

Non-atopic asthma in children is related to maternal bronchial hyperreactivity

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Data on the pathogenic mechanisms underlying the development of non-atopic asthma in children are scarce. Our aim was to evaluate the association and compare the atopic status, pulmonary functions, bronchial hyperresponsiveness and serum total immunoglobulin E (IgE) levels of parents of atopic and non-atopic asthmatic children by using objective methods. Fifty-one asthmatic children aged 4–16 yr and their parents were included into the study. Initially the American Thoracic Society's Respiratory Disease questionnaire inquiring data on symptoms of asthma, rhinitis and past medical history was filled in. Afterwards, skin prick test with aeroallergens, pulmonary function and methacholine bronchial provocation tests and serum sampling for total IgE level determinations were carried out. Bronchial hyperresponsiveness to methacholine was significantly more common in the mothers of non-atopic children compared to those of atopic ones, although no significant difference was observed in the skin prick test reactivity, pulmonary function test parameters and serum IgE levels. Questionnaire data revealed that the presence of asthmatic symptoms such as wheezing and phlegm and doctor-diagnosed asthma were more common in the mothers of non-atopic children. Meanwhile, asthmatic symptoms were also found to be significantly more common in fathers of non-atopic children. Logistic regression analyses revealed that maternal PC₂₀ was the only predictive factor for the risk of displaying non-allergic asthma in children. The results demonstrate that among the risk factors studied, maternal bronchial hyperreactivity was associated with the development of asthma in non-atopic children.

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Asthma is a chronic inflammatory disorder mainly associated with atopy and bronchial hyperreactivity (BHR) inherited with multifactorial genetic components. The nature of any relationship between BHR and atopy has been identified as a central issue in our understanding of asthma (1). Although various environmental factors affect the development of asthma, both

twin and family studies point to a strong genetic component which had been investigated for many years (2, 3).

Asthmatics with known atopy have positive skin prick test response to common environmental allergens, elevated serum total and allergen-specific immunoglobulin E (IgE) levels. There are studies demonstrating an association between high total serum IgE levels and BHR with allergy, and strong aggregation of high total IgE levels within the families suggesting the regulation of serum IgE by genetic effect (4). However, in some of the patients with asthma, it is not possible to detect allergen-specific IgE and

Abbreviations: ATS, American Thoracic Society; BHR, bronchial hyperreactivity; FEF₂₅₋₇₅, forced mid-expiratory flow rate; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HDM, house dust mite; PEF, peak expiratory flow rate; SPT, skin prick test.

a positive skin test, so called non-atopic. It is uncertain whether these two conditions represent with different mechanisms or similar immunopathology (5).

Bronchial hyperresponsiveness, considered as one of the key features in asthma, is heritable and closely related to serum IgE levels and airway inflammation. An elevated level of serum total IgE is co-inherited with a trait for bronchial hyperresponsiveness and the gene governing BHR is located near a major locus that regulates serum IgE levels on chromosome 5q (6, 7). Studies of the genetic basis of asthma have identified several genes that predispose to pathogenesis of BHR and airway wall remodeling, including regions of chromosomes 2, 5q, 11q, 12q, and 20p (8–10). Apart from chromosome 20 (ADAM33, A Disintegrin and Metalloproteinase 33) (8), the precise identity of the responsible genes remains unknown.

Family history of atopy was found to be a risk factor for the development of allergic diseases and asthma in population-based studies. Children of atopic and asthmatic parents are considered to be at greater risk of developing atopy and asthma compared with children of non-atopic parents (11). On the other hand, various studies have shown predisposition to atopy and asthma in the offspring of non-atopic asthmatic subjects. Maternal, rather than paternal, influence is thought to be the major factor in the development of asthma in the offspring (12).

In the current study, we aimed to evaluate whether there is any difference between the parents of atopic and non-atopic asthmatic children with respect to BHR, atopic status, serum IgE levels and symptoms of asthma and rhinitis.

Materials and methods

Subjects and study design

Parents of 51 asthmatic children (4–16 yr old) who were being followed up at the Pediatric Allergy and Immunology Division of Marmara University were invited to participate into the study. Diagnosis of childhood asthma was made according to the Global Initiative for Asthma guidelines (13). All children were evaluated for lung function tests, total serum IgE levels and skin prick testing (SPT). Among 27 children (female/male: 13/14), those who had a positive SPT to at least to one aeroallergen were considered as atopic, whereas another 24 were non-atopic (female/male: 14/10). Parents of children were interviewed by using the American Thoracic Society's (ATS) respiratory disease question-

naire, then underwent skin prick, lung function and methacholine bronchial provocation tests. A blood sample was also taken for determining serum total IgE level at the same visit. The diagnosis of parental asthma was made according to the criteria defined by the ATS (14).

