Gene-environment interaction for childhood asthma and exposure to farming in Central Europe

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Background: Asthma is a disease in which both genetic and environmental factors play important roles. The farming environment has consistently been associated with protection from childhood asthma and atopy, and interactions have been reported with polymorphisms in innate immunity genes.
Objective: To detect gene-environment interactions for asthma and atopy in the farming environment.
Methods: We performed a genome-wide interaction analysis for asthma and atopy by using 500,000 genotyped single nucleotide polymorphisms (SNPs) and farm-related exposures in 1708 children from 4 rural regions of Central Europe. We also tested selectively for interactions between farm exposures and 7 SNPs that emerged as genome-wide significant in a large meta-analysis of childhood asthma and 5 SNPs that had been reported previously as interacting with farm exposures for asthma or atopy.
Results: Neither the asthma-associated SNPs nor the SNPs previously published for interactions with asthma showed significant interactions. The genome-wide interaction study did not reveal any significant interactions with SNPs within genes in the range of interacting allele frequencies from 30% to 70%, for which our study was well powered. Among rarer SNPs, we identified 15 genes with strong interactions for asthma or atopy in relation to farming, contact with cows and straw, or consumption of raw farm milk.
Conclusion: Common genetic polymorphisms are unlikely to moderate the protective influence of the farming environment on childhood asthma and atopy, but rarer variants, particularly of the glutamate receptor, metabotropic 1 gene, may do so. Given the limited statistical power of our study, these findings should be interpreted with caution before being replicated in independent farm populations. (J Allergy Clin Immunol 2011;127:138-44.)

Key words: *Childhood asthma, atopy, GWAS, gene-by-environment interaction, candidate genes*

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Abbreviations used
aOR: Adjusted odds ratio
GAS7: Growth arrest specific 7
G*E: Interaction of environment and genotype
GRM1: Glutamate receptor metabotropic 1
GWAS: Genome-wide association study
IOR: Interaction odds ratio
logOR: Logarithm of the odds ratio
OR: Odds ratio
SNP: Single nucleotide polymorphism
TLR: Toll-like receptor

Genome-wide studies of common diseases and quantitative traits have shown that measured or imputed common single nucleotide polymorphisms (SNPs) explain only a small proportion of heritability.[1] For asthma, the situation is less clear, but a similar picture seems to emerge.[2] Complementary explanations for heritability may be found in copy number variation, epigenetic modification, gene expression, and genetic epistasis.[2] A further potential mechanism involves interaction of environment and genotype (G*E).[3] This can be considered a modification of environmental effects by the underlying genotype or modification of genetic effects by the environmental exposure.

In this article, we investigate childhood exposure to farming because its inverse relation to asthma has been consistently found in many populations all over the world.[4] Furthermore, various gene-environment interactions with farming or farm related exposures have been reported for asthma or allergic sensitization from the European Allergy and Endotoxin (ALEX) and Prevention of Allergy Risk factors for Sensitisation In children related to Farming and Anthroposophic Lifestyle (PARSIFAL) studies.[5-8] In addition to testing these previously reported G*E interactions, we assess interactions with farm exposures for asthma-related polymorphisms identified in a recent meta-analysis of genome-wide association studies for childhood-onset asthma[9] and perform the first genome-wide screen for SNPs that may modify the association of childhood asthma and atopy with environmental exposures on Central European farms.

METHODS

Population

The GABRIEL Advanced Studies were carried out as population surveys in 5 rural regions of Europe: Baden-Württemberg and Bavaria in Germany, North/Central Switzerland, Tyrol in Austria, and Lower Silesia in Poland. Because the farm exposure in Poland differed with respect to character and intensity from the other centers, the Polish center was not included in the present analysis. Children age 5 to 13 years were recruited through primary schools. A recruitment questionnaire was sent out to 132,518 children, of whom 79,888 (60.3%) returned their completed questionnaires (phase 1). Of these children, 34,491 were eligible for phase 2 as defined by a documented parental consent to dust sampling, blood sampling, and genetic analyses. To enrich informative observations, a stratified random selection process was applied in 2 steps. In a first step, 9668 children were selected for phase 2 within the 3 exposure strata per center (Fig 1). In the second step, 1708 children were selected for genotyping (genome-wide association study [GWAS] sample) within exposure and outcome strata per center (Fig 1).

