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

Oliver Fuchs, MD,[a,i]* Jon Genuneit, MD,[b]* Philipp Latzin, MD, PhD,[a] Gisela Büchele, PhD,[b] Elisabeth Horak, MD,[c] Georg Loss, MSc,[d,e] Barbara Sozanska, MD,[f] Juliane Weber, MD,[g] Andrzej Boznanski, MD,[f] Dick Heederik, PhD,[h] Charlotte Braun-Fahrländer, MD,[d,e] Urs Frey, MD, PhD,[a,i] Erika von Mutius, MD,[g] and the GABRIELA Study Group‡

Bern and Basel, Switzerland, Ulm and Munich, Germany, Innsbruck, Austria, Wroclaw, Poland, and Utrecht, The Netherlands

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*

Discuss this article on the JACI Journal Club blog: www.jaci-online.blogspot.com.

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

From [a]the Division of Respiratory Medicine, Department of Pediatrics, Inselspital, University of Bern; [b]the Institute of Epidemiology and Medical Biometry, Ulm University; [c]the Department of Pediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University; [d]the Swiss Tropical and Public Health Institute, Basel; [e]the University of Basel; [f]Wroclaw Medical University, 1st Department of Pediatrics, Allergology and Cardiology; [g]University Children's Hospital, Ludwig-Maximilians-University, Munich; [h]Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology; and [i]University Children's Hospital (UKBB), University of Basel.

*These authors contributed equally to this work.

‡The members of the GABRIELA Study Group are listed in alphabetical order in the acknowledgments section.

Supported by the European Commission as part of GABRIEL (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community), contract no. 018996, under the FP6-LIFESCIHEALTH Integrated Program LSH-2004-1.2.5-1. O.F. is the recipient of a Long-Term Research Fellowship by the European Respiratory Society (no. 675) and a Training Scholarship by the Austrian, German and Swiss Pediatric Respiratory Society.

Disclosure of potential conflict of interest: E. von Mutius is a consultant for Novartis, GlaxoSmithKline, ALK-Abelló, and Protectimmun; has received speaker's fees from InfectoPharm; and is a member of the expert panel for the UK Research Excellence Framework. B. Sozanska, J. Weber, D. Heederik, H. Danielewicz, M. Depner, C. Strunz-Lehner, and I. Wouters have received research support from the European Commission (EU). M. Ege has received research support from the European Commission and the Deutsche Forschungsgemeinschaft (DFG). M. Kabesch has received research support from Roxall, GlaxoSmithKline, Novartis, Sanofi Aventis, Allergopharma, AstraZeneca GmbH, DFG, BMBF, and the EU. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 10, 2011; revised April 19, 2012; accepted for publication April 27, 2012.

Available online June 28, 2012.

Corresponding author: Urs Frey, MD, PhD, University Children's Hospital (UKBB), Spitalstrasse 33, 4056 Basel, Switzerland. E-mail: urs.frey@ukbb.ch.

0091-6749/$36.00

doi:10.1016/j.jaci.2012.04.049

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

TABLE I. Overview of the GABRIEL Advanced Studies population and design

Study module	Year	Study region	Population	Total no.	Mean age (5th-95th percentile)	Exposure strata		
						Unexposed nonfarm children	Exposed nonfarm children	Farm children
Phase 1	2006	All 4 centers*	Parental informed consent to further analyses†	34,491	8.7 (6.5-11.1)	n = 21,292	n = 8,666	n = 4,533
Phase 2	2007	All 4 centers*	Random subsample stratified for exposure‡	9,668		n = 2,955	n = 3,236	n = 3,477
			Complete phase 2 dataset§	8,023	9.2 (7.1-11.5)	n = 2,386	n = 2,660	n = 2,977
Phase 3	2007-2008	Bavarian center	Random subsample stratified for exposure‖	895		n = 297	n = 300	n = 298
			Invited for lung function and FENO measurements¶	858		n = 290	n = 284	n = 284
			Valid lung function measurements#	711	9.9 (8.0-11.7)	n = 236	n = 242	n = 233
			Valid FENO measurements**	795	9.9 (8.0-11.7)	n = 264	n = 262	n = 269

*Austria (Tyrol), Germany (Baden-Wuerttemberg and Bavaria), and Switzerland (9 German-speaking cantons).
†Signed informed consent to the following analyses and to all additional investigations after completion of the first recruiting questionnaire (Bavaria: n = 11,183: 1,797/2,708/6,678).
‡Random selection of children from phase 1 for phase 2: detailed comprehensive questionnaire and blood sampling exclusively stratified for farm exposure (unexposed nonfarm children = control subjects, exposed nonfarm children = intermediate-exposure group, and farm children = highest-exposure group; Bavaria: n = 2,573:1,014/814/745).
§Participation in blood sampling and analysis of specific IgE levels; complete detailed questionnaire in phase 2.
‖Random selection from 2,573 Bavarian children (for logistic reasons) from phase 2 for phase 3: spirometric and FENO measurements, exclusively stratified for farm exposure.
¶Participation in any phase 3 study module before lung function and FENO measurements (including collection of milk samples, mattress dust, and settled and scooped dust in bedroom and stables [see Ege et al[2]]).
#Participation in spirometry and valid measurements before BDR according to guidelines.[13]
**Participation in FENO sampling and acceptable measurements according to guidelines.[14]

OR of 0.78 (95% CI, 0.64-0.96) comparing the highest-exposure group, farm children, with the unexposed reference group. However, except for the transient form (prevalence of 12.8% in the study population), all other wheeze phenotypes were significantly associated with atopic sensitization. Comparing children with atopic sensitization with children without atopic sensitization, adjusted ORs amounted to 2.15 (95% CI, 1.61-2.88) for persistent wheeze, 4.88 (95% CI, 3.65-6.51) for late-onset wheeze, and 3.30 (95% CI, 2.67-4.06) for current wheeze, with prevalences of 5.9%, 7.0%, and 14.8%, respectively. Because of this interrelation, it was not possible to discriminate between the effect of farm exposure on atopy and on wheeze phenotypes. Therefore all further analyses were modeled, including an interaction term between atopic sensitization and farm exposure. Consequently, all wheeze forms except transient wheeze revealed heterogeneous results when comparing atopic with nonatopic children.