The Ethics Committee of Marmara University approved the study protocol and consent was taken from the parents of children.

American Thoracic Society respiratory disease questionnaire

The ATS respiratory disease questionnaire inquiring data on asthmatic symptoms (cough, phlegm, wheezing, breathlessness, cold in the chest) as well as past medical history including symptoms of allergic rhinitis and smoking habits of parents were noted on a face to face interview mode. An independent person who translated the original English questionnaire into Turkish prepared the questionnaire. Parents were assumed to have symptoms of asthma if they had experienced two or more asthmatic symptoms according to the questionnaire. A positive response to a question 'was asthma diagnosis confirmed by a physician' substantiated as 'doctor-diagnosed asthma'.

Lung function and bronchial methacholine provocation test

Lung functions were assessed with a spirometer (Sensormedics, S3513; Sensormedics, CA, USA). All parents were free of acute respiratory tract infection for 4 wk and required no bronchodilator therapy for 3 days prior to pulmonary function and bronchial methacholine provocation tests. To induce bronchial provocation, the nebulizer was attached to a dosimeter (Mediprom FDC 88; Mediprom, Paris, France) that consisted of a breath-activated solenoid valve and a source of compressed air (pressure, 20 psi). The solenoid valve was set to remain open for 0.4 s when triggered by the subject's inspiratory effort. Methacholine challenge test was performed using ATS guidelines (15). Briefly, the baseline FEV₁ was measured, the subjects inhaled five breaths of saline solution diluents and the measurements were repeated after a 2-min interval. Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity via a mouthpiece. Starting from 0.031 mg/ml concentration, the subsequent methacholine dilutions were increased doubly until the provocative concentration of an inhaled agonist producing a 20% decrease in FEV₁ (PC₂₀) is achieved compared to the value recorded at baseline. At the end of each

experiment, the subjects were given two inhalations of albuterol to reverse any residual bronchoconstriction. A PC₂₀ value less than 16 mg/ml was considered as a positive for bronchial hyperresponsiveness.

Skin prick tests

Skin prick tests were performed with 20 common aeroallergens belonging to five groups – mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), molds (*Alternaria*, *Aspergillus* mix, *Penicillium* mix, *Candida albicans*), pollens (*Betulaceae*, *Aesculus hippo*, *Olea europea*, *Plantago*, *Artemisia*, *Parietaria*, *Secale cereale*, *Triticum vulgare*, *Zea mays*, mixture of five grasses), animal dander (feather mixture, cat hair, dog hair), and insects (cockroach) (Stallergenes, Antony, France). Histamine and saline were used as positive and negative controls, respectively. A drop of each allergen extract was placed on the volar surface of the left forearm and was penetrated with a separate lancet (Mizollen, H. Herenz GmbH, Hamburg, Germany). After 15 min, the wheal reaction was measured as the mean of the longest diameter and the diameter perpendicular to it. A wheal diameter of at least 3 mm greater than those of the negative controls was considered as positive. Subjects with one or more positive reactions were considered atopic.

Serum total IgE

A blood sample was taken from each patient and centrifuged at 2500 r.p.m. (1200 g) Serum samples were frozen at -20°C until the day of analysis. Total serum IgE was determined in subjects using the Immulite method.

Data analyses

One-way ANOVA was used to test differences between groups and subgroups. The chi-square analysis was performed to evaluate differences in frequency distributions of variables. Logistic regression analyses were performed to investigate the independent effects of different variables. PC₂₀ was transformed into logarithmic (log) values for regression analyses. Univariate regression models included were parental age, wheezing, breathlessness, smoking duration, BHR, log-transformed PC₂₀ values, phlegm production, asthmatic symptoms and doctor-diagnosed asthma. Those with a p-value ≤ 0.20 were included for the logistic regression model. A p-value ≤ 0.05 was considered to be statistically significant. Statistical analyses were performed using the Statistical Package

for the Social Sciences, SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

Results

All of the parents of atopic children (n = 27) accepted to participate while 22 mothers and 21 fathers of non-atopic children (n = 24) did so. Atopic and non-atopic children were comparable with respect to gender and age. Serum total IgE levels were significantly higher in atopic children when compared with non-atopics (p = 0.005). There was no significant difference between atopic and non-atopic children with respect to their parents either having BHR and/or atopy. Demographic characteristics and pulmonary function test results of the asthmatic children are presented in Table 1.

