Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies

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Background: Studies on the association of farm environments with asthma and atopy have repeatedly observed a protective effect of farming. However, no single specific farm-related exposure explaining this protective farm effect has consistently been identified.
Objective: We sought to determine distinct farm exposures that account for the protective effect of farming on asthma and atopy.
Methods: In rural regions of Austria, Germany, and Switzerland, 79,888 school-aged children answered a recruiting questionnaire (phase I). In phase II a stratified random subsample of 8,419 children answered a detailed questionnaire on farming environment. Blood samples and specific IgE levels were available for 7,682 of these children. A broad asthma definition was used, comprising symptoms, diagnosis, or treatment ever.
Results: Children living on a farm were at significantly reduced risk of asthma (adjusted odds ratio [aOR], 0.68; 95% CI, 0.59-0.78; *P* < .001), hay fever (aOR, 0.43; 95% CI, 0.36-0.52; *P* < .001), atopic dermatitis (aOR, 0.80; 95% CI, 0.69-0.93; *P* = .004), and atopic sensitization (aOR, 0.54; 95% CI, 0.48-0.61; *P* < .001) compared with nonfarm children. Whereas this overall farm effect could be explained by specific exposures to cows, straw, and farm milk for asthma and exposure to fodder storage rooms and manure for atopic dermatitis, the farm effect on hay fever and atopic sensitization could not be completely explained by the questionnaire items themselves or their diversity.
Conclusion: A specific type of farm typical for traditional farming (ie, with cows and cultivation) was protective against asthma, hay fever, and atopy. However, whereas the farm effect on asthma could be explained by specific farm characteristics, there is a link still missing for hay fever and atopy. (J Allergy Clin Immunol 2012;129:1470-7.)

Key words: *Asthma, hay fever, atopic dermatitis, atopic sensitization, childhood, farming, farm milk, early life*

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Asthma and allergies constitute complex diseases; their cause involves both genetic and environmental determinants. Moreover, both diseases frequently have their onset in childhood and thus appear to comanifest. However, recent results from the GABRIEL Surveys contradict this concept of interdependent phenotypes. The GABRIEL Surveys were designed to identify key factors in the development of asthma using the latest research across a variety of disciplines, including genetics, epidemiology, and immunology (see Table E1 in this article's Online Repository at www.jacionline.org).[1-6] A genome-wide association study within the GABRIEL Surveys found no overlap in genes associated with asthma and total IgE levels.[1] Furthermore, within the GABRIEL Surveys, discrepant results were also observed for the protective role of microbial diversity within a farming environment.[2] Whereas the protective farm effect on childhood asthma could be explained by the overall diversity of bacteria and fungi from dust of farm and nonfarm children, this did not hold for atopy.

Previous studies on the protective effect of growing up on a typical Central European farm were fairly consistent with respect to hay fever and atopy. In contrast, results for asthma were quite heterogeneous. This potentially indicates that not all farms are the same and that specific farm characteristics are possibly of greater effect than farm exposure in general.[7-10] These previous studies mainly used questionnaires assessing the farm's characteristics but not the child's exposure. The aim of the current epidemiologic GABRIEL Advanced Studies was an in-depth analysis of the protective exposures within a farming environment both on asthma

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Abbreviations used
aOR: Adjusted odds ratio
LCA: Latent class analysis

and atopy. This was based on a newly designed questionnaire aiming at disentangling the protective effect of a child's distinct farm exposures.

METHODS

Study design and population

The GABRIEL Advanced Surveys were conducted by 5 study centers in rural areas of southern Germany (Bavaria and Baden-Württemberg), Switzerland (9 German-speaking cantons), Austria (Tyrol), and Poland (Silesia) from winter 2006 to spring 2008.[5] Because of differences in study design, the Polish data will be reported separately. In the population-based phase I study a short recruiting questionnaire was distributed to parents of all schoolchildren through their elementary schools. In phase II stratified random samples of all children whose parents had given written informed consent to blood sampling, genetic analyses, and dust sampling were studied. Three 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, or cow's milk produced on a farm); and (3) unexposed nonfarm children.

In all centers the ethics committees of the respective universities and the data protection authorities approved the study.

Questionnaires

The recruitment questionnaire in phase I assessed the prevalence of respiratory and allergic symptoms and diagnoses, socioeconomic status, family history of atopy, maternal smoking, and farm characteristics comprising types of animal breeding, cultivation, and animal feeding. A comprehensive questionnaire was handed out to parents in phase II assessing characteristics of asthma and detailed information on the child's farm-related exposures. All farm-related exposures were assessed for 5 time periods (pregnancy; first, second to third, and fourth to fifth years of life; and past 12 months) and 5 frequency categories per time period (never/almost never, about once a month, about once a week, about once a day up to 15 minutes, and about once a day longer than 15 minutes). The following exposures were assessed: contact with animals (cats, dogs, cows, pigs, poultry, sheep, and horses), stay in animal sheds (cow, pig, and poultry), contact with animal feed (straw, hay, grain, corn, grass, silage, pellet feed, and sugar beet), presence during parental farming activities (harvesting/kibbling/ensiling corn, harvesting/handling hay, ensiling grass, harvesting/threshing/kibbling grain, fieldwork, manuring, and spraying pesticides), stay in barn or fodder storage room, and consumption of cow's milk produced on the farm.

Asthma and other allergic illnesses

Asthma was defined as either current wheeze (parental reporting of wheeze in the past 12 months), a positive answer to the question "Did your child ever use an asthma spray?," or a doctor's diagnosis of asthma at least once or of wheezy bronchitis more than once. Atopic and nonatopic current wheeze was defined as current wheeze with or without atopic sensitization (see the definition below), respectively, by using the children without current wheeze as a common reference group. Severe wheeze was defined as wheeze in the past 12 months with multiple triggers and asthma inhaler use ever.

Hay fever was defined as either nasal symptoms with itchy or watery eyes in the past 12 months or a doctor's diagnosis of hay fever ever. Atopic dermatitis was defined as a doctor's diagnosis ever.

All questionnaire-based outcomes were reported in phase I except for severe wheeze, which was assessed in phase II, and atopic and nonatopic current wheeze because atopic sensitization was also only assessed in phase II.

Atopic sensitization

Blood samples were collected, and serum IgE antibodies against inhalant (*Dermatophagoides pteronyssinus*, cat, grass mix [sweet vernal grass, rye grass, timothy grass, cultivated rye, and velvet grass], birch, and mugwort) and food (egg white, cow's milk, fish, wheat, peanut, and soybean) allergens were measured in one central laboratory at the Robert-Koch-Institute, Berlin, Germany, by using the UNICAP 1000 (Phadia AB, Uppsala, Sweden). Atopic sensitization was defined as specific IgE antibodies of at least 0.7 kU/L against *D pteronyssinus*, cat, or birch or a positive reaction (0.35 kU/L) to the grass mix.

Statistical analyses

For further information on statistical analyses, see the Methods section in this article's Online Repository at www.jacionline.org.