The inverse association of farming with current wheeze (Fig 1) was statistically significant only among the nonatopic study population (P_{int} = .023), with an adjusted OR of 0.45 (95% CI, 0.32-0.63) only for the comparison of the highest-exposure group, farm children, with the unexposed reference group. Indicating the increased risk among atopic children, there were generally higher adjusted ORs for current wheeze across all exposure strata compared with the nonatopic unexposed reference. We found the same pattern of association with farm exposure for persistent wheeze, with an adjusted OR of 0.38 (95% CI, 0.24-0.58) when comparing farm children with the unexposed reference group. We detected a significant effect of farm exposure also for late-onset wheeze (see Fig E1 in this article's Online Repository at www.jacionline.org). Restricting these analyses to the study population with valid lung function measurements only, we identified the same pattern of farm effects, although without statistical significance because of lower sample size (data not shown).

Farm exposures and objective outcomes: Lung function and FENO measurements

Some of the results of this study have been previously reported in the form of abstracts.[17,18]

Study participants with current wheeze had significantly worse lung function, demonstrated a positive BDR response more often, and also had increased FENO levels compared with never wheezers. The same was found for those with late-onset and persistent wheeze. Children with transient wheeze had normal lung function and FENO levels. Atopic study participants had significantly higher FENO levels and worse lung function than nonatopic subjects (see Table E3 in this article's Online Repository at www.jacionline.org). Consequently, we also analyzed the farm effect on lung function and FENO levels, including an interaction term between atopic sensitization and farm exposure.

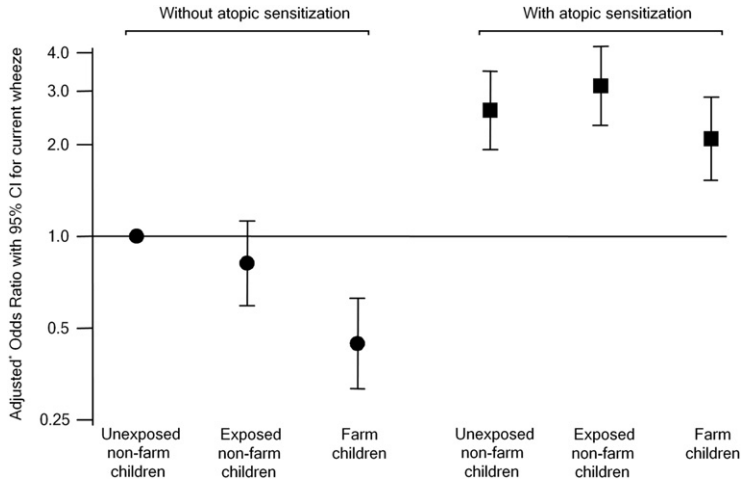

FIG 1. Farm exposure and current wheeze. This figure shows the association of farm exposure with current wheeze across levels of exposure to farming environments stratified by atopic sensitization in the phase 2 population. The *line* represents the level of unexposed nonfarm children without atopic sensitization as a reference. *Left (circles),* Children without atopic sensitization; *right (squares),* children with atopic sensitization. *Adjusted for center, sex, age, and family history of allergic diseases.

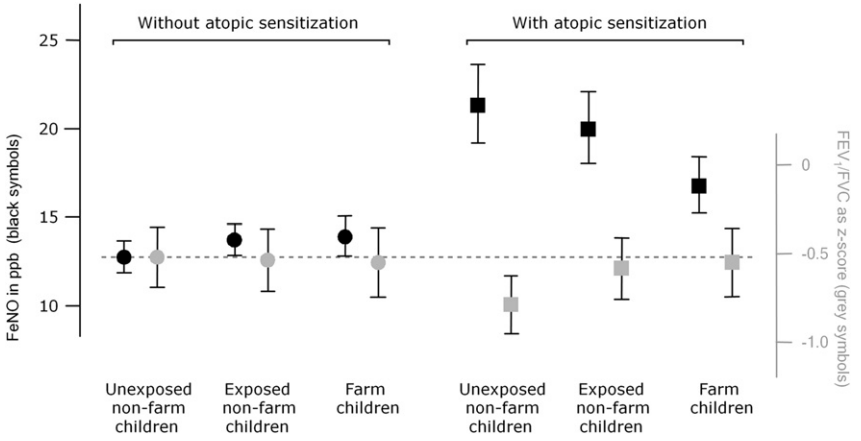

FIG 2. Farm exposure and objective outcomes. This figure shows the effect of farm exposure on crude estimates and their respective SEs for spirometry (*gray symbols* and *gray scale on right*, mean *z*-score FEV_1/FVC ratio) and of FENO levels (*black symbols* and *black scale on left*, geometric means) stratified by atopic sensitization in the phase 3 study population. The *dashed line* represents the level of unexposed nonfarm children without atopic sensitization as a reference. *Left (circles),* Children without atopic sensitization; *right (squares),* children with atopic sensitization. Valid spirometry before bronchodilator test, n = 711; FENO data, n = 795.

In the general study population farming was neither associated with lung function before or after bronchodilator, as well as with BDR, nor with FENO levels. Fig 2 depicts the crude FENO levels and FEV_1/FVC ratio *z*-scores together with their respective SEs across categories of exposure to farming environments and atopic sensitization. Significant farm effects on FENO levels were only seen among atopic subjects ($P_{int} = .0013$, Fig 2). Here FENO levels were significantly lower among farm children compared with those in the unexposed reference group, with a corresponding adjusted geometric mean ratio of 0.74 (95% CI, 0.63-0.86). Farming was also associated with better lung function among atopic subjects. Atopic farm children had an increased FEV_1/FVC ratio *z*-score by 0.32 (95% CI, 0.04-0.60) compared with that seen in the unexposed reference group. In contrast to FENO levels, this interaction with atopic sensitization did not reach statistical significance ($P_{int} = .200$). Other than lung function before bronchodilator and FENO levels, farming was not associated with BDR or postbronchodilator values among atopic or nonatopic children separately (data not shown).

The farm effect on the degree of atopic sensitization might explain the inverse association of farming with objective outcomes of lung function and FENO levels among atopic subjects. The levels of specific IgE against the respective allergen among sensitized subjects were correlated with FENO levels (data not shown). When we adjusted the farm effect on FENO levels among sensitized subjects for their degree of sensitization, the inverse association was attenuated. This suggests that the farm effect on FENO levels is partly attributable to an effect on the degree of atopic sensitization (Table II). In contrast to FENO levels, there was no correlation of lung function with degree of atopic sensitization.