Questionnaires and IgE measurements

The questionnaires covered items on general and respiratory health of the children and their families, socioeconomic background, and farm-related exposures. Children were considered "farm children" if they lived on a farm run by their family. All other children were "nonfarm children." The latter group was divided into 2 groups. The "exposed nonfarm children" were exposed at least once a week to animal sheds, hay ricks, or farm milk consumption. All other nonfarm children were coded "unexposed nonfarm children." Farm-related exposures such as contact with farm animals or animal feed were defined present if they occurred at least once a week during pregnancy or the first 3 years of life. Children exposed less frequently or only after the age of 3 years were included in the reference category for the respective exposure. Asthma diagnosis was defined as a reported diagnosis of asthma at least once or of asthmatic bronchitis at least twice established by a physician. The reference category for asthma was no reported diagnosis of asthma ever and a diagnosis of asthmatic bronchitis no more than once. Atopy was defined as detectable allergen specific IgE to *Dermatophagoides pteronyssinus* (d1), cat dander (e1), or birch pollen (t3) at a detection limit of 0.7 kU/L or to a grass mix (gx3) at a detection limit of 0.35 kU/L. IgE to seasonal allergens was made up of IgE to tree pollen such as birch or grass pollen at a detection limit of 0.35 kU/L. The respective reference categories were all children not fulfilling the criteria for the respective atopy outcomes—that is, nondetectable values for any of these IgE specificities. The IgE measurements were performed centrally at the Robert Koch Institute, Berlin, Germany. Atopic asthma was assumed when asthma and atopy were present. Nonatopic asthma was assumed if only asthma but not atopy was present. In both cases, the reference category was "no asthma" irrespective of atopy.

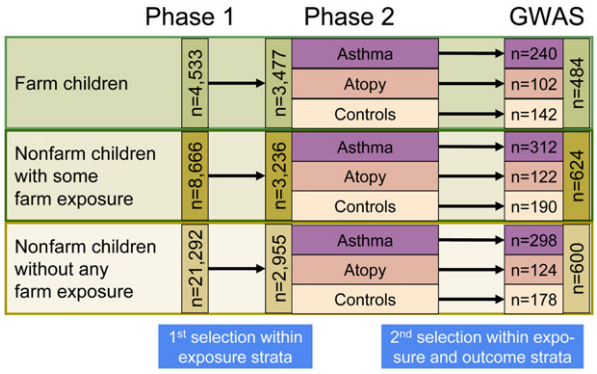

FIG 1. Selection of the study population.

Genotyping

For genotyping, the Illumina Human610 quad array (Illumina Inc, San Diego, Calif, http://www.illumina.com) was used.[9] Genotyping was carried out in a fully automated Illumina beadlab with Tecan robotics and iScans. Raw data were analyzed by using GTS Image with stringent quality control settings and extracted for statistical analysis. Family relationships were confirmed or revised on the basis of the results of an identity-by-state analysis.

The 5 SNPs previously reported in the literature to interact with farming for asthma or atopy were not measured. However, by using the linkage disequilibrium patterns available from the HapMap CEU SNP panel version 2 and the 1000 Genomes pilot 1 release, we identified proxy SNPs that were directly genotyped.

Gene symbols are explained in this article's Table E1 in the Online Repository at www.jacionline.org.

Statistical analysis

For statistical analyses, the programs R2.8.1 and STATA10.1 were used. The time-intensive calculations were performed on the National Supercomputer HLRB-II SGI Altix 4700 at the Leibniz-Rechenzentrum in Garching, Germany. Because of the disproportionate sampling design, stratified weighted analysis with robust standard errors was performed by using the package "survey" in R and the "svy" commands in STATA. Thus the exposure-stratified case-control

sample was weighted back to the original survey setting representing 34,491 children. Only SNPs with a minor allele frequency >0.05 and a *P* value for a Hardy-Weinberg equilibrium test $>10^{-4}$ in controls were tested, resulting in 502,255 SNPs for asthma and 497,294 SNPs for atopy. Each SNP was coded 0-1-2 for the number of copies of the minor allele and entered as a continuous variable in the models corresponding to an additive model. The disease outcomes and environmental exposures were coded as dichotomous variables. For the interaction analysis, 4 approaches were applied, as follows.

First, SNPs previously identified in a meta-analysis of GWASs for childhood-onset asthma were tested for interactions with farming and farm-related exposures in logistic regression with a product term coding the interaction. Second, SNPs retrieved from a literature search for interactions with farming and farm-related exposures were considered candidates. These first 2 approaches were not corrected for multiple testing because we assumed single *a priori* hypothesis testing.

Third, a genome-wide screen for interaction with farming and relevant farm-related exposures was performed by using a modification of the 2-step approach proposed by Murcray et al.[10] In the first step, a GWAS for associations between environmental exposure and genotypes was performed separately for cases and controls. The association estimates (logarithm of the odds ratio [logOR]) were averaged (unweighted) over cases and controls, and a χ^2 value (1 degree of freedom) derived. Also, a GWAS for associations between disease and genotypes was performed separately for exposed and unexposed individuals. Similarly, logOR estimates were averaged (unweighted) over exposed and unexposed individuals and a 1 degree of freedom χ^2 derived. The sum of these 2 independent χ^2 values was tested for significance as a χ^2 on 2 degrees of freedom. Only SNPs with *P* values below the square root of .05/number of SNPs tested (ie, $P < .00032$ for asthma and atopy) were selected for full interaction modeling in the second step. The second step corresponded to a logistic regression for the disease with the marginal effects of environment and genotype and a multiplicative interaction term. Because both steps are statistically independent, Bonferroni correction for multiple testing was applied only to the second step. Because the outcomes and environmental exposures under investigation are closely cross-correlated, Bonferroni correction was applied only to the number of SNPs.

