ was found between the two groups. The exposures to perennial allergens might have been too weak for a redistribution of MC, and the limited number of subjects in the two subgroups might also explain the absence of a group difference.

The proportion of allergic subjects with demonstrable MC was decreased at the 18-year follow-up (19/45) compared to the 18-month follow-up (33/64). This is probably explained by allergy to foods, which was common in during infancy, but resolved in most of the food allergic subjects in young adulthood. Nasal mast cells have been demonstrated in children with ongoing allergy to food only.[26]

A possible explanation for not detecting MC may also be false negative findings, overlooking single MC in dense areas of epithelial cells. The metachromatic staining procedure and analysis by light microscopy was the only method available at the start of our cohort study, and has been used in all of the three follow-ups. Improved information of MC infiltration would have been obtained by methods including specific antibodies to mast cell subtypes and basophils, as used in immunocytochemistry or laser scanning cytometry.[27,28] However, in a study on cellular infiltrates in allergic children based on findings of specific antibodies to mast cells and other cells in mucosal biopsies, the results were in agreement with our study, as unexpected absence of mast cells in the epithelium was found in a proportion of allergic children sensitized to aeroallergens.[29] In the future, microarray-based studies of genes and transcripts in cells will probably result in superior information on predictive biomarkers, as this technique has shown genes exclusively expressed in mast cells and other cells in subjects with current allergy.[13,30]

The family history of allergy is since long regarded as 'the golden standard' as a predictor in risk evaluation of allergy development. In our cohort study, the group with no allergy heredity was inadmissibly reduced at the detailed check-up of the family members[15] resulting in a small control group of individuals with a negative heredity but an unwanted group of subjects with possible heredity. However, the study showed, that the detailed family history of allergy was a good predictor of allergy in individuals with a positive heredity.

The over-all predictive value of heredity was higher compared to the over-all predictive value of $MC_{infancy}$ findings, due to the fact that negative cell findings did not identify a large proportion of the risk subjects. The best risk evaluation was achieved by combined information on positive heredity and positive $MC_{infancy}$ findings. Unfortunately, the risk could not be excluded with negative findings of these two factors in combination.

The development of allergy symptoms in our cohort followed the well-known changes in atopic manifestation from infancy to adulthood, described as 'the atopic march'.[31] Atopic dermatitis, being predominant during infancy[15] and pre-school age[17] had gradually resolved in many of the sufferers, and most of the 46 subjects with previous or current allergy had developed airway symptoms. Only five of them had suffered from atopic eczema with no airway symptoms. At the age of 18 years asthma manifestations were less pronounced in severity as compared to the previ-

ous follow-ups and allergic rhinitis was the most common symptom. Examinations outside the peak of their symptom period may explain why subjects reporting pollen related relevant symptoms in the lower airways did not reach the criteria for asthma diagnosis, and therefore were classified as having allergic bronchitis.

The ascertainment of correct diagnoses from case histories was provided by the Phadiatop® and SPT in those individuals attending the clinic. The information from the case history was in high agreement with the results from both of the allergy tests. Thus, it was assumed that the subjects evaluated at a distance without the availability of allergy tests were correctly classified into the group with current allergy and the healthy group, respectively.

The strength of this study is the long follow-up period, so that allergy development up to young adulthood could be followed in all of the included infants. Few cohort studies, with the aim of evaluating early biomarkers of allergic diseases in children, have followed individuals into adult life. Piippo-Savolainen *et al*. followed 83 children prospectively in order to evaluate if early sensitization can predict asthma.[32] Sixty-five percent of the children remained in their study at the age of 18-20 years, and they found that early sensitization to seasonal pollen predicts subsequent asthma among adolescents.

We conclude, that nasal $MC_{infancy}$, if detected, predicted allergy development with high accuracy. To our knowledge, the long interval found in many of the individuals between the detection of nasal MC during infancy and debut of allergy not before adolescence is not previously described. This time period might be used for important preventive measurements. However, due to negative $MC_{infancy}$ findings in a high proportion of subjects developing allergy, the over-all predictive value was lower than the over-all predictive value of a detailed family history of allergy. In spite of an increased risk evaluation by the combined information of heredity and cell findings, we do not regard the $MC_{infancy}$ as an adequate predictive biomarker of allergy development. Further studies, using improved methods of cell analyses or studies at the gene level, are needed to find biomarkers with high accuracy to predict and also to exclude the risk of allergy development.

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