TABLE II. Farm effect on FENO levels among sensitized children

	Unexposed nonfarm children	Farm children				
	Geometric mean (ppb)*	Geometric mean (ppb)*	aGMR*†	95% CI	aGMR*‡	95% CI
Dermatophagoides pteronyssinus	25.7	20.7	**0.78**	**0.62-0.98**	0.87	0.71-1.08
Cat	26.9	22.2	0.77	0.56-1.06	0.79	0.57-1.08
Rye	21.0	16.7	**0.75**	**0.63-0.90**	0.86	0.72-1.03
Timothy grass	21.1	17.1	**0.76**	**0.64-0.91**	0.88	0.73-1.05
Birch	22.5	18.2	**0.79**	**0.64-0.98**	0.86	0.69-1.06
Mugwort	21.9	16.5	**0.71**	**0.57-0.88**	**0.74**	**0.59-0.92**
Increased total IgE level	20.3	16.6	**0.79**	**0.67-0.93**	**0.83**	**0.71-0.98**

Association of farming with FENO levels among those sensitized against the respective allergen without† and with‡ adjustment for specific IgE levels is shown.
aGMR, Adjusted geometric mean ratio. The boldfaced text indicates significance.
*Because of their distribution, FENO levels were log-transformed. Results are therefore given as geometric means and adjusted geometric mean ratios.
†All models are adjusted for parental smoking and parental education.
‡All models are additionally adjusted for levels of the respective specific IgE.

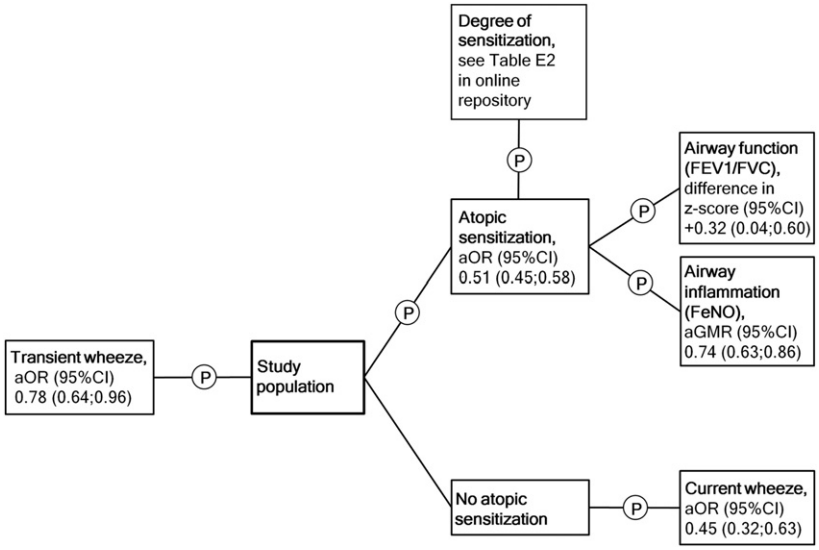

FIG 3. Different protective farming effects identified in the GABRIEL Advanced Studies population. This figure shows the protective farming effects *(P)* together with their respective magnitudes (1) on the prevalence of transient early wheeze and (2) on the prevalence of current wheeze among children without atopic sensitization in addition to the already known protective effect on the presence of atopic sensitization (3) also on its degree. Among children with atopic sensitization, we detected (4) less increased exhaled nitric oxide levels as a marker of reduced eosinophilic airway inflammation and (5) less impaired lung function. *aGMR,* Adjusted geometric means ratio; *aOR*, adjusted OR.

To corroborate our findings, we repeated the analyses using a single item ("Does your child live on a farm? [yes/no]") to alternatively define exposure to farming environments. Within these sensitivity analyses, the detected patterns of associations did not change (data not shown).

DISCUSSION

Traditional Central European farm environments in rural areas seem to exert several protective effects among children (Fig 3). The inverse association with atopy has been shown previously, but we were able to further demonstrate that this is also true for the degree of atopic sensitization. We found that children growing up on farms have a lower prevalence of all wheeze phenotypes. Furthermore, we demonstrated better lung function and lower FENO levels among atopic study participants growing up on farms, suggesting that atopic wheeze might be less severe among farm children.

The farm effect on the presence of atopy was replicated with an effect size comparable with that of previous studies and cannot be explained in our population by moving of families with an affected child to a nonfarming environment.[1,3,4,15,19] As with atopy, the inverse association with the degree of atopic sensitization might be related to the effect of farm exposures on immune response. Despite the recent finding that microbial diversity seems to play a lesser role for atopy than for asthma,[2] farms provide an environment that can be seen as a model of natural immunotherapy.[1,2]

Farm exposures have been shown to affect expression of pattern-recognition receptors, such as Toll-like receptors 2 and 4, and *CD14*. This results in higher levels of the T_H1 cell–associated cytokine IFN-γ and increased activation of regulatory T cells. These effects might balance adaptive immune responses and alleviate allergen-induced T_H2 cell–associated cytokine expression (eg, IL-4 and IL-13). Decreased IgE class-switching can also contribute to a lower degree of atopic sensitization.[1,2] Although not analyzed in this study, an important question is why some children have atopic sensitization despite being exposed to protective environments on farms. Genetic differences are known to explain a small part of atopic heritability. In addition to further explanations,

such as epigenetic modifications, another possibility is that genetic influences might be modified by farm environments within gene-environment interactions.[20] In other words, a certain genotype might lead to unchanged or increased rates of atopy among farm-exposed children, whereas the other genotype might result in decreased risk. However, the gene-environment interaction analyses recently performed in this population remained inconclusive.[20]

We have defined the different forms of childhood wheeze to better assess a protective effect of farm exposures on childhood asthma, which probably resembles a complex syndrome rather than a single disease.[5,7] Independent of atopy, we found a strong protective farm effect on all childhood wheeze phenotypes. The notion of farm environments conferring a protective effect among nonatopic subjects has already been implied in 2 other studies, but different phenotypes according to current ERS/ATS guidelines had not been assessed.[12,21]

Although our study represents a retrospective assessment with the risk of recall bias, we used comprehensive self-reported information on childhood wheeze to carefully define wheeze phenotypes according to current guidelines as outcomes.[7] Yet the possible drawback of recall bias has to be kept in mind when interpreting our data.

