

Determinants of atopic sensitization in Turkish school children: Effects of pre- and post-natal events and maternal atopy

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Emergence of new environmental risk factors, and/or loss of protective factors of a traditional lifestyle may explain the increase, or variations in prevalence of allergic diseases. The aim of this study was to delineate the prevalence and spectrum of, and to reveal the causal and/or protective factors for atopic sensitization among a heterogeneous cohort of Turkish children, for the first time in our country. The study design adhered to International Study of Asthma and Allergies in Childhood (ISAAC) phase II protocol. A self-administered parental questionnaire about demographic characteristics and detailed risk factors, and skin-prick test with 13 allergens were employed in a clustered random sample of 8–11-yr-old Turkish school children. Atopy was defined as the presence of at least one positive skin reaction to any allergen tested. The association between a total of 78 risk factors and different aspects of atopy were analyzed in 1144 children with multivariate logistic regression analysis. The overall prevalence of atopy was 20.6%. Most common sensitizations were to grass pollens, *Dermatophagoides pteronyssinus* and *Blatella germanica*. Day care attendance, high paternal education level, male gender and maternal asthma were significant risk factors for atopy. Breastfeeding more than 6 months (compared with 0–6 months), maternal smoking during pregnancy and a birth weight under 2500 g were inversely related to (or protective factors for) atopic sensitization. Maternal atopic disease had significant effects on risk factors pattern. In children with a maternal atopy history a low birth weight, day care attendance and maternal smoking during the first year of life independently increased the risk of atopic sensitization. Gender, breastfeeding and paternal education did not show any association with atopy in this group of children. A history of measles and low gestational age were significant protective factors for mite sensitization. This study showed that children of atopic mothers showed a different profile of risk factors associated with atopic sensitization, when compared with other children. Prenatal and early childhood events had important associations with atopic sensitization.

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In recent decades, there has been a great and worldwide scientific interest in the epidemiologic characteristics of allergic diseases. One major finding of the studies is that, there are substantial variations (up to 60-fold) in the prevalence rates of these diseases, not only between countries but

also within the same country, as shown by the unique International Study of Asthma and Allergies in Childhood (ISAAC) phase I (1). The other important observation is the rising trend in the prevalence rates of allergic phenotypes in many industrialized countries, based

mostly on questionnaire data, but also more objective measurements such as skin-prick testing (SPT) or specific immunoglobulin E (IgE) measurements (2).

Genetic factors alone are insufficient to explain the substantial differences in prevalences between populations of similar ethnic background, or the increase in prevalence rates of allergic diseases. Therefore, the expression of allergic diseases should be strongly influenced by environmental factors. During the last decade, two general hypotheses have been proposed to explain the increase in atopic diseases, (i) new risk factors associated with environmental exposure, nutrition, or lifestyle have emerged; and/or (ii) protective factors of a more traditional lifestyle have been lost with a resultant increase in the susceptibility to atopic diseases (3). A long list of suggested environmental and/or lifestyle factors associated with allergies include indoor and outdoor air pollution (4, 5), passive smoking (6), nutrition (7, 8), allergen exposure (9), socio-economic status (SES) (10), infections/family size (11, 12), and gestational/perinatal factors (13, 14). However, most of these factors have not showed a clear-cut and consistent association with atopic phenotypes in the epidemiologic studies, so far.

The atopic phenotypes in childhood include atopic dermatitis, atopic asthma, allergic rhinoconjunctivitis, skin test reactivity to specific allergens, and elevated allergen-specific IgEs and total IgE in serum (3). As different phenotypes may be modulated by different genetic, or environmental factors, it is more rational to focus on a single phenotype in epidemiological studies. The aim of this study, which was conducted as part of an ISAAC phase II study in Ankara, was to delineate the prevalence and spectrum of atopic sensitization (skin test reactivity to allergens) and to reveal the causal and/or protective factors for atopic sensitization among this heterogeneous cohort of Turkish children, for the first time among the child population in our country.