For phase I, categorical variables are presented as relative frequencies; P values are based on the Pearson χ^2 test. A latent class analysis (LCA) was used to derive different types of farming, the association of which with outcomes was then analyzed by using logistic regression analysis. For phase II, all questionnaire-based farm-related exposures were dichotomized into presence or absence of the exposure based on an exposure frequency of at least once a week in a specific time period. Early-life exposure was then defined as the presence of the exposure in pregnancy or the first 3 years of life. Correlation between these farm-related exposure variables was assessed by using the Kendall tau-b correlation coefficient. Diversity of farm exposures was defined by summing up all dichotomous farm exposures and division into quartiles based on the weighted distribution in the study sample. Categorical variables are presented as weighted relative frequencies and compared over categories by using the Rao-Scott χ^2 test. Weighted logistic regression models were used to calculate associations between outcomes and farm-related exposures. Stepwise logistic regression analyses were calculated to assess final models containing the most relevant exposures. Combined effects of all dichotomized farm-related exposure variables defined as 4-level categorical variables were included in this process. All models were adjusted for farming, center, and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). Statistical analysis was performed with SAS 9.2 software (SAS Institute, Inc, Cary, NC), and a P value of .05 was considered significant. Because of the exploratory character of the analysis, corrections for multiple testing were not performed.

RESULTS

In phase I, 132,518 recruitment questionnaires were distributed, of which 79,888 (60.3%) were returned. Of those, 34,491 (43.2%) parents provided written informed consent for blood sampling, genetic testing, and dust sampling. Their children were eligible for phase II (Fig 1); mean age was 8.7 ± 1.4 years. Of these, 9,668 were randomly selected for phase II by exposure stratum (ie, farm children, exposed nonfarm children, and unexposed nonfarm children), and 8,419 (87%) returned the detailed phase II questionnaire. Of these participants, 7,682 (91%) provided blood samples for measurements of specific IgE levels. Families participating in phase II were of higher education and had more allergic illnesses in the family, as also observed in other studies.[11]

A lower prevalence of asthma, hay fever, atopic dermatitis, and atopic sensitization was found among farm children compared with nonfarm children in phases I and II (Table I), with the exposed nonfarm children having intermediate prevalences. After adjusting for confounding variables, the adjusted odds ratios (aORs) for asthma, hay fever, and atopic sensitization with farming status (farm vs nonfarm) were as follows: 0.68 (95% CI, 0.59-0.78; $P < .001$), 0.43 (95% CI, 0.36-0.52; $P < .001$), and 0.54 (95% CI, 0.48-0.61; $P < .001$), respectively. For atopic dermatitis, the farm effect only amounted to an aOR of 0.80 (95% CI,

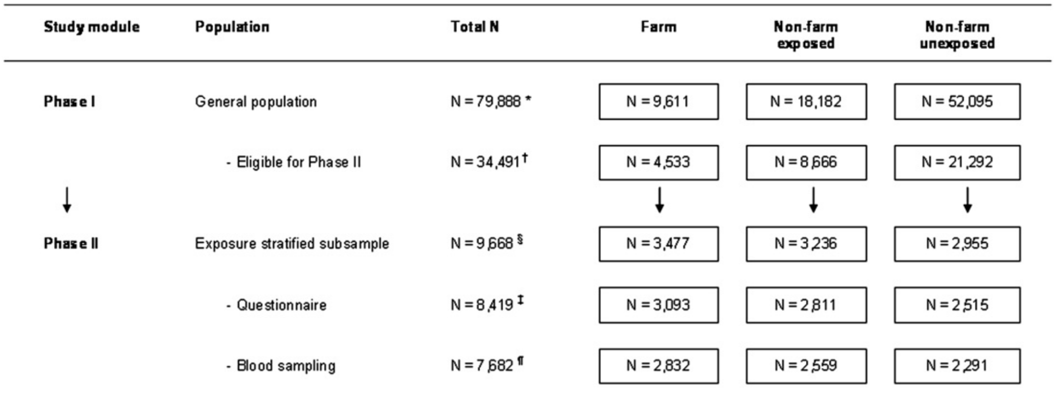

FIG 1. Study population and design. *Completed phase I recruiting questionnaire. †Completed phase I recruiting questionnaire and signed a consent form for analyses and all additional investigations in phase II. §Random selection stratified for exposure. ‡Completed phase II questionnaire. ¶Completed phase II questionnaire and participated in blood sampling and analysis of specific IgE levels.

TABLE I. Prevalence of asthma, other allergic illnesses, and atopic sensitization, as well as specific farm exposures among farm children compared with exposed and unexposed nonfarm children

		Nonfarm children		
	Farm children	Exposed	Unexposed	
Phase I*				
Atopic dermatitis	10.6%	14.4%	14.5%	†
Hay fever	4.8%	10.5%	14.7%	†
Asthma	11.4%	15.8%	18.3%	†
Current wheeze	6.7%	9.7%	11.7%	†
Phase II‡				
Atopic dermatitis	12.8%	17.3%	18.0%	‖
Hay fever	6.4%	11.6%	18.2%	‖
Atopic sensitization§	24.5%	35.5%	43.1%	‖
Asthma	14.1%	20.0%	22.2%	‖
Current wheeze	8.8%	12.6%	15.0%	‖
Atopic§	4.7%	7.5%	8.7%	‖
Nonatopic§	3.5%	5.2%	6.3%	‖
Severe wheeze	1.7%	2.9%	3.6%	‖
Phase II‡¶				
Contact with cows	70.7%	31.0%	5.2%	‖
Stay in cow shed	67.6%	24.3%	2.7%	‖
Contact with straw	64.9%	24.1%	3.8%	‖
Stay in barn	73.4%	28.5%	4.0%	‖
Stay in storage room	30.6%	7.0%	0.8%	‖
Consumption of farm milk	70.9%	51.1%	5.3%	‖

*Phase I population: n = 79,888.
†$P < .001$ of the Pearson χ^2 test for farm versus nonfarm children.
‡Phase II population: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
§Reduced phase II population: n = 7,682 because of reduced sample size for blood sampling; analyses weighted to eligible subjects for phase II (n = 34,491).
‖$P < .001$ of the Rao-Scott χ^2 test for farm versus nonfarm children.
¶Farm exposures in pregnancy and the first 3 years of life assessed in the phase II questionnaire.

0.69-0.93; $P = .004$). The protective farm effect was seen for all asthma phenotypes: asthma, current wheeze, current atopic wheeze, current nonatopic wheeze, and severe wheeze.

In phase I farm characteristics with respect to animal breeding, cultivation, and animal feeding were assessed within the group of farm children. By using LCA, 3 types of farms were identified (Fig 2). The first type comprised farms without dairy cows or cattle breeding. These farms typically kept other animals, such as pigs, poultry, or horses, combined with cultivation of grain and feeding of grain shred. The second type of farming comprised farms with dairy cows and cattle breeding but nearly no cultivation. In contrast, the third farm type typically comprised those that kept dairy cows and bred cattle combined with cultivation, mostly of grain and corn. Farmers of the latter group also typically fed corn silage and grain shred to their animals. When assessing the association of the 3 types of farming with asthma, hay fever, atopic dermatitis, and atopic sensitization within the group of farm children, a protective effect of the third type of farming on asthma, hay fever, and atopic dermatitis was observed (Table II). For atopic sensitization, only a nonsignificant protective trend was observed, potentially because of the reduced sample size in phase II.