All spirometric and FENO measurements were performed according to the highest standards of the ERS and ATS.[16,17] High response rates and shares of valid data were accomplished despite age difficulties and a nonclinical setting. We achieved 82.9% acceptable spirometric data before and 76.0% valid pairs before and after bronchodilator and another 92.7% acceptable FENO measurements. An initial hypothesis to perform spirometry in this study was that farm exposures might alter lung volume, especially airway size, and mechanics, resulting in less obstruction. Contrary to our expectations, we did not uncover a general farm effect on spirometric outcomes also when taking BDR into account. These findings suggest that there are no beneficial farm effects on lung or airway size.

Nevertheless, atopic patients with wheeze demonstrated better lung function and lower FENO levels when growing up on farms. Possibly because of sample size and power, in contrast to FENO results, the interaction of lung function results with atopic sensitization did not reach statistical significance. According to our data, the farm effect on FENO levels can at least partly be attributed to the known effect on atopy. In the context of allergic airway inflammation, this would also explain the association with spirometry among atopic subjects. The farm effect on allergic airway inflammation and especially lung function among atopic subjects is functionally small and might not be clinically important. However, lung function in this cross-sectional setting might instead represent a proxy for severity of wheeze rather than a proxy for its prevalence. The small decrements in spirometric results might also explain the absence of effect on BDR.

Our study is not the first survey on the effect of farm exposure on objective measures of airway mechanics. A study among 1199 Canadian adolescents 12 to 19 years old[22] and one from rural Iowa among 644 children with a mean ± SD age of 9.6 ± 5.0 years[23] have done the same. Similar to our results, these 2 studies did not demonstrate a farm effect on FEV_1/FVC ratio in the general population.[22,23] Yet the Canadian study found a farm effect on airway hyperresponsiveness after methacholine challenge.[22] We neither performed bronchial provocation nor assessed bronchial hyperresponsiveness and cannot contribute to this observation.

A common ground for both effects on transient and nonatopic wheeze might be protection against lower respiratory tract infections, most likely because of viral triggers. Susceptibility to viral infections has indeed been related to innate immunity.[24] As described earlier, farm exposures upregulate factors related to innate immunity, thus possibly also conferring a better protection against respiratory tract viruses.[1,2] Because genetic variations in components of innate immunity pathways have also been associated with asthma phenotypes, these might furthermore constitute a link between lower respiratory tract infection and asthma risk being modulated by farm exposure.[25] Unfortunately, we cannot further substantiate this notion because modern techniques for the detection of viral infections were not included in the study design.

The mechanisms underlying nonatopic asthma have not been clarified, and data collected among adults are conflicting.[26] One study revealed no difference between *IL4* and *IL5* expression levels from bronchial biopsy specimens of atopic and nonatopic asthmatic patients.[27] However, in another study both groups revealed diverse patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage fluid.[28]

Few studies have been performed in children given the ethical constraints for the use of invasive techniques, such as bronchoscopy and bronchoalveolar lavage, in this age group. Recent data for 55 children aged 2 to 10 years suggested that in bronchial biopsy specimens there are no differences between nonatopic and atopic children in airway pathology. Also the number of eosinophils and *IL4* and *IL5* expression levels did not vary significantly between both groups in comparison with those seen in healthy control subjects, at least in the setting of multitrigger wheeze.[29] Given the lack of knowledge about the type of airway inflammation in subjects with nonatopic wheeze, we can only speculate about the mechanisms underlying the protective farm effect on nonatopic wheeze. In addition to a potential antiviral effect discussed above, immune responses can be altered by farm exposures, hindering the development of airway inflammation. Alternatively, microbial colonization of airways can play a significant role. As shown recently, the diversity of environmental microbial exposures significantly reduces the risk of asthma but not atopy in this population.[2] Such microbial exposures might affect the airway microbiome and thereby prevent the development of wheeze and asthma.[30] Furthermore, the airway microbiome can affect the susceptibility of asthmatic subjects to viral infections.

Childhood exposure to farm environments not only protects against atopy but also against wheeze independently of atopy. This protective farm effect is not attributable to improved lung volume, airway size, and lung mechanics among children growing up on farms in the entire study population. The underlying mechanisms are unknown, but farm exposures might affect airway inflammation through antiviral properties and alterations of the airway microbiome. Among atopic subjects, farm environments might furthermore act through the degree of atopic sensitization and thus FENO metabolism. Here the effect on lung function might be secondary to the attenuated airway inflammation and subsequently milder airway obstruction.

The members of the GABRIELA Study Group are listed in alphabetical order:

Silvia Apprich, PhD,[g] Andrzej Boznanski, MD, PhD,[j] Charlotte Braun-Fahrländer, MD,[d,e] Gisela Büchele, PhD,[c] William Cookson, MD, DPhil,[a] Paul Cullinan, MD,[a] Hanna Danielewicz, MD,[j] Anna Dębińska,[j] Martin

Depner, PhD,[b] Markus Ege, MD,[b] Urs Frey, MD, PhD,[k] Oliver Fuchs, MD,[k] Jon Genuneit, MD,[c] Dick Heederik, PhD,[f] Elisabeth Horak, MD,[l] Anne Hyvärinen, PhD,[h] Sabina Illi, PhD,[b] Michael Kabesch, MD,[m] Katalin Kovacs,[j] Aleksandra Kosmęda, PhD,[j] Wolfgang Kneifel, PhD,[g] Philipp Latzin, MD, PhD,[k] Roger Lauener, MD,[o] Georg Loss, MSc,[d,e] Stephanie MacNeill, MSc,[a] Bernhard Morass, MD,[l] Anne-Cécile Normand, PhD,[p] Renaud Piarroux, MD, PhD,[p] Helena Rintala, PhD,[h] Mascha K. Rochat, MD,[b] Nikolaos Sitaridis,[c] Barbara Sozanska, MD,[j] David Strachan, MD,[n] Christine Strunz-Lehner MPH,[b] Bertrand Sudre, MD, PhD,[i] Erika von Mutius, MD, MSc,[b] Marco Waser, PhD,[d,e] Juliane Weber, MD,[b] and Inge Wouters, PhD[f]

From [a]Imperial College London, National Heart and Lung Institute London, United Kingdom; [b]LMU Munich, University Children's Hospital, Munich, Germany; [c]Ulm University, Institute of Epidemiology and Medical Biometry, Ulm, Germany; [d]the Swiss Tropical and Public Health Institute, Basel, Switzerland; [e]the University of Basel, Basel, Switzerland; [f]Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, Utrecht, The Netherlands; [g]BOKU Vienna, University of Natural Resources and Life Sciences, Department of Food Science and Technology, Vienna, Austria; [h]THL Kuopio, National Institute for Health and Welfare, Kuopio, Finland; [i]Université de Franche-Comté, Département de Parasitologie/Mycologie, Besançon, France; [j]Wroclaw Medical University, 1st Department of Paediatrics, Allergology and Cardiology, Wroclaw, Poland; [k]the Division of Pulmonology, Department of Paediatrics, Bern University Hospital, Bern, Switzerland; [l]the Department of Pediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University, Innsbruck, Austria; [m]Hannover Medical School, Clinic for Paediatric Pneumology and Neonatology, Hannover, Germany; [n]St George's, University of London, London, United Kingdom; [o]High Mountain Hospital Davos, Davos-Wolfgang, Switzerland; and [p]the Department of Parasitology and Mycology, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, Marseille, France.