Methods

Study area

The study was conducted in Ankara, the capital of Turkey, between October 1999 and April 2000. Ankara is located in central Anatolia and is the second most crowded metropolitan city in Turkey with approximately 4,007,860 inhabitants according to the 2000 national census. This metropolitan city receives a substantial number of immigration from mostly rural portions of

Anatolia every year. There are many ghettos within the central urban districts of the city harboring thousands of people of the low SES. The climate is dry, with a hot summer and snowy winter. In the last few years, air pollution has substantially increased, especially in some underprivileged districts of the city, owing to the usage of bad quality charcoal for indoor heating. Additionally, heavy car traffic with motor vehicle exhaust emissions contribute much to the air pollution during winter and summer. According to the formal data provided by the Refik Saydam Hygiene Center in Ankara, mean annual (1999) concentrations of sulfur dioxide (SO₂) and particulate matter (PM) were 57.8 and 76.4 µg/m³ for Ankara as a whole, while those annual figures reached to 77.7 and 98.7 µg/m³, respectively, in some heavily polluted districts of the city. As individual exposures of the subjects to pollution could not be determined, districts of the city were categorized into three groups according to the degree of pollution; heavily, moderately, and mildly polluted. District of habitation was used as a rough measure of air pollution.

Study population and design

The study design adhered to the ISAAC phase II protocol (15). The sampling method was two stepped. In the first step of the study (prevalence study), in order to be representative, the eight administrative districts of Ankara were accepted as the strata and weighted number of schools were selected in a stratified random manner from a complete list of primary schools in Ankara. All school children attending the fourth grade in 22 schools (n = 3426) were selected, primary schools being the sampling unit. A self-administered parental questionnaire and SPT were obtained in this population.

In the second step (case-control study), the children were stratified according to the presence or absence of wheezing in the last 12 months based on questionnaire responses. One hundred and seventy-five children from 'current wheezers' stratum and 175 children from 'current non-wheezers' stratum were randomly selected by block randomization method. These 350 children were recruited to blood sampling for serum-specific and total IgE measurements, spirometric measurements and bronchial challenge tests (case-control study).

Questionnaires

The ISAAC phase II questionnaire modules including questions about demographic characteristics

(age, sex, nationality, maternal and paternal education, income per month) and risk factors were employed (15). The questionnaires were translated into Turkish by one translator familiar with asthma and allergy terminology, and then back translated. The risk factor questionnaire included 31 questions about gestational characteristics, feeding practices, family history, siblings, childhood infections, household conditions (heating, dampness, bedroom, etc.), animal contacts, tobacco smoke exposure (pre- and post-natal), for both current time and first year of life. Parents of 3056 (89.2%) children returned the questionnaire. Among those returned, 3041 (88.7%) questionnaires were taken up for evaluation.

Skin-prick tests and IgE measurements

Skin-prick tests were performed with six 'core' allergen extracts recommended in ISAAC phase II module (15); *Dermatophagoides pteronyssinus*, *D. farinae*, *Alternaria alternata*, cat epithelium, mixed grasses (*Phleum pratense*, *Poa pratensis*, *Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Avena eliator*), and mixed trees [mixed tree standard (St): *Betula verrucosa*, *Alnus glutinosa*, *Coryllus avellana*]. Seven additional allergens of local relevance were also added; *Parietaria officinalis*, *Cladosporium herbarum*, *Olea europea*, mixed feathers, mixed weeds (*Artemisia vulgaris*, *Chenopodium*, *Plantago*, *Salsola kali*), mixed local trees [mixed tree (L): *Quercus alba*, *Ulmus americana*, *Platanus*, *Salix*, *Populus*], and *Blatella germanica*. Histamine (10 mg/ml) and diluent were used as positive and negative controls, respectively. The standardized core allergen extracts and controls were provided by ALK-Abello (Horsholm, Denmark), and standardized local allergens were provided by ALK-Abello, Allergopharma (Reinbek, Germany), and Center Pharmaceuticals, Inc. (Port Washington, NY, USA).

Skin-prick testing was performed similar to that recommended in ISAAC phase II module (15). All antihistaminic drugs were withheld 10 days prior to skin testing. One drop of each SPT solution was placed on the volar surfaces on the right and left forearms and were penetrated with a separate ALK lancet. Reactions to skin test solutions were measured after 15 min. The contours of each wheal were outlined with a fine filter tip pen and then transferred to the record sheet by means of a translucent tape. The size of each wheal was documented as the mean of the longest diameter and the diameter perpendicular to it. A positive skin reaction was defined as a

wheal size ≥ 3 mm, after subtraction of the negative control. Atopy was defined as the presence of at least one positive skin reaction to any allergen tested. Written consent for the tests were obtained from guardians in 2858 (92.5%) children. Of the eligible children skin prick tests were performed and taken into evaluation in 2774 (97.1%). Children with a histamine wheal size ≤ 3 mm were not taken into statistical evaluation ($n = 5$). Only 15 students showed complications during the test procedures, which were mostly vasovagal reactions. No anaphylactic reaction was observed.

