Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide

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Background: Previous studies have demonstrated that children raised on farms are protected from asthma and allergies. It is unknown whether the farming effect is solely mediated by atopy or also affects nonatopic wheeze phenotypes.

Objective: We sought to study the farm effect on wheeze phenotypes and objective markers, such as lung function and exhaled nitric oxide, and their interrelation with atopy in children.

Methods: The GABRIEL Advanced Studies are cross-sectional, multiphase, population-based surveys of the farm effect on asthma and allergic disease in children aged 6 to 12 years. Detailed data on wheeze, farming exposure, and IgE levels were collected from a random sample of 8023 children stratified for farm exposure. Of those, another random subsample of 858 children was invited for spirometry, including bronchodilator tests and exhaled nitric oxide measurements.

Results: We found effects of exposure to farming environments on the prevalence and degree of atopy, on the prevalence of transient wheeze (adjusted odds ratio, 0.78; 95% CI, 0.64-0.96), and on the prevalence of current wheeze among nonatopic subjects (adjusted odds ratio, 0.45; 95% CI, 0.32-0.63). There was no farm effect on lung function and exhaled nitric oxide levels in the general study population.

Conclusions: Children living on farms are protected against wheeze independently of atopy. This farm effect is not attributable to improved airway size and lung mechanics. These findings imply as yet unknown protective mechanisms. They might include alterations of immune response and susceptibility to triggers of wheeze, such as viral infections. (J Allergy Clin Immunol 2012;130:382-8.)

Key words: *Asthma, atopy, children, exhaled nitric oxide, farming, hay fever, protection, protective environments, pulmonary function test, wheeze*

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Numerous studies have observed that farms provide a protective environment for the development of hay fever and atopy, pointing to early modulation of innate and adaptive immune responses. This might be mediated by timely and intense exposure to microbes.[1,2]

Reports of the farm effect on childhood asthma have been less consistent in comparison with atopy.[3,4] Yet recent findings indicate that the microbial diversity conferred by farm exposures seems to play a stronger role for asthma than for atopy.[1,2]

Childhood asthma is not one disease but rather a syndrome including many wheeze phenotypes.[5] This might further explain the thus far inconsistent findings in studies assessing the protective effect of exposure to farm environments on childhood asthma. Thus farm exposures might exert diverse effects not only on asthma and atopy but also on different wheeze phenotypes. The phenotypes of transient, persistent, and late-onset wheeze in childhood were initially described in the Tucson Children's Respiratory Study.[6] These phenotypes were associated with distinct patterns of lung function changes.[7] Underlining their continued usefulness for epidemiologic research, recent attempts to phenotype preschool wheeze by using modern mathematic techniques resulted in quite similar entities.[8] Furthermore, risk factors have been shown to differ for the various wheeze phenotypes that underline the importance of investigating these phenotypes separately.[7,9-12] However, the comanifestation of wheeze and atopy hampers the identification of individual determinants, such as those for wheeze in the absence of atopy. A possible solution to this dilemma is to stratify the population into atopic and nonatopic subjects.

Within the large, population-based, cross-sectional, multiphase GABRIEL Advanced Studies, we set out to close this gap of

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Abbreviations used
ATS: American Thoracic Society
BDR: Bronchodilator response
ERS: European Respiratory Society
FENO: Fraction of exhaled nitric oxide
FVC: Forced vital capacity
OR: Odds ratio

missing or inconclusive evidence. We aimed at studying the effect of exposure to farming environments on childhood atopy, different wheeze phenotypes defined according to current guidelines, and objective markers of lung mechanics and airway inflammation both in the general population and for atopic and nonatopic children separately.

METHODS

Study population

Table I[2,13,14] provides an overview of the study design and population of the GABRIEL Advanced Studies. In addition to what is displayed in brief here, a more detailed description of the study population and methods is provided in the Methods section in this article's Online Repository at www.jacionline.org. The study design is further described in more detail elsewhere.[15]

During phase 1 in 2006, 34,491 children 6 to 12 years old were recruited in rural areas of Austria, southern Germany, and Switzerland. A short questionnaire assessed asthma or allergic diseases and farm exposures. Three exclusive exposure strata were defined: (1) farm children (ie, children living on a farm run by the family); (2) exposed nonfarm children (ie, children not living on a farm but regularly exposed to stables, barns [at least once a week over 6 months], or unprocessed cow's milk consumed directly from a farm ever in life); and (3) unexposed nonfarm children as a reference group. For phase 2 in 2007, random samples stratified for exposure (n = 9,668) were selected. Of these, 8,023 study participants provided detailed data on wheeze and farm exposure with a comprehensive questionnaire and blood samples for IgE measurements. In 2007-2008, phase 3 was conducted only in Bavaria for logistic reasons. Here a further random subsample of 895 children was selected for fraction of exhaled nitric oxide (FENO) and lung function measurements, of whom 858 were invited for phase 3 measurements. The ethics committees of the respective universities, as well as the data protection authorities, approved the study.