We thank Dr Wulf Thierfelder and Michael Thamm from the Robert-Koch-Institute, Berlin, Germany, for their cooperation and the measurement of total and specific IgE levels that was used in the definition of atopic subjects. We also thank Ruedi Isler from Ecomedics, Duernten, Switzerland, and Joerg Egger from ndd, Zürich, Switzerland, for their technical assistance, as well as Janet Maccora for proofreading of the manuscript. Furthermore, we thank the participating children, their families, and the fieldworkers of the GABRIEL Advanced Studies.

Key messages

- **Growing up on a farm confers protection against all childhood wheeze phenotypes.**
- **This farm effect on clinical symptoms, such as wheeze, is independent of the effect on atopy and not attributable to improved airway mechanics in this population.**
- **Activation of innate immune responses resulting in decreased vulnerability to viral infections, the main triggers of wheeze, might play a role.**

REFERENCES

1. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol 2010;10:861-8.
2. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.
3. von Mutius E, Radon K. Living on a farm: impact on asthma induction and clinical course. Immunol Allergy Clin North Am 2008;28:631-47, ix-x.
4. Tse K, Horner AA. Defining a role for ambient TLR ligand exposures in the genesis and prevention of allergic diseases. Semin Immunopathol 2008;30:53-62.
5. Bush A, Menzies-Gow A. Phenotypic differences between pediatric and adult asthma. Proc Am Thorac Soc 2009;6:712-9.
6. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
7. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J 2008;32:1096-110.
8. Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. Thorax 2008;63:974-80.
9. Court CS, Cook DG, Strachan DP. Comparative epidemiology of atopic and non-atopic wheeze and diagnosed asthma in a national sample of English adults. Thorax 2002;57:951-7.
10. Garcia-Marcos L, Castro-Rodriguez JA, Suarez-Varela MM, Garrido JB, Hernandez GG, Gimeno AM, et al. A different pattern of risk factors for atopic and non-atopic wheezing in 9-12-year-old children. Pediatr Allergy Immunol 2005;16:471-7.
11. Kurukulaaratchy RJ, Fenn M, Matthews S, Arshad SH. Characterisation of atopic and non-atopic wheeze in 10 year old children. Thorax 2004;59:563-8.
12. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol 2007;119:1140-7.
13. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med 2007;175:1304-45.
14. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
15. Genuneit J, Büchele G, Waser M, Kovacs K, Debinska A, Boznanski A, et al. The GABRIEL Advanced Surveys: study design, participation, and evaluation of bias. Paediatr Perinat Epidemiol 2011;25:436-47.
16. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-68.
17. Fuchs O, Genuneit J, Latzin P, Frey U, Braun-Fahrländer C, Horak E, et al. The influence of farming on lung function in school-age children—the GABRIEL advanced surveys. Eur Respir J 2009;34:758s.
18. Genuneit J, Latzin P, Fuchs O, Frey U, Braun-Fahrlander C, Horak E, et al. Measurement of exhaled nitric oxide in school-age children in rural areas—the GABRIEL advanced surveys. Eur Respir J 2009;34:758s.
19. von Mutius E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. Immunobiology 2007;212:433-9.
20. Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol 2011;127:138-44, e1-4.
21. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129-33.
22. Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. Am J Respir Crit Care Med 2000;161:1563-6.
23. Merchant JA, Naleway AL, Svendsen ER, Kelly KM, Burmeister LF, Stromquist AM, et al. Asthma and farm exposures in a cohort of rural Iowa children. Environ Health Perspect 2005;113:350-6.
24. Gern JE. Rhinovirus and the initiation of asthma. Curr Opin Allergy Clin Immunol 2009;9:73-8.
25. Sly PD, Kusel M, Holt PG. Do early-life viral infections cause asthma? J Allergy Clin Immunol 2010;125:1202-5.
26. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet 2006;368:804-13.
27. Humbert M, Durham SR, Ying S, Kimmitt P, Barkans J, Assoufi B, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and non-atopic asthma: evidence against "intrinsic" asthma being a distinct immunopathologic entity. Am J Respir Crit Care Med 1996;154:1497-504.
28. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. Allergic and non-allergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am Rev Respir Dis 1992;146:109-15.
29. Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. Am J Respir Crit Care Med 2008;178:476-82.
30. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, et al. Disordered microbial communities in asthmatic airways. PLoS One 2010;5:e8578.

METHODS

This is a detailed description of the study population and all described methods and definitions, as already provided in the main document. The study design is further described elsewhere.[E1]

Study population

As shown in Table I, the GABRIEL Advanced Studies are cross-sectional, population-based multiphase surveys that have been conducted to assess the effect of exposure to farming environments on asthma and allergic disease in children.

During phase 1 in 2006, a short recruitment questionnaire was distributed to parents of 79,888 schoolchildren 6 to 12 years old in rural areas of Austria (Tyrol), southern Germany (Bavaria and Baden-Württemberg), and 9 German-speaking cantons in Switzerland.

The prevalence of farm characteristics, farm-related exposures, and asthma and allergic diseases were assessed, along with potential confounders and indicators of potential participation bias for later analyses. Three exclusive farm-exposure strata were defined based on data collected during phase 1: (1) farm children (ie, children living on a farm currently 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 the remainder. Unexposed nonfarm children were used as a reference group. All children whose parents had provided written informed consent to blood and dust sampling and also genetic analyses were eligible for phase 2 (n = 34,491).

Of these, in 2007, informative random samples (n = 9668) disproportionately stratified for farming exposure were drawn. Disproportionate stratified sampling was applied to reduce the numbers of unexposed children because of economic constraints. The phase 2 questionnaire comprehensively assessed details of the child's farm-related exposures at various ages and doses. Between April and July 2007, in total, 8023 study participants provided detailed data on wheeze and farming exposure, as well as blood samples for measurements of specific IgE levels.