Serum total IgE levels were measured in all 350 children forming the case-control group by fluoroenzymeimmunoassay method (UniCAP, Pharmacia and Upjohn, Uppsala, Sweden). As these children were selected in a stratified random manner according to wheezing status, weighted prevalence values were calculated.

Levels of serum specific IgE for *D. pteronyssinus*, *D. farinae*, cat, cockroach, grass mix, tree mix, weed mix, olea and *Cladosporium* were measured by fluoroenzymeimmunoassay method (UniCAP, Pharmacia and Upjohn) in 80 children which were selected at random from those with and without a positive SPT. The SPT results were compared with the serum specific IgE results. The correlation between SPT and specific IgE was best for mite and grass allergens (81.2%, 93.8% and 89.6%, respectively), while the lowest values were for weed mix (59.4%) and cat (62.6%) allergens. The specificity of SPT in relation to specific IgE measurements was over 90% for all antigens.

Field work and quality control

The parental questionnaires were distributed through the schools. All the tests were conducted in the schools during the study period of 6 months by three teams of field workers; each of the first and second teams included one skin tester and one skin test reader. Third team included two workers performing spirometry and bronchoprovocation tests. All the field workers were trained by one of the principal investigators (S.K.) and also supervised by the same investigator throughout the field work. Histamine training studies were performed before field work. Reproducibility of SPTs were monitored by the coefficient of variation of histamine reaction sizes, which was expected to be under 20% for each field worker. The mean coefficient of variation values for the whole study period were 15.8% for the first, and 13.4% for the second field worker who performed skin tests.

Statistical analysis

The SPSS software package version 10.0 was used for all calculations. Comparisons were performed using Student's *t*-test for continuous variables and the chi-squared test for proportions. Test for trends was performed using the Mantel–Haenszel test statistic.

The association between a total of 78 risk factors and different aspects of atopic sensitization were analyzed with chi-squared test, and univariate and multivariate logistic regression analysis in 1144 children having complete risk factor data. The sociodemographic characteristics including age, sex, number of siblings and family income distribution of this cohort were very similar to the original study population. Only paternal education distribution showed a 10% higher rate of children with fathers from high school or university. A positive reaction to any one allergen (denoted 'atopy' or 'atopic sensitization'), any pollen ('pollen sensitization'), and any mite ('mite sensitization') were defined as dependent variables. Major independent variables were: district of habitation (as a measure of environmental pollution, three groups); maternal and paternal atopic disease histories (asthma, allergic rhinitis and/or eczema, separately for each); maternal and paternal education levels (four categories: primary, secondary, high school, university); family income (two categories: <350 and ≥350 \$/month); total number of siblings, number of older siblings and younger siblings (three categories: 0, 1, 2+ siblings for each); birth weight (two categories: <2500 g, ≥2500 g); month of birth (two categories: pollen season, winter months); gestational age (three categories: 3 wk earlier than the calculated date, within 3 wk of the calculated date, 3 wk later than the calculated date); duration of breastfeeding (three categories: none, 1–6 months, >6 months, or two categories: 0–6 months, >6 months); attendance at day care; current passive smoking; maternal smoking during pregnancy; maternal smoking during the first year of life; measles history; current and first 12 month exposures to heating systems, bedding/pillows, mold/dampness at home, pets, cockroaches, poultry and livestock. Initially, the crude association between risk factors and dependent variables was analyzed. Variables that were associated with the outcomes in the univariate analysis at a *p*-value of <0.25 were examined in the multivariate logistic regression models. A backward reduction modeling strategy was used. Non-significant variables were dropped if their removal did not result in a

change of more than 10% of any other variables. The size of the effect of each of the risk factors was measured by using the odds ratios (ORs) and 95% confidence intervals (CIs).