Definitions of childhood wheeze

Wheeze definitions were based on current recommendations of the European Respiratory Society (ERS).[7] From retrospective questionnaire information, *transient wheeze* was defined as wheeze before the age of 3 years but not at school age. *Persistent wheeze* was defined as onset of wheeze before age 3 years and wheeze at school age. *Late-onset wheeze* was defined as onset of wheeze at or after the age of 3 years. The latter 2 categories were combined into the *current wheeze* category to increase the sample size and thereby the statistical power.

Atopy

Serum IgE antibodies against individually tested allergens (*Dermatophagoides pteronyssinus*, cat, rye, timothy, birch, and mugwort) were measured in one laboratory at the Robert-Koch-Institute, Berlin, Germany (UNICAP 1000; Phadia AB, Uppsala, Sweden).

Any *allergen-specific sensitization* was generally defined as a specific serum IgE antibody level of at least 0.35 kU/L against the respective allergen. *Atopic sensitization* was defined as allergen-specific sensitization against at least 1 of the 6 tested allergens. *Monosensitization* was defined as allergen-specific sensitization against only 1 of the 6 tested allergens, and *polysensitization* was defined accordingly as allergen-specific sensitization to more than 1 of the 6 tested allergens.

FENO measurements

Before spirometry, trained fieldworkers collected exhaled air with an offline kit (EcoMedics AG, Duernten, Switzerland) in triplicate in Mylar-coated bags (Quintron, Cedar Rapids, Iowa) and measured FENO levels within 12 hours by using a rapid-response chemiluminescence analyzer (CLD 88; EcoMedics AG), according to current guidelines of the ERS and the American Thoracic Society (ATS).[14]

Lung function measurements

Trained fieldworkers performed spirometry with a mobile spirometer (EasyOne; ndd, Zurich, Switzerland), according to current ERS/ATS standards,[13] before and after bronchodilator tests (400 μg of salbutamol). Outcomes were FEV_1, forced vital capacity (FVC), the FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC. A positive bronchodilator response (BDR) was defined as a relative change in FEV_1 at least 12% from baseline values.[16]

Statistical analyses

Accounting for the stratified sampling design, phase 2 and phase 3 data were analyzed by using stratified weighted statistical methods, with the Taylor series method to estimate variances. We calculated mean differences, geometric mean ratios, and odds ratios (ORs), each with their 95% CIs, using linear and logistic regression. All models were adjusted for study center, sex, age, and further relevant confounders (family history of allergic disease, parental smoking, and parental education), as further described in the Online Repository at www.jacionline.org.

P values for trend were labeled as P_{trend}. Statistical interaction was modeled with multiplicative interaction terms between 2 dummy variables for the 3 farming categories and atopy. The corresponding *P* value was labeled as P_{int}. Statistical analyses were performed with SAS 9.2 software (SAS Institute, Inc, Cary, NC).

RESULTS

Farm exposures and atopic sensitization

We replicated the inverse association of farm exposures with atopic sensitization also for levels of total and specific IgE (see Table E1 in this article's Online Repository at www.jacionline.org). Farm children had significantly lower adjusted ORs for atopic sensitization. The same pattern, although weaker and not always statistically significant, was found for exposed nonfarm children compared with the unexposed reference group. This effect was observed for both seasonal and perennial inhalant allergens and total IgE levels. In sensitivity analyses these inverse associations were even stronger at higher than at lower cutoff levels (≥3.5 vs <0.35 kU/L, data not shown).

For sensitized subjects, we analyzed the association of farming with levels of specific IgE against the individual allergens, with levels of total IgE, and with the number of allergens to which subjects were sensitized. We observed an inverse association of farming both with the degree of sensitization for all allergens separately, and with levels of total IgE (see Table E2 in this article's Online Repository at www.jacionline.org). Furthermore, among those sensitized to any allergen (cutoff at 0.35 kU/L), farm children and exposed nonfarm children were more often monosensitized than polysensitized compared with the unexposed reference group ($P_{trend} < .0001$ and $P_{trend} = .0195$, respectively).

Farm exposures and wheeze phenotypes

Farm exposures were inversely associated with childhood wheeze phenotypes. For transient wheeze, we found an adjusted