In contrast to phase I assessing farm characteristics irrespective of whether the child itself was actually exposed, in phase II the child's exposure to specific farm characteristics was assessed. First contact with these farm exposures typically occurred early in life, especially in pregnancy and the second to third year of life (Fig 3). Therefore in all subsequent analyses the timing of farm exposures relates to the period from pregnancy to the third year of life. Many of these exposures, such as contact with cows, other farm animals, or animal fodder, the consumption of cow's milk produced on a farm, and the child's presence in stables, barns, or fodder storage rooms, were inversely related to asthma, hay fever, atopic dermatitis, and atopic sensitization, even when adjusting for farming (Table III). Children were often exposed to several factors, although correlations between different factors were only moderate, with somewhat higher correlations for exposure to grass, hay, and straw (tau-b correlation coefficient, ≥0.7; data not shown). Still, many of the assessed exposures showed a strong overlap (eg, 75% of the children that "were present while the parents are manuring" also had contact with both cows and straw), requiring multivariate selection procedures to identify relevant exposures.

Therefore a stepwise variable selection process was performed. In the resulting final multivariate models, only few farm exposures remained inversely related to asthma, hay fever, atopic

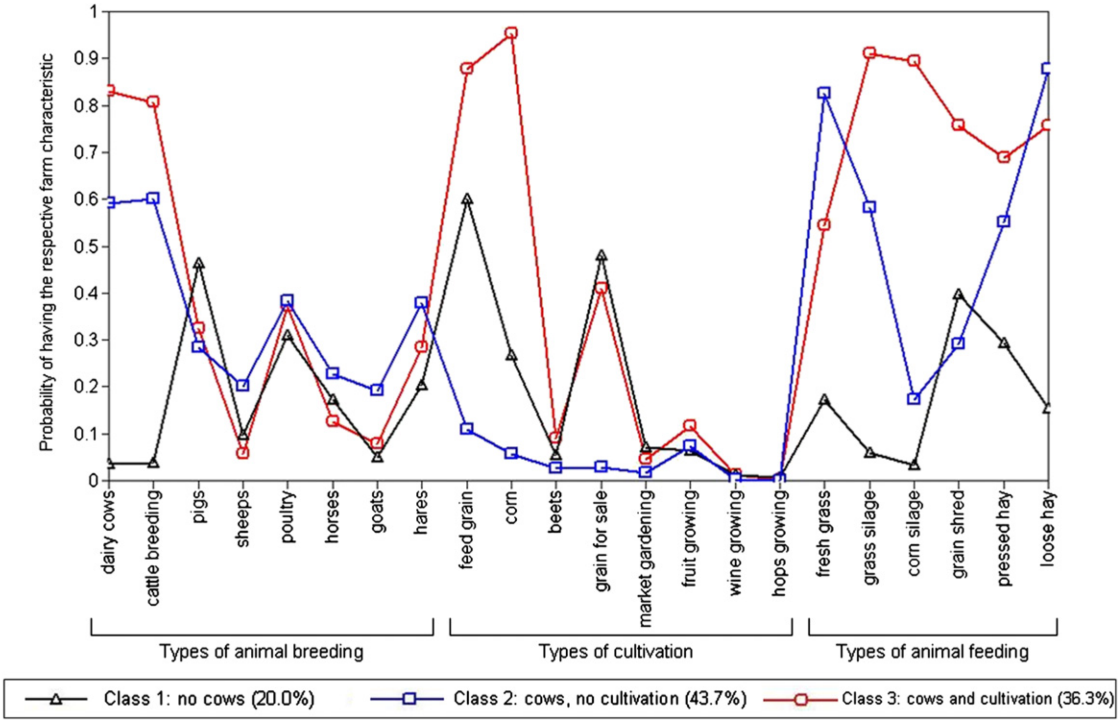

FIG 2. Types of farms based on farm characteristics. Results of LCA with 3-class solution are shown. Farm characteristics assessed in the phase I recruitment questionnaire are shown (n = 9611 farm children).

TABLE II. Types of farms and risk of asthma, hay fever, atopic dermatitis, and atopic sensitization

	Asthma*			Hay fever*			Atopic dermatitis*			Atopic sensitization†		
Farm type	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value	aOR‡	95% CI	*P* value
No cows	1.00	—	—	1.00	—	—	1.00	—	—	1.00	—	—
Cows, no cultivation	0.84	0.69-1.03	.09	0.94	0.70-1.26	.68	0.78	0.64-0.96	.02	1.01	0.78-1.32	.94
Cows and cultivation	0.79	0.65-0.95	.01	0.70	0.53-0.94	.02	0.75	0.62-0.91	.004	0.82	0.64-1.06	.13

*Outcomes assessed in phase I recruitment questionnaire (n = 9611 farm children).
†Outcome assessed in phase II blood sampling (n = 2832 farm children).
‡Adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking, and parental education).

dermatitis, and atopic sensitization (Fig 4; data are shown in Table E2 in this article's Online Repository at www.jacionline.org). Concurrent contact with cows and straw and the consumption of cow's milk produced on the farm were independent protective factors for asthma. The farm effect aORs increased from 0.68 (95% CI, 0.59-0.78) to 0.89 (95% CI, 0.75-1.06) after inclusion of the relevant farm exposures, suggesting that they accounted for most of the farm effect. When stratifying the analysis into atopic and nonatopic children, the variables selected into the final model remained unchanged in the group of nonatopic subjects, whereas only farm milk remained in the model as a significant protective factor for asthma among atopic children. "Being present while the parents are manuring" showed the lowest odds ratios for all outcomes except atopic sensitization. However, in the multivariate model, when including contact with cows and with straw, manuring was no longer significant.

Similarly to asthma, protective farm exposures remaining in the final multivariate model for hay fever were contact with cows and consumption of farm milk. However, in contrast to asthma, contact with straw was no longer significant in the final model, even in combination with concurrent contact with cows. The farm effect aOR increased from 0.43 (95% CI, 0.36-0.52) to only 0.68 (95% CI, 0.55-0.84), indicating the presence of additional undetected protective exposures in the farming environment.

For atopic sensitization, contact with straw and the consumption of cow's milk produced on the farm were significant independent protective determinants in the final model (Fig 4). Similarly to hay fever, the aOR for farming only increased from 0.54 (95% CI, 0.48-0.61) to 0.74 (95% CI, 0.64-0.86). Exposure to poultry and dogs early in life additionally contributed to the model when defining atopic sensitization at a higher cutoff (≥3.5 kU/L).