Relation between risk factors and atopic sensitization was further analyzed according to maternal history of atopy. Each of three atopic diseases and their combinations were tested. Maternal atopy was defined with a history of asthma and/or eczema based on questionnaire data, as these indices showed the best interaction with risk factors.

Ethical considerations

All study methods had been approved by the Ethics Committee of the Turkish Ministry of Health and the Ethics Committee of Hacettepe University, Faculty of Medicine. Informed written consent was obtained from the parents for all tests at one time at the beginning of prevalence study.

Results

The study group included children aged 8–11 yr, 92.7% of whom were 9–10 yr of age. Gender distribution was 50.4% males and 49.6% females. University education was reported in 11.8% of mothers and 20.1% of fathers. More than one-third (37.1%) of families had three or more children. Pet ownership was present in only 2.2% of homes, while presence of cockroaches was reported in 7.9%. Exposure to passive smoking was present in 61.1% of children. Family income per month was reported to be under 350\$ in 75.8% of families.

The overall prevalence of atopy (at least one positive SPT reaction) was 20.6% (Table 1). The monosensitization (only one positive SPT) and polysensitization (more than one reaction) rates were 11.2% and 9.4%, respectively. The most common sensitizations were to grass pollens, *D. pteronyssinus*, cockroach (*Blattella germanica*) and weed pollens, respectively. The prevalence of sensitization to mixed tree pollens of local relevance [mixed tree (L)] was five times higher than standard mixed tree pollens [mixed tree (St)] (4.5% and 0.9%, respectively). The prevalences of *Cladosporium*, *Alternaria*, *Parietaria* and mixed feather sensitizations were under 1%. The weighted geometric mean value of serum total IgE for the study population was 43.8 kU/l. The mean value in SPT positive subjects was more than two times higher than the value in SPT negatives.

Table 1. Prevalence rates of atopic sensitization to inhalant allergens and levels of total IgE among 8–11-yr-old Turkish school children

	n (%)
Positive skin prick test (N = 2774)	
Mixed grass pollen	245 (8.8)
<i>D. pteronyssinus</i>	160 (5.8)
Cockroach	161 (5.8)
Mixed weed pollen	136 (4.9)
Mixed tree pollen (L)	124 (4.5)
<i>D. farinae</i>	79 (2.8)
Cat epithelium	70 (2.5)
<i>Olea</i>	61 (2.2)
Any pollen	351 (12.7)
Any mite	177 (6.4)
Any mold	22 (0.8)
Any allergen	57 (20.6)
Total serum IgE [N = 350; geometric mean (kU/l)]	
Whole population*	43.87
SPT (+)	84.2
SPT (–)	38.7

Mixed tree pollen (L), local tree pollens; N, number of children included in the analyses; n, number of children with a positive result; SPT, skin-prick test.

*Weighted value.

Risk factors for atopic sensitization in multivariate logistic regression analyses

Significant ($p < 0.25$) factors from the univariate analysis were taken into multivariate logistic regression analysis in order to assess the independent effects of risk factors on atopy. As shown in Table 2 maternal asthma, male gender, day care attendance and high paternal education level (high school and university) were significant risk factors for atopy. Breastfeeding more than

6 months (compared with 0–6 months), maternal smoking during pregnancy and a birth weight under 2500 g were inversely related to (or protective factors for) atopy. Total number of siblings, number of older or younger siblings, and district of habitation did not have a significant association with atopy in multivariate models.

Association of maternal atopic disease and risk factors for atopic sensitization in the offspring

In order to see the effect of maternal atopic disease on risk factors pattern, children with a maternal history were analyzed separately with multivariate logistic models. In children without a maternal atopy history, male gender and higher paternal education levels (high school and university) were independently and positively associated with atopy. A low birth weight (LBW), maternal smoking during pregnancy, breastfeeding > 6 months (compared with 0–6 months), wool pillow usage and bedroom sharing during the first year of life (of borderline significance) were inversely related to atopic sensitization in this group of children. In children with maternal atopic disease a LBW, day care attendance and maternal smoking during the first year of life increased the risk of atopic sensitization (Table 3). This genetic tendency group differed from the first group in that, gender, breastfeeding, parental education and wool pillows did not show any association with atopy. Interestingly, the independent effects of birth weight (and also maternal smoking) on atopic sensitization were in the

Table 2. Associations of risk factors with atopy (at least one positive skin-prick test) in separate analyses and mutually adjusted† analysis in Turkish children