Phase 3 was conducted in 2007-2008 to collect in-depth exposure and data on objective outcomes of allergic airway disease. This study phase was conducted only in Bavaria for logistic reasons. Of 1903 eligible Bavarian children, a further random subsample of 895 children was selected. Of those, 858 who had already consented to and participated in any phase 3 study module before lung function and FENO measurements (including collection of milk samples, mattress dust, and settled and scooped dust in bedroom and stables) were recontacted and then participated in phase 3 FENO and lung function measurements between April and July 2008. Among them, a further questionnaire on respiratory symptoms and medication was distributed.

The ethics committees of the respective universities, as well as the data protection authorities, approved the study. These were the Bavarian Medical Association and Ulm University for the 2 German centers of Bavaria and Baden-Württemberg; the cantons Luzern, Zurich, and Thurgau for the Swiss study region; and the Medical University of Innsbruck for the Austrian study center.

Definitions of childhood wheeze

For outcomes in phases 2 and 3, we defined the following wheeze phenotypes based on current recommendations by the ERS[E2]:

1. *Never wheeze*—subjects who answered no to the question on wheezing ever in life and who did not report an age at onset of wheeze. This was the reference category for all the following wheeze phenotypes.
2. *Transient wheeze*—subjects who reported an onset of wheeze before age 3 years but no wheeze in the past 12 months during phases 1 or 2.
3. *Current wheeze*—subjects who reported age at onset of wheeze and wheeze in the past 12 months in phase 2, with onset of wheeze either before or after the age of 3 years.
4. *Persistent wheeze*—subjects with "current wheeze" who reported an onset of wheeze before the age of 3 years.
5. *Late-onset wheeze*—subjects with "current wheeze" who reported an onset of wheeze at or after the age of 3 years.

Atopy

After collection of blood samples, total and specific serum IgE antibody levels against the individually tested allergens *Dermatophagoides pteronyssinus* (d1), cat dander (e1), timothy grass (g6), cultivated rye (g12), common silver birch (t3), and mugwort (w6) were measured in a central laboratory at the Robert-Koch-Institute, Berlin, Germany, by using the UNICAP 1000 fluorescence immunoassay (Phadia AB).

Any allergen-specific sensitization was generally defined as specific serum IgE antibody levels 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 individually tested allergens. Monosensitization was defined as allergen-specific sensitization against only 1 of the 6 individually tested allergens, and polysensitization was defined accordingly as allergen-specific sensitization to more than 1 of the 6 individually tested allergens.

Anthropometric measurements

Body weight and height were measured according to World Health Organization guidelines (seca 862, seca 214, and seca 200; Seca GmbH & Co KG, Hamburg, Germany).

FENO measurements

Before spirometry, exhaled air was collected from 826 children in Mylar-coated bags (Quintron) with an offline kit (EcoMedics AG), according to current guidelines by the ERS and the ATS.[E3] Contamination of FENO measurements by ambient nitric oxide was avoided by using nitric oxide–free air for inspiration through a scrubber used to inhale room air and attached to the offline kit. We aimed for a triplicate measurement per study participant, which was achieved in 90% of cases. Within 12 hours after collection, FENO levels were measured with a rapid-response chemiluminescence analyzer (CLD 88, EcoMedics AG). Dr Fuchs performed fieldworker training and provided regular support through telephone and personal meetings with fieldworkers, including regular feedback on data quality. After exclusion of 29 participants who reported intake of oral or inhaled steroid medication in the week before the field visit (10 farm children, 11 exposed nonfarm children, and 8 unexposed nonfarm children, all of whom were asthmatic as in the stratum definition), 795 of 858 participants remained with acceptable FENO measurements, resulting in a rate for valid data of 92.7% according to current guidelines.

Lung function measurements

Spirometry was performed with a validated mobile spirometer (EasyOne), according to current ERS/ATS standards.[E4] Measurements before and after bronchodilator tests (with 400 μg of a short-acting β-agonist) were taken by trained fieldworkers strictly adhering to a standard operating procedure including all measurement procedures according to current ERS/ATS standards.[E4]

Before spirometry, study participants and their parents were asked not to inhale short-acting β-agonists at least 4 hours before the test or long-acting β-agonists at least 12 hours before the test, as well as not to take any leukotriene receptor antagonists at least 12 hours before the test. Otherwise, measurements would have been excluded for analyses of BDR. Outcome parameters before and after bronchodilator tests were FEV_1, FVC, the FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC. A positive BDR result was defined as a relative change in FEV_1 after versus before short-acting β-agonists of at least 12%.[E5] Dr Fuchs prepared measurement devices, performed fieldworker training, assessed adherence to the standard operating procedure or to ERS/ATS standards, and provided regular support through telephone and personal meetings with fieldworkers, including regular feedback on data quality. Here Dr Fuchs performed extensive quality control by visually reanalyzing the flow-volume and volume-time traces of each measurement for adherence to ERS/ATS standards of lung function measurements in children.[E4] With 711 acceptable measurements before bronchodilator testing and 652 acceptable pairs of measurements before and after bronchodilator testing, this results in a rate for valid data among 858 children invited for spirometry of 82.9% and 76.0%, respectively, according to current guidelines. None of the measurements had to be excluded for analyses due to inhalation

or intake of short-acting β-agonists, long-acting β-agonists, or leukotriene receptor antagonists within the given timeframes, as mentioned above. Outcomes were standardized by calculating *z*-scores with recently published reference data for spirometry.[E6]

Statistical analyses

All data collected in phases 2 and 3 were analyzed by using stratified, weighted statistical methods to account for the stratified sampling design. Weights were calculated as the ratio of the children eligible for sampling to the children contributing to the analyses per center and strata for each analysis separately. Thus all analyses were weighted to the total number of the study population of phase 1 eligible for phase 2 (n = 34,491). We used the Taylor series method to estimate variances. We calculated mean differences, geometric mean ratios, and 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). Family history of allergic disease included reports of hay fever or atopic eczema for parents or siblings ever in life. Parental smoking was assumed if either the mother or father reported current or past smoking, including maternal smoking during pregnancy. Parental education was assessed in categories of schooling and dichotomized into "eligibility for university entrance" (yes/no) as appropriate for each study country. Age was taken as age at the phase of interest in the analyses.