Maternal symptoms, lung functions and atopy

Maternal asthmatic symptoms such as cough, phlegm, wheezing, breathlessness, and cold in the chest were reported to be present in 24%, 42%, 32%, 34%, and 36% of all mothers, respectively. Twenty percent of mothers had been diagnosed as having asthma by a physician, although 24% of mothers declared symptoms of rhinitis. Among mothers, the current smoking ratio was 32%, and 8% of them were ex-smokers. The mean duration of smoking was 6.3 ± 8.7 yr. FVC%, FEV₁%, FEF_{25–75}% and PEF% mean values of pulmonary function tests were 110.3%, 96.9%, 82.9%, and 89.1%, respectively. When methacholine bronchial provocation tests were evaluated, 27.3% of the mothers had a PC₂₀ level ≤ 16 mg/ml, with a mean ± s.d. log PC₂₀ of 1.15 ± 0.3 mg/ml. Skin prick test reactivity was positive in 21.7%, whereby 6.5% were monosensitized to house dust mite (HDM), 2.2% to pollens and 4.3% to molds while 8.7% were polysensitized. The mean of total serum IgE levels was 114.6 ± 368.2 IU/ml.

Comparison of mothers of atopic and non-atopic children

On comparing atopic and non-atopic children's mothers based on data from questionnaires and results of pulmonary function and methacholine bronchial provocation tests, skin prick test reactivity and determination of total serum IgE levels showed that presence of phlegm, wheezing, doctor-diagnosed asthma and bronchial hyperresponsiveness were significantly more common, while log PC₂₀ values were significantly less in mothers of non-atopic children compared with atopic children (p = 0.002, 0.027, 0.015, 0.017, and 0.015, respectively) (Table 2).

Table 1. Demographic characteristics and pulmonary function tests of asthmatic children

	Atopic children (n = 27)	Non-atopic children (n = 24)	p-Value
Demographics			
Age (yr)	11.2 ± 2.9	9.6 ± 3.1	0.650
Gender (female/male) (n)	13/14	14/10	0.470
Lung functions			
FVC%	100.7 ± 12	100.9 ± 15.5	0.900
FEV ₁ %	92.5 ± 11.5	102 ± 8	0.011
FEF _{25–75} %	85.6 ± 22	94.7 ± 24.5	0.208
PEF%	88.3 ± 17.6	90.6 ± 26	0.728
Atopy			
Skin prick test positivity (%)	100	0	
IgE (IU/ml) [mean (range)]	525.8(594)	134(202)	0.005
Both parents having			
Bronchial hyperresponsiveness (%)	0	6	0.457
Atopy (%)	8	0	0.414

All values are mean ± s.d. unless otherwise stated. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF, forced mid-expiratory flow rate; PEF, peak expiratory flow rate; IgE, immunoglobulin E.

Paternal symptoms, lung functions and atopy

Results of the ATS questionnaire revealed that fathers reported cough, phlegm, wheezing, breathlessness, and cold in the chest in 25%, 47.9%, 29.2%, 20.8%, and 22.9% of them respectively. Doctor-diagnosed asthma was 2%. Forty-four percent of the fathers were smokers with a mean smoking duration of 13.3 ± 11.2 yr. Symptoms of rhinitis were present in 18.8%. They had a mean FVC% value of 95.9%, whereas means of FEV₁%, FEF_{25–75}% and PEF% values were 92.8%, 82.0% and 91.2%, respectively. Methacholine bronchial provocation tests revealed that 8.1% of the fathers had a PC₂₀ level ≤ 16 mg/ml, with a mean log PC₂₀ of 0.23 ± 1.03 mg/ml. Skin prick test was found to be positive in 24.4% of fathers, whereby 11% were sensitive to HDM, 2% to pollen and 11% were polysensitized. The mean total serum IgE level was 217.9 ± 345.2 IU/ml.

Comparison of fathers of atopic and non-atopic children

On comparing fathers of atopic and non-atopic children, asthmatic symptoms were the only statistical significant finding, being more in fathers of non-atopic asthmatic children (p = 0.005) (Table 3).