With respect to atopic dermatitis, only very few distinct questionnaire-based farm exposures were significantly protective after adjusting for farming and potential confounders. Of these, only staying in a fodder storage room remained in the final model, with the effect of farming being no longer significant. In contrast to the other phenotypes, onset of atopic dermatitis typically occurs in infancy, with an increased potential role of exposures in pregnancy. We thus repeated all analyses for exposures in pregnancy only. The maternal exposures inducing the greatest change in the effect of farming on atopic dermatitis were staying in a cow shed and manuring during pregnancy. In contrast, when

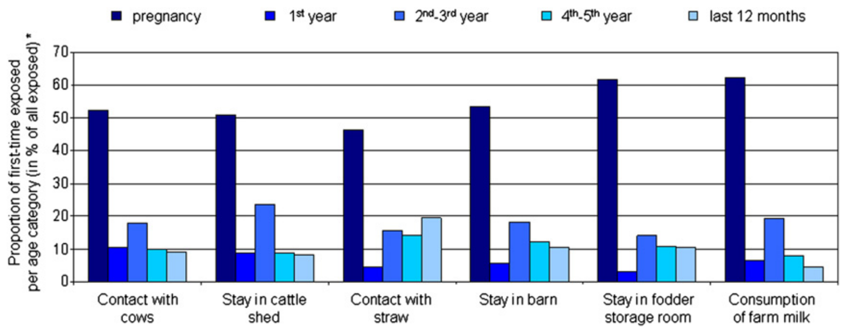

FIG 3. Timing of the first exposure to farm characteristics. Most children experienced their first exposure through their mothers in pregnancy. *Computations are based on 5 groups of children ever exposed to cows, cow sheds, straw, barns, fodder storage rooms, or farm milk. The *bars* show the proportion of children with first-time contact with the respective farm exposure per age category. Proportions of the 5 age categories add up to 100%.

including only maternal exposures during pregnancy for asthma, hay fever, and atopic sensitization, factors remaining in the final models were unchanged. Atopic dermatitis was merely defined as a doctor's diagnosis because this was assessed in phase I, whereas corresponding symptoms were only assessed in phase II. When using an outcome variable combining diagnosis and symptoms, the final multivariate models remained unchanged, except that manuring additionally remained in the model.

For the assessment of the diversity of exposures, a score was generated by summing up all dichotomous farm exposures. This diversity score was significantly associated with atopic sensitization: aORs of 0.79 (95% CI, 0.65-0.97; $P = .03$) for 2 to 4 exposures (third quartile) and 0.65 (95% CI, 0.52-0.80; $P < .001$) for 5 to 23 exposures (fourth quartile) versus no exposure (first quartile). However, when adjusting the final models for diversity, it was no longer significant, and the association of contact with straw and consumption of farm milk with the outcome remained basically unchanged (see Table E3 in this article's Online Repository at www.jacionline.org).

Sensitivity analyses investigating the individual contribution of prenatal and postnatal exposure for asthma, hay fever, and atopic sensitization showed some differences between factors but in general suggested that both periods were of importance, showing similar effects for exposure in pregnancy and in the first 3 years of life (data not shown). Furthermore, a dose-response relationship was seen (ie, stronger protection with increased frequency of exposures; see Fig E1 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Children growing up on farms in Germany, Austria, and Switzerland are protected against asthma, hay fever, and atopic sensitization. Only 3 distinct farm exposures assessed by means of questionnaire (ie, the pregnant mother's and subsequently the toddler's exposure to cows and straw and the consumption of cow's milk produced on the farm) accounted for the farm effect on asthma and partially on hay fever and atopic sensitization.

The protective effect of a farm environment on atopic dermatitis was much less pronounced than for the other outcomes. This discrepancy has already been observed in previous studies on farming and is in line with results from the German International Study of Asthma and Allergies in Childhood, in which atopic dermatitis showed no strong associations with environmental factors, indicating that the hygiene hypothesis might not hold for atopic dermatitis as much as for respiratory allergic diseases.[10,12]

The definition of asthma for population-based studies has been vividly debated. We used a broader asthma definition, including diagnosis, symptoms, and treatment, to also include milder and nonatopic phenotypes, as well as more specific definitions of current, atopic and nonatopic, and severe wheeze. Farm children were at lower risk of any of these phenotypes compared with nonfarm children, potentially indicating antiviral properties of the protective exposures. When using the International Study of Asthma and Allergies in Childhood's definition (doctor's diagnosis of asthma or recurrent wheezy bronchitis), as in previous farm studies, the farm effect remained unchanged (6.5%, 9.0%, and 10.5% in farmers and exposed and unexposed nonfarmers, respectively; $P < .001$) and was of similar magnitude as in the previous farm studies ALEX (Allergy and Endotoxin) and PARSIFAL (Prevention of Allergy–Risk Factors for Sensitization In Children Related to Farming and Anthroposophic Lifestyle) (see Table E4 in this article's Online Repository at www.jacionline.org).[8,13,14]

In cooperation with farmers and field workers coming from a farm environment, we developed an extensive questionnaire to assess the large spectrum of potential exposures that a child might encounter on a farm over the first years of life. The most relevant farm exposures were then selected into a final multivariate model through a stepwise statistical procedure based on the change in estimate of farming: the closer to the null effect the overall farming effect became when a specific farm exposure was additionally included in the model, the more likely it was to account for this farm effect. This method was very robust with respect to the selection of the final set of exposure variables. The standard stepwise variable selection procedure that merely uses the P value as an inclusion criterion resulted in the same final models, irrespective of whether farming and potentially confounding variables were forced into the model in the selection process.

In this newly developed comprehensive questionnaire, the child's contact with all types of animal feeding was assessed. The strongest protective effect on all outcomes except atopic dermatitis was seen for contact with straw. Straw is an agricultural byproduct of cereal plants (ie, the dry stalks after the grain has been removed) and is mostly used as bedding material for animals

TABLE III. Farm exposures (pregnancy to age 3 years) associated with decreased risk of asthma, hay fever, atopic dermatitis, and atopic sensitization†