For confounders to be included in the final models, the point estimate of the main explanatory variable had to change by at least 10% after addition of the confounder to the model. Family history of asthma (including reports of asthma for any parent or sibling ever in life) and number of siblings did not show a confounding effect and were thus not included. Because phase 3 was only conducted in Bavaria, no adjustment for study center was necessary in analyses of this phase. Standardizing spirometric indices according to recently published normative data for spirometry at the appropriate age range by Stanojevic et al[E6] made any further adjustment of models, including lung function measurements for sex, age, or body length, unnecessary.

The total serum IgE level was dichotomized at 100 kU/L or analyzed as a continuous log-transformed variable because of the skewed distribution. To assess effects on the degree of sensitization, we analyzed specific and total IgE levels as continuous, log-transformed variables among those with specific IgE levels of at least 0.35 kU/L (100 kU/L for total IgE) to the respective allergen. Because of their skewed distribution, data from FENO measurements were log-transformed as well.

P values for trend were labeled P_{trend}. Statistical interaction was modeled with a multiplicative interaction term 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).

REFERENCES

E1. Genuneit J, Büchele G, Waser M, Kovacs K, Debinska A, Boznanski A, et al. The GABRIEL Advanced Surveys: study design, participation, and evaluation of bias. Paediatr Perinat Epidemiol 2011;25:436-47.

E2. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J 2008;32:1096-110.

E3. ATS/ERS recommendations for standardized procedures for the online and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;(171):912-30.

E4. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med 2007;175:1304-45.

E5. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-68.

E6. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, et al. Reference ranges for spirometry across all ages: a new approach. Am J Respir Crit Care Med 2008;177:253-60.

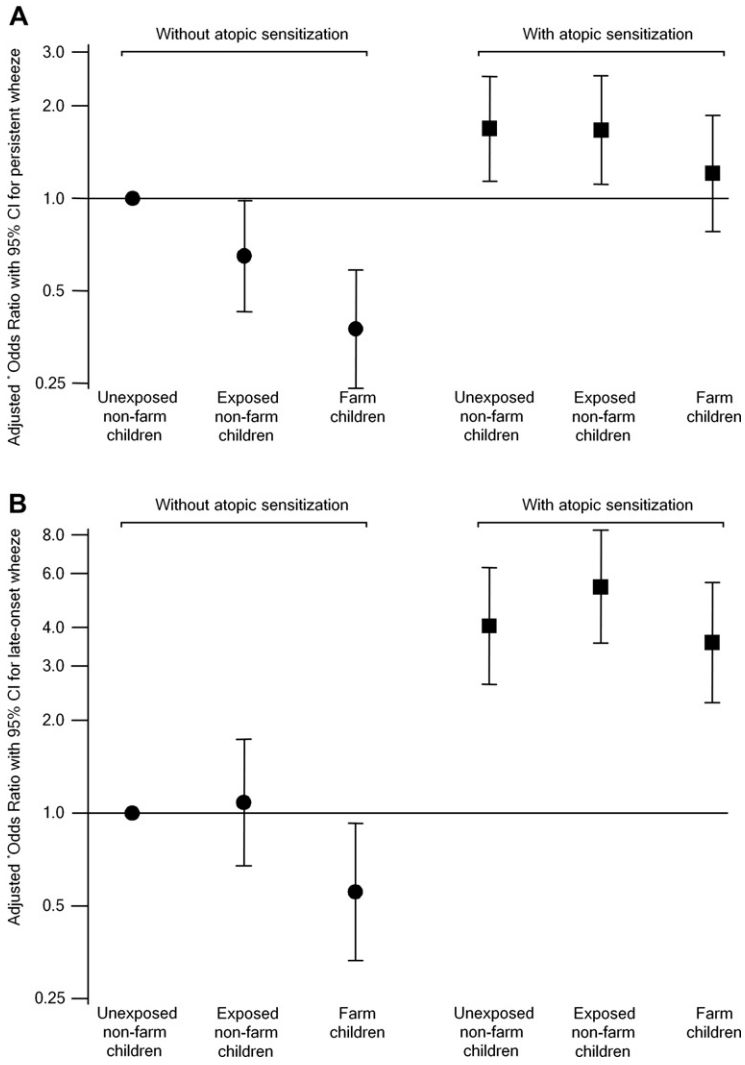

FIG E1. Farm exposure and persistent or late-onset wheeze. This figure shows the association of farm exposure with persistent wheeze (**A**; P_{int} = .098) and late-onset wheeze (**B**; P_{int} = .126) across levels of exposure to farming environments stratified by atopic sensitization in the phase 2 population. The *line* represents the level of unexposed nonfarm children without atopic sensitization as a reference. *Left (circles)*, Children without atopic sensitization; *right (squares)*, children with atopic sensitization. *Adjusted for center, sex, age, and family history of allergic diseases.

TABLE E1. Farm effect on atopic sensitization (phase 2)

	Unexposed nonfarm children (n = 2386)		Exposed nonfarm children (intermediate-exposure group [n = 2660])			Farm children (highest-exposure group [n = 2977])		
	Percent*	aOR‖	Percent*	aOR‖	95% CI	Percent*	aOR‖	95% CI
Atopic sensitization†	45.5	1.00	37.3	**0.72**	**0.64-0.81**	28.5	**0.51**	**0.45-0.58**
Dermatophagoides pteronyssinus‡	19.8	1.00	17.3	0.87	0.75-1.01	12.7	**0.65**	**0.56-0.76**
Cat‡	13.0	1.00	10.7	**0.82**	**0.69-0.99**	6.9	**0.56**	**0.46-0.68**
Rye‡	35.9	1.00	28.4	**0.71**	**0.62-0.80**	19.3	**0.45**	**0.39-0.51**
Timothy grass‡	36.4	1.00	28.1	**0.69**	**0.61-0.78**	18.3	**0.42**	**0.37-0.48**
Birch‡	22.9	1.00	16.7	**0.69**	**0.59-0.80**	11.7	**0.49**	**0.42-0.57**
Mugwort‡	22.8	1.00	17.7	**0.73**	**0.63-0.85**	14.2	**0.61**	**0.52-0.70**
Increased total IgE level§	37.0	1.00	35.1	0.93	0.82-1.05	29.9	**0.78**	**0.69-0.88**

Association of farming with prevalences* of atopic sensitization,† sensitization against separate tested allergens,‡ and increased total IgE levels§ is shown.
aOR, Adjusted OR. The boldfaced text indicates significance.
*Prevalences are unadjusted and pooled across centers.
†Specific IgE level of 0.35 kU/L or greater against at least 1 of the following: *Dermatophagoides pteronyssinus*, cat, rye, timothy, birch, or mugwort.
‡Specific IgE level of 0.35 kU/L or greater.
§Total IgE level of 100 kU/L or greater.
‖All models are adjusted for center, sex, age, and family history of allergic diseases.