Variable	Category	Number	Crude OR (95% CI)	Adjusted OR (95% CI)
Gender	Female*	560	1.0	1.0
	Male	584	1.62 (1.23–2.14)	1.54 (1.15–2.05)
Maternal asthma	No*	1116	1.0	1.0
	Yes	28	2.21 (1.02–4.77)	2.45 (1.07–5.61)
Birth weight (g)	<2500	112	0.53 (0.30–0.91)	0.57 (0.32–0.99)
	>2500*	1032	1.0	1.0
Duration of breastfeeding (months)	0–6*	393	1.0	1.0
	>6	751	0.62 (0.47–0.83)	0.65 (0.47–0.88)
Attendance at day care	No*	839	1.0	1.0
	Yes	305	2.01 (1.49–2.69)	1.45 (1.04–2.02)
Paternal education	Primary*	246	1.0	1.0
	Secondary	179	1.51 (0.88–2.56)	1.41 (0.82–2.42)
	High school	392	2.02 (1.30–3.13)	1.76 (1.12–2.76)
	University	327	3.29 (2.13–5.106)	2.45 (1.53–3.91)
Maternal smoking (pregnancy)	No*	1022	1.0	1.0
	Yes	122	0.63 (0.38–1.04)	0.54 (0.32–0.91)

*Reference categories.

†Adjusted for age, district, maternal rhinitis, paternal atopy, family income, number of siblings, maternal education, month of birth, bedroom sharing, animal exposure, measles history, indoor environmental factors and factors listed in the table.

OR, odds ratio; CI, confidence interval.

Table 3. Risk factors for atopic sensitization among school children with maternal atopy

Variable	Category	Number (N = 107)	Crude OR (95% CI)	Adjusted OR (95% CI)†
Birth weight (g)	<2500	8	2.94 (0.68–14.2)	7.87 (1.1–53.9)
	≥2500*	99	1.0	1.0
Attendance at day care	No*	75	1.0	1.0
	Yes	32	4.77 (1.91–11.9)	6.80 (2.31–19.9)
Maternal smoking (f)	No*	82	1.0	1.0
	Yes	25	3.57 (1.37–9.09)	4.75 (1.54–14.66)

CI, confidence interval; (f), first year of life.

*Reference categories.

†Adjusted for age, district, gender, duration of breastfeeding, maternal and paternal education, family income, maternal smoking in pregnancy, bedroom share, animal exposure, month of birth, number of siblings and indoor environmental factors.

inverse directions for children with and without an atopic mother.

Risk factors for mite and pollen sensitizations

In multivariate regression analysis male sex, maternal asthma and a paternal education of university were independent risk factors for mite sensitization. Measles history (OR 0.56, 95% CI 0.34–0.92) and a low gestational age (>3 wk earlier than the estimated date of birth) were independent protective factors. In addition to family atopy and male gender, lack of, or short (<6 months) duration of breastfeeding, increasing levels of paternal education, and mold spots at home, significantly increased the risk of pollen sensitization.

Discussion

The present ISAAC phase II study revealed that a number of factors independently increased or decreased the risk of atopic sensitization in 8–11-yr-old Turkish children. One of the major findings of this study was the relationship between birth weight, atopic sensitization in the offspring and maternal atopy. A birth weight <2500 g was inversely associated with atopic sensitization and a delivery date more than 3 wk before the estimated date decreased the risk of mite sensitization at 9–11 yr of age. Although a LBW is not synonymous with low gestational age, it is a more reliable index for early delivery than an estimated date of birth in a questionnaire survey of our population. Moreover, early delivery showed similar trends with LBW in the analyses, but not reaching statistical significance (data not shown). A body of evidence showed

that there is an inverse relationship between gestational age (13, 16), or birth weight (14, 16) and SPT reactivity (13, 14), or atopic disease (16). The latter study included 148,000 conscripts for military service and found that a birth weight under 2500 g, or a gestational age under the 36th week was associated with a lower risk of atopy (16). In an earlier risk factor study gestational age below 38 wk was independently and positively associated with SPT positivity (17). However, none of these studies examined the effects of maternal atopic disease on these relationships. In the present study maternal atopic history had a significant inverse interaction with the birth weight–atopy relation. While LBW significantly decreased the risk of atopy in children without a maternal history, the risk increased in LBW babies whose mothers were atopic. When the children were analyzed according to parental atopy, no significant effect was encountered (data not shown).