	Asthma‡				Hay fever‡				Atopic dermatitis‡				Atopic sensitization§			
	aOR‖	95% CI	P value		aOR‖	95% CI	P value		aOR‖	95% CI	P value		aOR‖	95% CI	P value	
Contact with animals																
Cat	0.90	0.77-1.04	.16	#	0.92	0.77-1.10	.37	#	0.85	0.72-1.01	.06	¶	**0.81**	**0.71-0.93**	**.003**	#
Dog	0.99	0.84-1.15	.86		0.90	0.74-1.10	.31	#	0.88	0.74-1.05	.15		**0.85**	**0.74-0.97**	**.02**	#
Cow	**0.74**	**0.62-0.89**	**.002**	*,¶,#	**0.52**	**0.41-0.66**	**<.001**	*,¶,#	0.87	0.71-1.06	0.17	¶,#	**0.75**	**0.65-0.88**	**<.001**	*,¶,#
Pig	0.89	0.70-1.14	.36	¶	0.76	0.53-1.07	.12	¶,#	0.98	0.77-1.26	.89		0.87	0.70-1.07	.18	
Poultry	0.95	0.77-1.17	.63		**0.72**	**0.54-0.95**	**.02**	*,¶,#	0.95	0.76-1.17	.61		**0.76**	**0.64-0.91**	**.003**	#
Sheep	0.79	0.62-1.02	.07		0.74	0.52-1.05	.09	#	0.91	0.69-1.21	.53		0.84	0.68-1.04	.12	
Horse	1.13	0.89-1.43	.30		0.95	0.69-1.29	.73		**1.33**	**1.05-1.70**	**.02**		**0.79**	**0.63-0.99**	**.04**	
Stay in animal sheds																
Cow	**0.79**	**0.65-0.95**	**.01**	*,¶	**0.66**	**0.52-0.85**	**.001**	*,¶,#	0.82	0.67-1.01	.06	¶,#	**0.78**	**0.67-0.92**	**.003**	*,¶,#
Pig	1.04	0.81-1.33	.78		0.72	0.51-1.02	.06	¶,#	0.99	0.76-1.29	.92		0.85	0.68-1.06	.14	
Poultry	0.92	0.74-1.15	.48	¶	0.89	0.66-1.20	.44	#	0.91	0.72-1.15	.42	¶	0.84	0.69-1.01	.06	#
Contact with animal feed																
Straw	**0.79**	**0.66-0.95**	**.01**	*,¶	**0.61**	**0.47-0.80**	**<.001**	*,¶,#	0.83	0.67-1.02	.07	¶,#	**0.61**	**0.52-0.72**	**<.001**	*,¶,#
Hay**	0.87	0.73-1.04	.14	¶,#	**0.78**	**0.63-0.98**	**.03**	*,¶,#	0.91	0.76-1.10	.35	¶,#	**0.74**	**0.63-0.86**	**<.001**	*,¶,#
Grain**	0.93	0.76-1.14	.49		0.91	0.68-1.21	.52	#	0.91	0.73-1.14	.43	¶,#	**0.72**	**0.61-0.86**	**<.001**	*,¶,#
Corn**	0.86	0.67-1.09	.21	¶,#	0.88	0.64-1.20	.41	#	0.84	0.66-1.06	.14	¶	**0.78**	**0.64-0.95**	**.01**	#
Corn silage**	0.81	0.61-1.07	.14	¶,#	0.72	0.47-1.09	.12	¶,#	**0.70**	**0.54-0.93**	**.01**	*,¶,#	0.84	0.67-1.04	.11	#
Grass	0.95	0.79-1.13	.56		0.82	0.65-1.03	.09	¶,#	0.86	0.70-1.04	.11	¶,#	**0.82**	**0.70-0.96**	**.01**	
Grass silage**	0.96	0.76-1.21	.72	#	0.73	0.52-1.02	.07	¶,#	0.79	0.62-1.01	.06	¶,#	**0.79**	**0.65-0.95**	**.01**	*,¶,#
Pellet feed	0.84	0.68-1.05	.13		0.77	0.54-1.09	.13	#	0.98	0.77-1.25	.85		**0.75**	**0.62-0.92**	**.005**	#
Sugar beet	1.19	0.82-1.73	.36		0.97	0.52-1.81	.92	#	0.95	0.62-1.46	.82		0.76	0.53-1.09	.14	
Stay in —																
Barn	0.87	0.72-1.04	.13		**0.62**	**0.48-0.80**	**<.001**	*,¶,#	0.86	0.70-1.05	.14	¶,#	**0.70**	**0.59-0.82**	**<.001**	*,¶,#
Fodder storage room	0.94	0.72-1.23	.65	#	0.72	0.49-1.07	.10	¶,#	**0.72**	**0.55-0.93**	**.01**	*,¶	**0.79**	**0.64-0.98**	**.03**	
Present while parents are —																
Doing field work	1.09	0.82-1.44	.57		0.91	0.59-1.41	.68		0.85	0.62-1.19	.35	¶	0.83	0.65-1.06	.14	
Manuring	**0.65**	**0.47-0.90**	**.01**	#	**0.51**	**0.33-0.80**	**.003**	*,¶	**0.66**	**0.45-0.96**	**.03**	*,¶,#	0.85	0.65-1.11	.23	
Spraying pesticides	1.22	0.32-4.62	.77		1.00	0.12-8.14	1.00		0.74	0.27-2.07	.57		1.45	0.52-4.02	.48	
Consumption of —																
Farm milk	**0.77**	**0.66-0.90**	**.001**	*,¶,#	**0.64**	**0.53-0.77**	**<.001**	*,¶,#	0.89	0.76-1.06	.18	¶	**0.73**	**0.64-0.84**	**<.001**	*,¶,#

Significant results are shown in boldface.
*Variable included in subsequent stepwise analyses for the respective outcome (criteria: significant results and ≥10% change in aOR of farming toward the null effect).
†Farm exposures assessed in phase II questionnaire.
‡Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
§Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).
‖Adjusted for center, farming, and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).
¶Ten percent or greater change in aOR of farming toward the null effect.
#Significant aOR within strata of farmer's children.
**Combination of several questionnaire items: *Hay*, contact with forage hay or present while parents were harvesting or handling hay; *Grain*, contact with forage grain or present while parents were harvesting, threshing, or kibbling grain; *Corn*, contact with forage corn or present while parents were harvesting or kibbling corn; *Corn silage*, contact with forage corn silage or present while parents are ensiling corn; *Grass silage*, contact with forage grass silage or present while parents are ensiling grass.

in the study areas. Children are exposed either in barns or in the stable when litter is placed and aerosolized or removed. However, children exposed to straw were often also exposed to hay, grass, and manure. Therefore individual effects of grass, hay, manure, and straw could not be disentangled with certainty. Recent experimental studies have shown that the oligosaccharide arabinogalactan from grass and hay protects mice against allergic asthma.[15] Cereal, the source material of straw, also contains arabinogalactan, suggesting that exposure to this plant-derived oligosaccharide might protect children against asthma and atopy.[16] Alternatively or additionally, thus far unidentified microbial exposures associated with hay and straw might explain the effect. Straw has been shown to be contaminated with a high variety of fungi and bacteria.[17]

Consumption of cow's milk produced on the farm also showed a consistently strong inverse relation with 3 of the outcomes: asthma, hay fever, and atopy. This corroborates previous findings.[8,18,19] Refined analyses on the handling of milk samples by parents (boiling or skimming) and content of microbes, fat, protein, and various enzymes have been reported separately.[6] It is important to note that the effect of consumption of cow's milk produced on the farm was independent of the protective effect of contact with cows, potentially indicating different pathways: whereas milk exerts its effect through the gut, contact with cows might potentially be an inhaled exposure affecting the airway mucosa.