TABLE E2. Farm effect on the degree of sensitization (phase 2)

	Unexposed nonfarm children (n = 2386)	Exposed nonfarm children (intermediate-exposure group [n = 2660])		Farm children (highest-exposure group [n = 2977])	
	aGMR‡	aGMR‡	95% CI	aGMR‡	95% CI
*Dermatophagoides pteronyssinus**	1.00	0.98	0.81-1.19	**0.79**	**0.64-0.97**
Cat*	1.00	0.92	0.78-1.09	**0.82**	**0.69-0.98**
Rye*	1.00	**0.73**	**0.63-0.85**	**0.65**	**0.56-0.76**
Timothy grass*	1.00	**0.78**	**0.67-0.91**	**0.66**	**0.57-0.76**
Birch*	1.00	**0.72**	**0.62-0.85**	**0.63**	**0.53-0.74**
Mugwort*	1.00	**0.86**	**0.77-0.98**	**0.84**	**0.74-0.94**
Increased total IgE level†	1.00	0.92	0.84-1.00	**0.88**	**0.80-0.95**

Association of farming with levels of specific IgE against separate tested allergens among those sensitized to the respective allergen with a specific IgE level of 0.35 kU/L or greater or for total IgE with levels of 100 kU/L or greater (all as adjusted geometric mean ratios‡ of IgE levels as continuous log-transformed variables). The boldfaced text indicates significance.

aGMR, Adjusted geometric mean ratio.

*Specific IgE level of 0.35 kU/L or greater.

†Included in calculations if 100 kU/L or greater.

‡All models are adjusted for center, sex, age, and family history of allergic diseases.

TABLE E3. Association of wheeze phenotypes and atopic sensitization with lung function and exhaled nitric oxide level (phase 3)

	Never wheeze	Transient wheeze		Current wheeze		Persistent wheeze		Late-onset wheeze	
Wheeze	**Mean**	**Mean difference†**	**95% CI**	**Mean difference†**	**95% CI**	**Mean difference†**	**95% CI**	**Mean difference†**	**95% CI**
Spirometry before bronchodilator*									
FEV_1	−0.51	0.11	−0.23 to 0.45	**−0.40**	**−0.69 to −0.11**	**−0.50**	**−0.90 to −0.11**	−0.32	−0.70 to 0.06
FVC	−0.23	0.11	−0.22 to 0.43	−0.13	−0.39 to 0.12	−0.04	−0.35 to 0.28	−0.20	−0.54 to 0.14
FEV_1/FVC ratio	−0.59	0.05	−0.25 to 0.34	**−0.39**	**−0.63 to −0.14**	**−0.65**	**−1.01 to −0.28**	−0.20	−0.50 to 0.10
FEF_{25-75}	−0.72	0.10	−0.28 to 0.48	**−0.54**	**−0.88 to −0.20**	**−0.83**	**−1.29 to −0.36**	−0.33	−0.77 to 0.10
BDR‡	**Percent**	**aOR†**	**95% CI**	**aOR†**	**95% CI**	**aOR†**	**95% CI**	**aOR†**	**95% CI**
Positive	15.4	0.68	0.24 to 1.91	**2.30**	**1.21 to 4.36**	2.18	0.94 to 5.08	**2.27**	**1.05 to 4.92**
Exhaled inflammatory marker§	**Geometric mean§**	**aGMR†,§**	**95% CI**	**aGMR†,§**	**95% CI**	**aGMR†,§**	**95% CI**	**aGMR†,§**	**95% CI**
FENO (ppb)	15.0	0.91	0.80 to 1.05	**1.55**	**1.30 to 1.87**	1.19	0.92 to 1.53	**1.91**	**1.54 to 2.37**

	Nonatopic subjects (n = 333)	Atopic subjects (n = 377)	
Atopic sensitization‖	**Mean**	**Mean difference†**	**95% CI**
Spirometry before bronchodilator*			
FEV_1	−0.45	−0.17	−0.38 to 0.04
FVC	−0.21	−0.03	−0.22 to 0.16
FEV_1/FVC ratio	−0.53	**−0.19**	**−0.35 to −0.03**
FEF_{25-75}	−0.64	**−0.25**	**−0.47 to −0.02**
BDR‡	**Percent**	**aOR†**	**95% CI**
Positive	15.6	1.02	0.59 to 1.75
Exhaled inflammatory marker§	**Geometric mean§**	**aGMR†,§**	**95% CI**
FENO (ppb)	13.2	**1.55**	**1.42 to 1.68**

aGMR, Adjusted geometric mean ratio; *FEF_{25-75}*, forced expiratory flow between 25% and 75% of FVC. The boldfaced text indicates significance.

*Results for spirometry (FEV_1, FVC, FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC) presented as *z*-scores after standardization to normative data for spirometry[E6] in children of appropriate age. Numbers of study participants with valid test results before bronchodilator for different wheeze phenotypes are as follows: never wheeze, n = 371; transient wheeze, n = 67; current wheeze, n = 116 (sum of persistent wheeze [n = 49] and late-onset wheeze [n = 67]); nonatopic subjects, n = 333; and atopic subjects, n = 377.

†Adjusted for parental smoking and parental education.

‡A positive BDR result is defined as the relative change in FEV_1 before/after bronchodilator of at least 12%. Numbers of study participants with valid test results before and after bronchodilator for different wheeze phenotypes are as follows: never wheeze, n = 336; transient wheeze, n = 62; current wheeze, n = 112 (sum of persistent wheeze [n = 48] and late-onset wheeze [n = 64]); nonatopic subjects, n=302; and atopic subjects, n = 349.

§Because of their distribution, FENO levels had to be log-transformed. Results are therefore given as geometric means and adjusted geometric mean ratios. Numbers of study participants with valid FENO measurements for different wheeze phenotypes are as follows: never wheeze, n = 441; transient wheeze, n = 77; current wheeze, n = 92 (sum of persistent wheeze [n = 36] and late-onset wheeze [n = 53]); nonatopic subjects, n = 387; and atopic subjects, n = 408.

‖Specific IgE level of 0.35 kU/L or greater against at least 1 of the following: *Dermatophagoides pteronyssinus*, cat, rye, timothy, birch, or mugwort.