Multivariate analysis

The following items were included in the logistic regression model: maternal age, presence of asthmatic symptoms, doctor-diagnosed asthma,

Table 2. Comparison of mothers of asthmatic and non-asthmatic children

	Mothers of atopic children (n = 27)	Mothers of non-atopic children (n = 22)	p-Value
Maternal characteristics			
Demographics			
Age (yr) (mean ± s.d.)*	38.6 ± 4.6	36.0 ± 6.1	0.095
Current smokers (%)†	25.9	39.1	0.318
Ever smokers (%)†	26	39	0.986
Smoking duration (yr) (mean±s.d.)*	4.7 ± 7.0	8.1 ± 10.0	0.187
Lung functions			
FVC% (mean ± s.d.)*	102.1 ± 12.2	98.4 ± 15.2	0.347
FEV ₁ % (mean±s.d.)*	99.6 ± 11.5	93.9 ± 14.9	0.184
FEF _{25–75} % (mean ± s.d.)*	85.6 ± 18.5	80.0 ± 20.5	0.444
PEF% (mean±s.d.)*	91.9 ± 14.1	86.2 ± 15.3	0.306
BHR to methacholine (%)†	8.0	36.4	0.017
log PC ₂₀ mg/ml (mean ± s.d.)*	1.3 ± 0.3	1.0 ± 0.3	0.015
Questionnaire			
History of cough (%)†	22.2	26.1	0.756
History of phlegm (%)†	22.2	65.2	0.002
History of wheezing (%)†	18.5	47.8	0.027
History of breathlessness (%)†	25.9	43.5	0.199
History of cold in the chest (%)†	25.9	47.8	0.112
Asthmatic symptom (%)†	37.0	78.3	0.003
Symptoms of rhinitis (%)†	22.2	26.1	0.756
Doctor-diagnosed asthma (%)†	7.4	38	0.015
Atopy			
Skin prick test positivity (%)†	23.1	20	0.802
IgE (IU/ml) [mean (range)]	63.0 (7.2–282)	198.4 (6.6–2415)	0.814

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF, forced mid-expiratory flow rate; PEF, peak expiratory flow rate; BHR, bronchial hyperreactivity; IgE, immunoglobulin E.

*One-way analysis of variance.

†Chi-square.

smoking duration, and log-transformed PC₂₀ as well as paternal age, presence of asthmatic symptoms and log-transformed PC₂₀. Logistic regression analysis revealed that maternal PC₂₀ is predictive for the risk of displaying non-allergic asthma in children [OR: 1.86; 95% confidence interval (CI) 0.99–3.5].

Discussion

This study demonstrated that the presence of maternal BHR, maternal and paternal asthmatic symptoms were statistically more common in non-atopic children's parents compared with atopic asthmatic children's parents. Moreover, parental PC₂₀ values of non-atopic asthmatic children were significantly less than their atopic peers. The other finding that extends our knowledge regarding connections among mothers and children's asthmatic illness was that lower maternal PC₂₀ increases the risk of a child developing non-atopic asthma.

Sensitization to common environmental allergens is a major cause of asthma and rhinitis in

Table 3. Comparing fathers of atopic and non-atopic asthmatic children

Paternal characteristics	Fathers of atopic children (n = 27)	Fathers of non-atopic children (n = 21)	p-Value
Demographics			
Age (yr) (mean±s.d.)*	43.0 ± 5.3	40.3 ± 6.4	0.126
Current smokers (%)†	44.4	38.1	0.497
Ever smokers (%)†	44.4	38.1	0.412
Smoking duration (yr) (mean ± s.d.)*	12.2 ± 10.3	14.5 ± 12.1	0.523
Lung Functions			
FVC% (mean±s.d.)*	97.1 ± 14.7	94.7 ± 9.6	0.538
FEV ₁ % (mean ± s.d.)*	95.6 ± 17.3	90.1 ± 13.8	0.259
FEF _{25–75} % (mean±s.d.)*	88.7 ± 28.3	75.4 ± 27.4	0.130
PEF% (mean ± s.d.)*	91.6 ± 17.4	90.8 ± 19.4	0.881
BHR to methacholine (%)†	5.3	11.1	0.528
log PC ₂₀ mg/ml (mean±s.d.)*	1.3 ± 0.2	1.1 ± 0.5	0.085
Questionnaire			
History of cough (%)†	18.5	33.3	0.249
History of phlegm (%)†	40.7	57.1	0.269
History of wheezing (%)†	18.5	42.9	0.068
History of breathlessness (%)†	11.1	33.3	0.064
History of cold in the chest (%)†	14.8	33.3	0.136
Asthmatic symptom (%)†	22.2	61.9	0.005
Symptoms of rhinitis (%)†	22.2	14.3	0.495
Doctor-diagnosed asthma (%)†	0	5	0.463
Atopy			
Skin prick test positivity (%)†	24	25	0.435
IgE (IU/ml) [mean (range)]	222.8 (10.8–2000)	210.9 (12.5–780)	0.912

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF, forced mid-expiratory flow rate; PEF, peak expiratory flow rate; BHR, bronchial hyperreactivity; IgE, immunoglobulin E.