This notion of different pathways is supported by the fact that contact with cows only exerted a strong effect on outcomes involving the airways (ie, on asthma, including nonatopic asthma [data not shown] and on hay fever). No such effect was observed for atopy in the final model. The effect of contact with cows on hay fever was independent of other protective exposures, such as

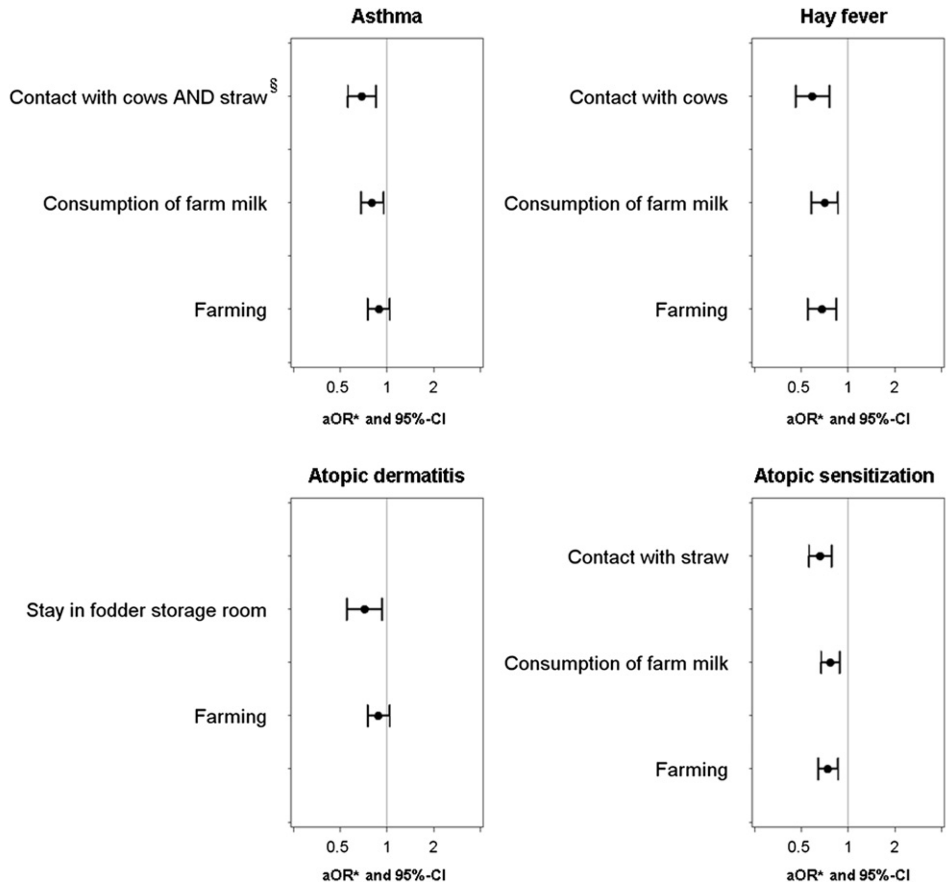

FIG 4. Specific farm exposures that best explain the overall effect of farming. Results of multivariate stepwise weighted regression models. *Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). §Compared with the reference group (neither contact with cows nor straw). Odds ratios for intermediate categories (contact with cows or straw) are shown in Table E2.

contact with straw. This was in contrast to the effect on asthma: contact with cows was only inversely associated with asthma if the child also had contact with straw, potentially reflecting a specific type of farming accounting for this combined protective effect. No such interaction was observed for hay fever or atopy. This might explain why previous surveys on children from dairy farms have come to similar and homogeneous results for hay fever and atopy but have shown conflicting results with respect to protection against asthma[10,20]: perhaps specific combinations of exposures not investigated in previous studies are essential to exert a protective effect on asthma. Interestingly, similar effects were observed irrespective of whether characteristics of the farm were assessed or farm exposures of the child: both approaches resulted in a combination of cows and products of cultivation (eg, straw) as factors best explaining the overall farm effect. These results are not only observed in Alpine but also in other European areas, eg, in the Polish arm of the GABRIEL Advanced Studies (results will be reported separately). This points toward a protective effect of the traditional way of farming as it has been pursued for centuries, comprising cows, their products (eg, milk), and cultivation of grains both for alimentation and bedding material. From an evolutionary perspective, mankind has been exposed to these since settling down. Immune responses adapted to this prevailing environment might thus induce tolerance. Therefore it is not surprising that some of the farm effects observed in Central Europe are not seen in the United States because the type of farming differs greatly between continents.

The detailed questionnaire not only assessed the type of exposure but also both its time period and frequency. For most exposures, first contact in the child's life most frequently occurred during pregnancy and the second to third years of life, indicating mothers working on a farm. Furthermore, when analyzing the association of timing and outcomes, the effects of exposures early in life (ie, from pregnancy up to age 3 years, as shown in this article) showed much stronger effects than current exposure at the time of outcome assessment (data not shown). This correlates with findings from other studies that observed an effect of farm exposure in pregnancy on specific IgE levels and cytokine responses in cord blood, indicating a protective farm effect as early as *in utero*.[21-23]

Our results show that protective mechanisms differ for asthma and atopy. The exhaustive questionnaire assessed the child's farm exposures in as detailed a manner as possible. In contrast to asthma, the farm effect on atopy, although about half of it was explained by the questionnaire items, was not completely accounted for by these or their diversity, indicating a link was still missing. This is in line with previous results from the GABRIEL study group observing differing genes involved in the cause of asthma and atopy and discrepant results for the role of microbial diversity: whereas the diversity of bacteria and fungi from dust of farm and nonfarm children accounted for the protective farm effect on asthma, this did not hold for atopy, indicating a potential role of an unknown, ubiquitous protective exposure on farms.[1,2] This unexpected finding is as puzzling as the very consistent protective effect of sibship size on atopy, which has not yet been completely explained by the hygiene hypothesis either.

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Key messages

- **Specific types of farms with cows and cultivation exerted a protective effect on asthma, hay fever, and atopic sensitization.**
- **This protective farm effect on asthma, hay fever, and atopic sensitization was determined by 3 specific early-life exposures of the child, namely contact with cows and straw and consumption of farm milk, thereby narrowing down the farm effect.**
- **Whereas the farm effect on asthma could be explained by contact with cows, straw, and farm milk, this was not the case for hay fever and atopic sensitization, indicating differing underlying protective mechanisms.**

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METHODS

Statistical analyses

For the analysis of the farm effect, exposed and unexposed nonfarm children were combined as nonfarm children and compared with farm children.

All questionnaire-based farm-related exposures were assessed for 5 time periods and 5 frequency categories per time period. For statistical analysis, these data were dichotomized into the presence or absence of the exposure based on an exposure frequency of at least once a week in a specific time period. Early-life exposure was then defined as the presence of the exposure in pregnancy or the first 3 years of life. The correlation between the dichotomized farm-related exposure variables was assessed by using the Kendall tau-b correlation coefficient. For assessment of the diversity of exposures, a score was generated by summing up all dichotomous farm exposures depicted in Table III and dividing the sum into quartiles based on the weighted distribution in the study sample.

Data from phase II were analyzed by using weighted statistical methods, taking the specific stratified sampling design into account. Fixed *a priori* weights were calculated as the inverse of the ratio of selected to eligible children per center and strata. All analyses were weighted to the total *n* value of the study population of phase I eligible for phase II. Missing values in selected children led to slightly diminished numbers per analysis. For the final logistic regression models, a sensitivity analysis was performed by using weights additionally adjusted for missing values in the variables included in the respective model, thus truly weighting the assessed data to the total *n* value. However, results remained unchanged (data not shown).

For phase I, categorical variables are presented as relative frequencies; *P* values are based on the Pearson χ^2 test. For phase II, categorical variables are presented as weighted relative frequencies and compared over categories by using the Rao-Scott χ^2 test, which applies a design effect correction to the Pearson χ^2 statistic computed from the weighted frequencies.