The underlying reasons for the relationship between LBW and atopic sensitization are not clear. Moreover, different mechanisms may operate in children of atopic mothers. Prematurity probably reflects the changes in duration and timing of environmental exposures which play a role in atopic sensitization. It has been shown that pregnancy is accompanied by an immune deviation toward the Th2-cytokine pattern (18). A pre-term delivery may be the result of a failure of maternal immune system to sustain a Th2-dominant immune response which is suggested to be necessary for maintenance of pregnancy. It can be hypothesized that a maternal Th1/Th2 balance biased towards Th1 response during pregnancy will be associated with an increased frequency of pre-term labor and also a Th1 polarized immunity that lowers the risk of atopy in the fetus. However, if the mother is atopic LBW may also be expected as a complication of a severe disease (19). In infants of atopic mothers, high levels of cord blood IgG antibodies to inhalant allergens were inversely associated with development of atopic disease (20). A pre-term delivery before the transplacental IgG antibodies peak in the fetus may result in waning of protective effects of these antibodies (21). These may partly explain why a LBW was related to an increased risk of atopy in children of atopic mothers in our study. There might be other operative factors for low-risk children. There is strong evidence that LBW infants show a significantly increased susceptibility to infections, especially diarrhea and respiratory infections, probably as a result of immune dysfunction (22). One of the contributing factors is impairment of

materno-fetal transfer of antibodies to infectious agents in LBW babies (23). Reduction in antibody transfer may further predispose these already vulnerable neonates to bacterial and viral infections. In the absence of genetic propensity, increased frequency of infections in early life may polarize the immune system towards Th1 type responses, thus inhibiting atopy (24).

We showed that a breastfeeding duration longer than 6 months significantly decreased the risk of atopic sensitization and also, there was a linear inverse relation between the duration of breastfeeding and risk of pollen sensitization. The role of breastfeeding in the prevention of allergic diseases is still disputed. Some studies showed that prolonged (6 months to 1 yr) breastfeeding was associated with a lower risk of eczema (25) and mite sensitization (26). On the other hand, MAS-90 study showed that each additional month of breastfeeding increased the risk of atopic eczema in high risk children (27). However, none of these studies explored the interaction in relation to maternal atopic status, instead of family history. In the present study, we showed that protective effects of prolonged breastfeeding was evident only in children whose mothers did not have an atopic history. It was shown that the association of breastfeeding to child atopy showed paradoxical results according to maternal IgE levels (8). Among children of mothers with lower IgE levels, breastfeeding was associated with diminished levels of total IgE in the child at 6 and 11 yr of age; however, among children of mothers with high IgE levels, breastfeeding of 4 months or longer was associated with higher IgE levels in the child. There is evidence for difference in the composition of milk from atopic and non-atopic mothers (28). Another explanation is that a strong genetic predisposition conferred by the mother and a shared common environment by the mother and her baby can result in the removal of the beneficial effects of breastfeeding on atopic sensitization. Nevertheless, it is clear that prolonged breastfeeding by non-atopic mothers is associated with a diminished risk of atopy in the offspring, as shown by the present, and other studies.

We found that maternal smoking, as a possible risk factor for atopic sensitization, acted in inverse directions in the presence or absence of maternal atopy. There was a significant inverse relationship between smoking in pregnancy and atopy in the offspring of atopic mothers. The MAS study found a significantly higher risk of sensitization to food allergens in infancy among children who were exposed to pre- and post-natal smoking (29). One British cohort study (10) and

a smaller study (30) found an inverse relation between SPT positivity in children and pre- (10) and post-natal (30) maternal smoking. In a comprehensive meta-analysis of 36 studies, Cook and Strachan (6) concluded that the balance of evidence did not support a positive association between allergic sensitization and pre- and post-natal parental smoking. It is suggested not to consider smoking habits and atopic disease in parents as independent variables in atopy risk factor studies of children because there was a significant association between current exposure to tobacco smoke and a low risk for atopic disorders in both smokers themselves and their offspring (31). We found an increased risk of atopic sensitization among children of atopic mothers who smoked during the first year of life (and also in pregnancy, but not statistically significant). Two other studies conducted in high risk (positive family history) children showed a similar positive association between maternal smoking and atopic disease in the offspring (32, 33). These point out that pre- and post-natal exposure to tobacco smoke may make an adjuvant effect for atopic sensitization in the context of a genetic risk, more specifically a maternal atopic disease.