*One-way analysis of variance.

†Chi-square.

children (16, 17). On the contrary, some epidemiological studies demonstrated that non-atopic asthmatic children are relatively equal to atopic asthmatic children, thus suggesting the overestimation of atopy as a cause of asthma (18–20). However, the majority of studies comparing parental asthma at risk and their offspring were concentrating mostly on IgE levels and atopy status of the family rather than on BHR (21, 22). Our aim was to study the differing parental features and risk factors associated with the development of atopic and non-atopic asthma in children. We used objective methods directly obtained from parents rather than collecting reported information, which we believe improved the reliability of the study.

In the current study, mothers and fathers were almost equally sensitized to aeroallergens, suggesting that there was no statistical difference or correlation between the sensitized parents and atopic or non-atopic asthmatic children. The

non-atopic children had a mean age of 9.5 yr at the time of the study. We believe that this time can be considered as an appropriate time for evaluation of atopic status in this group. These findings do not indicate that parental atopy may contribute to an equal risk of their child being atopic. Contrary to these results, several studies reported a proportional increase in the development of allergy in children with an increase in the number of sensitized parents (21, 23).

Although clinical presentation, age at onset, and family history of allergy differ between atopic and non-atopic asthmatics, there is some evidence that atopic and non-atopic asthma have similar pathologic mechanisms (24–26). Burrows et al. showed a strong relationship between high level of total serum IgE and asthma which persisted in the subgroup of non-allergic asthmatic children – a feature also found in our study (24). Several studies compared the molecular immunopathology of atopic and non-atopic asthma by demonstrating high levels of allergen-induced Th2 cytokines in the peripheral blood and infiltration of the bronchial mucosa with cells expressing Th2 type cytokines in individuals with either atopic or non-atopic asthma, providing further evidence for similarities in the immunopathogenesis of these clinically distinct forms of asthma (27, 28). Our results seem to be in accordance with previous studies in that the prevalence of SPT positivity and serum total IgE level were similar in parents of both atopic and non-atopic asthmatic children.

Gray et al. found that the risk of a non-atopic child having BHR was four times greater if either parent had BHR, suggesting that the risk of a non-atopic child having BHR is independent of parental atopy (29). Epidemiologic studies revealed that there is a genetic component in the regulation of BHR with an autosomal dominant inheritance pattern, suggesting that atopy and BHR are under separate genetic control (30). We have found that mothers of non-atopic asthmatic children were more hyper-responsive to methacholine challenge compared with their counterparts, otherwise there was no difference in terms of atopy.

Supporting this concept, Kim et al. found that the prevalence of BHR was higher among parents of asthmatic children. In addition, positive SPT response was higher among the parents of atopic asthmatic, non-atopic asthmatic and atopic control children than the parents of non-atopic control children. Total serum IgE levels were also higher among parents of atopic and non-atopic asthmatic children than those of non-atopic control children (31). In

another study, Clifford et al. demonstrated an increased incidence of BHR to methacholine in children of asthmatic parents than in the general population. Their findings indicate that children who have one parent with asthma have roughly doubled the chance of developing clinical features of asthma compared with the general population (32). Our results seem to be in accordance with previous studies in that the prevalence of SPT positivity and serum total IgE level were similar in parents of both atopic and non-atopic asthmatic children.

Mothers of non-atopic asthmatic children tend to smoke more often with a longer duration and indeed contribute to passive exposure to cigarette smoke which may predispose their children to BHR and asthmatic symptoms by spending more time together and both being similarly exposed. Other factors which were not determined in this study such as recurrent chest infections, low socio-economic status and non-allergenic factors such as air conditioning, pollutants and strong smells might have contributed to the development of non-allergic asthma in children. van Bever et al. reviewed the prognostic factors in childhood asthma, concluding that non-atopic asthma is frequently triggered by viral infections, and cigarette exposure worsens the prognosis (33). Combinations of genetic and environmental factors are believed to underlie the development of BHR and asthma. We found that low maternal PC₂₀ is a strong predictive factor of displaying non-allergic asthma in children. Several studies also demonstrated an increased incidence of BHR to methacholine in children of asthmatic parents than in the general population (32).

In conclusion, the results of this study demonstrated that the development of non-atopic asthma in children is related to low maternal PC₂₀, in other terms the severity of maternal BHR. Consequently, the maternal component of BHR suggests that the influence of genetic factors may be transmitted from one generation to the next.

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