In phase I LCA was used to derive different types of farming.[E1] LCA is a statistical method for finding subtypes of related subjects (latent classes) from multivariable categorical data. Farmers of our study population were clustered into a number of discrete latent classes based on the pattern of response to various questions on farm characteristics (types of animal breeding, cultivation, and animal feeding), as assessed in the phase I questionnaire. The posterior probability of each subject belonging to a particular class was estimated, and from these data, logistic regression was used to estimate associations of the respective classes or "farm types" with asthma, hay fever, atopic dermatitis, and atopic sensitization.

In phase II, weighted logistic regression models using the Taylor series method to estimate variances were used to calculate associations between dichotomous outcomes and farm-related exposures. All models were adjusted for farming, center, and potential confounders differing between farm and nonfarm children (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education). Stepwise logistic regression analyses were calculated to assess final models containing the most relevant exposures to detect specific exposure variables underlying the overall farm effect. The aim of this procedure was to explain the farm effect, and thus all exposure variables that were significant and that induced a change of at least 10% in the effect of farming toward the null-effect in farm- and confounder-adjusted analysis were included in this process. At each forward step of this model-building procedure, the exposure inducing the largest change in estimate for farming was additionally included in the multivariate model if significant. In a backward step variables were removed from the model if no longer significant. The model building ended if no additional exposure was significant if included in the model. Combined effects of all dichotomized farm related exposure variables were defined as 4-level categorical variables to detect exposures that only exert an effect if occurring concurrently with another exposure: (− −), both variables negative (reference category for statistical analysis); (+ −)/(− +), 1 variable positive; and (+ +), both variables positive. If these combined exposures induced a change of 10% or greater in the farm effect and if only the (+ +) category was significant in farm- and confounder-adjusted analysis, as well as the overall type III *P* value, this categorical variable was included in the stepwise procedure based on the type III *P* value and the change in farm effect. For phase I and phase II analyses within the group of farm children, unweighted center- and confounder-adjusted logistic regression models using the same method to estimate variances as for weighted analyses were applied. aORs and 95% CIs are reported.

Statistical analysis was performed with SAS 9.2 software (SAS Institute, Inc); a *P* value of .05 was considered significant. Because of the exploratory character of the analysis, corrections for multiple testing were not performed.

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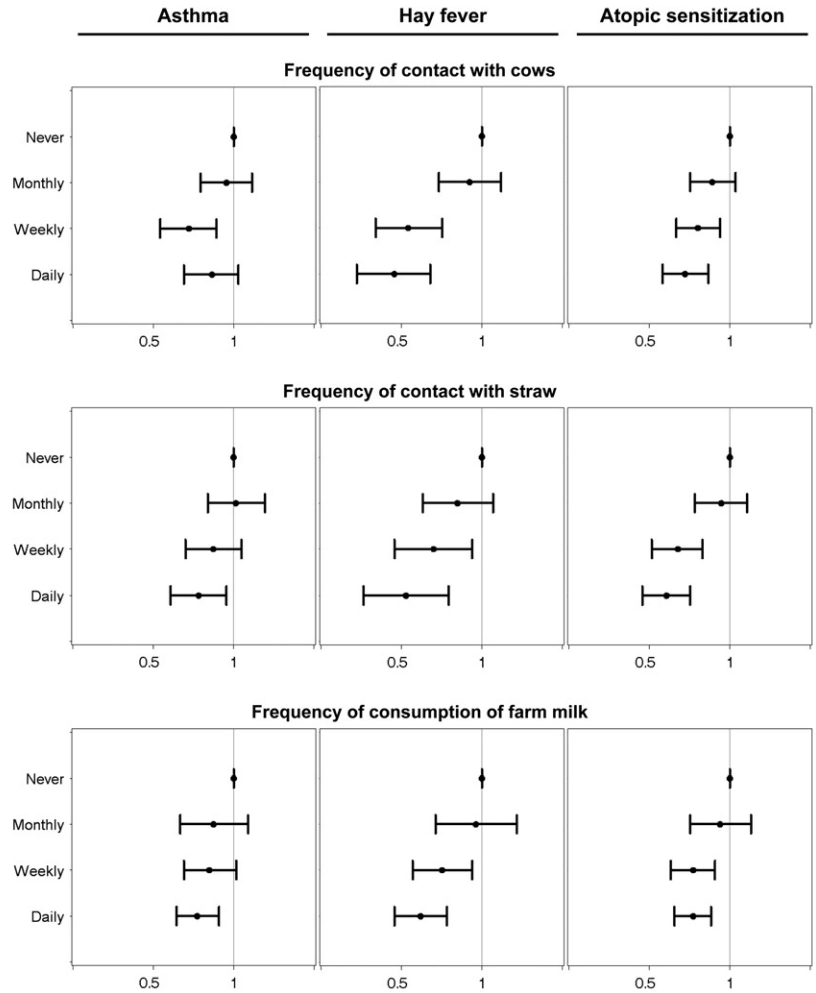

FIG E1. Frequency of exposure and risk of asthma, hay fever, and atopic sensitization. Frequency of exposure is defined as the maximum exposure of the 3 time periods (pregnancy, first year of life, and second to third year of life). aORs and 95% CIs are adjusted for farming and potential confounders.

TABLE E1. Previous publications from the GABRIEL Study Consortium

Publication	Title/key message
Moffatt et al,[E2] N Engl J Med 2010	A large-scale, consortium-based, genome-wide association study of asthma
	→ This genome-wide association study found little overlap between the principal loci that confer susceptibility to asthma and those that regulate total serum IgE levels. This suggests that an increase in IgE level is probably an inconstant secondary effect of asthma rather than its cause.
Ege et al,[E3] N Engl J Med 2011	Exposure to environmental microorganisms and childhood asthma
	→ Children living on farms were exposed to a wider range of microbes than were children in the reference group. This exposure explained a substantial fraction of the inverse relation between asthma and growing up on a farm. In contrast, atopy was only weakly associated with the diversity of microbes.
Ege et al,[E4] J Allergy Clin Immunol 2011	Gene-environment interaction for childhood asthma and exposure to farming in Central Europe
	→ A genome-wide interaction analysis revealed several novel interaction candidate genes for asthma and atopy in a farming environment. In turn, the top single nucleotide polymorphisms of a meta-analysis for childhood asthma did not interact with farming. Previously published interactions with farming-related exposures for asthma and atopy were not replicated.
Normand et al,[E5] Occup Environ Med 2011	Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings
	→ Microorganisms are transported from animal sheds and barns into farm dwellings. Therefore children living in these environments are exposed when indoors and when visiting animal sheds and barns. Indoor exposure might also contribute to the protective effect of the farm environment.
Genuneit et al,[E6] Paediatr Perinat Epidemiol 2011	The GABRIEL Advanced Surveys: study design, participation, and evaluation of bias
	→ The GABRIEL Advanced Surveys are one of the largest studies to shed light on the protective "farm effect" on asthma and atopic disease. Bias with regard to the main study question was able to be ruled out by representativeness and high participation rates in phases 2 and 3. The GABRIEL Advanced Surveys have created extensive collections of questionnaire data, biomaterial, and environmental samples, promising new insights into this area of research.
Loss et al,[E7] J Allergy Clin Immunol 2011	The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study
	→ Questionnaire-reported consumption of unboiled but not boiled farm milk was inversely associated with asthma, hay fever, and atopy. Higher levels of the whey proteins BSA, α-lactalbumin, and β-lactoglobulin in milk samples were associated with a reduced risk of asthma but not atopy. Neither total viable bacterial counts nor total fat content of milk were related to asthma or atopy.
MacNeill et al, Allergy 2011, submitted	Asthma and allergies: Is the farming environment (still) protective in Poland? The GABRIEL Advanced Studies
	→ This cross-sectional survey of schoolchildren in rural Poland showed that living on certain types of farms is significantly protective against atopic sensitization. Early-life exposure to grain might explain part of this effect.
Fuchs et al, J Allergy Clin Immunol 2011, in revision	Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide
	→ The protective farm effect on wheeze prevalence is independent of atopy and not attributable to improved airway size and lung mechanics. Underlying protective mechanisms include alterations of immune response and susceptibility to likely viral triggers of childhood airway disease also affecting airway inflammation.
Illi et al, J Allergy Clin Immunol 2012	Protection against childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies
	→ Specific types of farms with cows and cultivation exerted a protective effect on asthma, hay fever, and atopic sensitization. This protective farm effect on asthma, hay fever, and atopic sensitization was determined by 3 specific early-life exposures of the child, namely by contact with cows and straw and consumption of farm milk, thereby narrowing down the farm effect. However, whereas the farm effect on asthma could be completely explained by these, this was not the case for hay fever and atopic sensitization, indicating differing underlying mechanisms in spite of comanifestation of these outcomes.