Higher paternal education level was the strongest risk factor showing a linear positive relationship with atopic sensitization (both general and allergen-specific) rate in this study. In all models, paternal education was more determinative than maternal education and family income, as a marker of SES. However, in separate analysis according to maternal atopy history, paternal education was not a significant independent risk factor in children of atopic mothers. This shows that environmental and lifestyle factors which are represented by paternal education level play a determinative role in child atopy, mostly in the absence of a genetic risk. There are substantial data that support the positive association between the prevalence of allergic disorders or SPT positivity and higher SES (10, 12, 34), cultural adaptation in Turkish immigrants (35), or parental education status (36). It was shown that the increase in the risk of atopic sensitization with parental SES was independent of and more powerful than family size and birth order (10, 34).

In separate analyses of allergen specific sensitizations, a measles history was found to be a significant factor that decreased the risk of mite sensitization. Shaheen et al. (26) found that wild measles infection was associated with a large reduction in the risk of SPT positivity to mites. Subsequently, one study found a protective effect

of both measles infection and vaccination in children with multiple older siblings (37), while others did not find any (11) or found a positive association (38) between measles and atopy. Measles may be an indicator of overall exposure to infectious diseases that may reduce the risk of atopy.

In the present study, day care attendance significantly increased the risk of atopic sensitization, especially among children whose mothers had atopic disease. Although day care attendance is accepted as an expression of exposure to other children outside the house and resultant cross infections, this may not be the case for Turkish children, most of whom already had exposed to siblings and child partners before day cares. In our study population only 26% of children, mostly from higher parental education level, had attended at day cares and 81% these children had begun after 2 yr of age (data not shown). Kramer et al. (39) showed that among children from small families (no sibling) the risk of SPT positivity among children who started to nursery after 1 yr of age was three times higher when compared with children who first attended at 6–11 months of age, while there was no effect on atopy among children from larger families. Older age of entry to day care and larger family size of our children may explain the lack of a protective effect of day care attendance on atopy. On the contrary, most of the day care centers may increase the risk as a result of heavy exposure to a burden of indoor and outdoor allergens with their characteristic features of small rooms with wall to wall fitted carpets, basement flats and dampness and plant gardens to play in. A recent study in Brazil revealed that day care centers were important sources of exposure to especially house dust mites (40). As shown in the present study, attendance to day care after the critical period of infancy and especially in the presence of a genetic basis, may increase the risk of atopic sensitization in the child, probably because of a high level of allergen exposure. Most of the studies in the literature found no significant effect, or more rarely, an increased risk of atopy with day care attendance (10, 12, 41, 42).

The 'hygiene hypothesis' which was proposed a decade ago, and revisited recently by Strachan (12) postulates that the protection against the development of allergic disease provided by the presence of a large family size and/or older siblings was mediated by infection acquired from contact with siblings. Over the last decade, it appeared that there is a rather consistent and statistically independent protective effect of larger family size, domestic crowding and low SES

on hay fever and atopic sensitization (3, 10, 12–14, 34, 36, 43). However, they may also be regarded as indirect measures of some other factors which increase in the face of a low SES and/or a large family size. According to our study results, birth weight, home care (instead of day care) in early childhood, longer duration of breastfeeding, bedroom sharing, measles infection (and other infections), wool pillow usage, and other undetermined factors may play such a role.

In conclusion, this study showed that intra-uterine and early childhood factors are important in the development of atopic sensitization in school age. Risk factors pattern of atopic sensitization changes significantly if the mother has atopic disease. Low birth weight, female gender, prolonged breastfeeding and prenatal maternal smoking showed an inverse association with atopic sensitization only in children of non-atopic mothers. In the face of mother atopy, these effects disappeared or acted in inverse directions. In children without a genetic risk, familial SES, especially increasing paternal education level was the strongest risk factor for atopic sensitization. The incoherence of some earlier studies with regard to environmental risk factors related to childhood atopy may be resolved if maternal atopic status is taken into account and analyzed separately.

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