TABLE E2. Specific farm exposures that best explain the overall effect of farming on asthma, hay fever, atopic dermatitis, and atopic sensitization, as identified in multivariate stepwise regression models*

	aOR§	95% CI	*P* value
Asthma†			
− Contact with cows, − contact with straw	1.00	—	—
− Contact with cows, + contact with straw	1.00	0.76-1.32	1.00
+ Contact with cows, − contact with straw	0.94	0.73-1.21	.63
+ Contact with cows, + contact with straw	0.68	0.54-0.85	<.001
Consumption of farm milk	0.81	0.68-0.96	.02
Farming	0.89	0.75-1.06	.20
Hay fever†			
Contact with cows	0.59	0.46-0.76	<.001
Consumption of farm milk	0.71	0.58-0.86	<.001
Farming	0.68	0.55-0.84	<.001
Atopic dermatitis†			
Stay in fodder storage room	0.72	0.55-0.93	.01
Farming	0.88	0.75-1.04	.12
Atopic sensitization‡			
Contact with straw	0.66	0.56-0.78	<.001
Consumption of farm milk	0.77	0.67-0.88	<.001
Farming	0.74	0.64-0.86	<.001

*Weighted logistic regression models with stepwise variable selection for asthma, hay fever, atopic dermatitis, and atopic sensitization based on the largest change in estimate for farming after adjusting for confounding variables. All significant exposure variables (pregnancy to age 3 years) from previous farm- and confounder-adjusted analyses that induced a change in estimate of farming of 10% or greater toward the null effect were included in the selection process.
†Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
‡Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).
§Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).

TABLE E3. Final multivariate models for asthma, hay fever, atopic dermatitis, and atopic sensitization adjusted for diversity score*

	aOR§	95% CI	*P* value
Asthma†			
− Contact with cows, − contact with straw	1.00	—	.08
− Contact with cows, + contact with straw	0.90	0.60-1.37	.64
+ Contact with cows, − contact with straw	0.90	0.64-1.26	.53
+ Contact with cows, + contact with straw	0.66	0.46-0.96	.03
Consumption of farm milk	0.72	0.59-0.89	.002
Farming	0.97	0.80-1.18	.76
Diversity score*			
0	1.00	—	.66
1	1.09	0.86-1.37	.47
2-4	1.17	0.91-1.51	.21
≥5	1.13	0.77-1.66	.53
Hay fever†			
Contact with cows	0.57	0.42-0.77	<.001
Consumption of farm milk	0.62	0.50-0.78	<.001
Farming	0.71	0.55-0.92	.009
Diversity score*			
0	1.00	—	.42
1	1.11	0.85-1.44	.45
2-4	1.24	0.94-1.64	.13
≥5	1.28	0.90-1.82	.16
Atopic dermatitis†			
Stay in fodder storage room	0.72	0.52-0.98	.04
Farming	0.98	0.79-1.21	.84
Diversity score*			
0	1.00	—	.29
1	0.89	0.69-1.14	.35
2-4	1.02	0.80-1.31	.87
≥5	0.82	0.63-1.06	.14
Atopic sensitization‡			
Contact with straw	0.66	0.53-0.83	<.001
Consumption of farm milk	0.77	0.65-0.91	.002
Farming	0.79	0.67-0.94	.009
Diversity score*			
0	1.00	—	.57
1	0.96	0.78-1.18	.69
2-4	0.86	0.69-1.06	.17
≥5	0.92	0.70-1.20	.53

*The diversity score is defined as the number of exposures divided into quartiles based on the weighted distribution in the study sample.
†Outcomes assessed in phase II questionnaire: n = 8,419; analyses weighted to eligible subjects for phase II (n = 34,491).
‡Outcome assessed in phase II blood sampling: n = 7,682; analyses weighted to eligible subjects for phase II (n = 34,491).
§Mutually adjusted and additionally adjusted for center and potential confounders (family atopy, ≥2 siblings, sex, maternal smoking in pregnancy, and parental education).

TABLE E4. Prevalence of asthma, hay fever, and atopic dermatitis diagnoses and atopic sensitization in farm studies

	ALEX*		PARSIFAL†		GABRIEL phase I		GABRIEL phase II‖	
	Farm children‡	Nonfarm children	Farm children§	Nonfarm children	Farm children§	Nonfarm children	Farm children§	Nonfarm children
Asthma diagnosis ever	5.4%	11.8%	6.3%	9.1%	6.5%	10.1%	8.3%	12.1%
Hay fever diagnosis ever	5.9%	15.9%	1.3%	4.4%	3.0%	9.5%	3.9%	10.6%
Atopic dermatitis diagnosis ever	—	—	7.1%	9.9%	10.6%	14.5%	12.8%	17.8%
Atopic sensitization¶	17.9%	32.9%	22.7%	34.7%	—	—	24.7%	40.8%

*Riedler et al.[E8]
†Alfven et al.[E9]
‡Farm children were defined as children with contact with farm milk or stables ever.
§Farm children were defined as children currently living on a farm run by the child's family.
‖Weighted prevalences (weighted to GABRIEL phase I).
¶ALEX: IgE ≥3.5 kU/L for house dust/storage mites, cat, grass, birch, and cow; PARSIFAL: IgE ≥0.35 kU/L in Phadiatop or mix of common food allergens (fx5); GABRIEL: IgE ≥0.70 kU/L for house dust mite, cat, and birch or IgE ≥0.35 kU/L for grass mix.