As a fourth approach, we used 2.5 Mio SNPs imputed with the use of Markov chain-based haplotyper.[11] The statistical analysis was similar to that in the third approach. For selection to the second step of the above described 2-step procedure, we used a *P* value threshold of $P < .00028$ corresponding to 651,550 genome-wide independent SNPs as estimated by Dudbridge and Gusnanto.[12]

For a power simulation study, 1000 random data sets were created on the basis of the disease and exposure frequencies and the environment-disease odds ratio (OR) detected in the original GWAS data set. Nine SNPs with interacting allele frequencies from 0.1 to 0.9 were constructed for various interaction types such as extreme crossover, mild crossover, full effect concentration, partial effect concentration, no interaction, partial effect concentration in the opposite direction, and so forth until extreme cross over in the opposite direction.

RESULTS

The GWAS sample was made up of 1708 children including 850 cases of asthma (Fig 1), thereby accounting for a prevalence of asthma in the original survey of 13%. Frequencies of environmental exposures and potential confounders are given in Table I separately for cases of asthma and controls. Because none of the potential confounders changed the logOR estimate of farming on asthma by more than 10%, all subsequent models were adjusted only for center.

The top hits of a previous meta-analysis of GWAS data on childhood asthma were tested for interactions with farming. As shown in Table II, none of these SNPs interacted with farming. We then investigated SNPs previously published to interact with farming or related exposures for asthma or atopy (Table III). Because none of these SNPs were measured by the Illumina Human610 quad array, we selected the closest proxies as shown in Table III ($r^2 > 0.95$). For the Toll-like receptor (TLR) 2 (*TLR2*) SNP, no direct proxy was found, but imputation by using 2 related SNPs resulted in satisfactory accuracy ($r^2 = 0.75$).

TABLE I. Population characteristics for cases of asthma and controls

Characteristic	Cases (%)	Controls (%)	OR†	*P* value†
Farm-related exposures				
Living on a family-run farm	9	14	0.63	<.001
Mother grew up on a farm	24	28	0.84	.177
Regular consumption of raw farm milk*	7	11	0.55	<.001
Regular contact with cows*	15	20	0.69	.004
Regular contact with straw*	12	16	0.75	.018
Regular contact with hay*	17	21	0.76	.026
Coincidence of cow and straw exposure*	7	11	0.62	<.001
Potential confounders				
Mother smoking ever	38	34	1.21	.153
Mother smoked during pregnancy	12	9	1.52	.043
Breast-feeding at least 6 mo	50	55	0.80	.092
Family history of asthma	31	12	3.08	<.001
Parental education—elementary school	16	15	1.17	.382
At least 2 older siblings	45	48	0.87	.280
Atopy‡	62	38	2.63	<.001

*At least once a week during pregnancy or the first 3 years of life.
†Adjusted for center.
‡Specific IgE to mite, cat, or birch ≥0.7 kU/L or to a grass mix ≥0.35 kU/L.

The *TLR2* SNP did not significantly interact with farming for asthma or atopy. For the *TLR4* SNP, we detected a crossover interaction with contact with cows ($P = .013$) and of borderline significance with farming ($P = .088$). Children exposed to cows had a lower risk for asthma if carrying the major allele (adjusted OR [aOR] with 95% confidence intervals in square brackets, 0.60 [0.39-0.94]), whereas for unexposed children, the same genotype carried a slightly increased risk (aOR,1.21 [0.90-1.62]). For *CD14/-1721*, a protective effect concentration for atopy in farm children was noted. In this group, there was an inverse association for the SNP (aOR, 0.64 [0.41-1.00]), but not in the nonfarm children (aOR, 1.10 [0.86-1.39]). For *CD14/-260*, no interactions with contact with pets (data not shown) or with contact to cows or with farming (Table III) were found. The *NOD1* SNP interacted significantly with contact with cows for asthma and with borderline significance for atopy. It was inversely associated with asthma (aOR, 0.66 [0.41-1.06]) in the exposed group but not in the unexposed group (aOR, 1.04 [0.78-1.39]).

In the genome-wide screen, 97 SNPs emerged to interact with farming or related exposures for asthma with Bonferroni-corrected step 2 *P* values below .05 (Fig 2). For atopy, 65 interacting SNPs were detected (Fig 3); none of them interacted with asthma as well. For asthma, 8 genes were related to at least 2 of these SNPs; in analogy, 7 genes were detected for atopy. To assess the interaction type, we compared the associations of genotype and asthma (see this article's Table E2 in the Online Repository at www.jacionline.org) or atopy (see this article's Table E3 in the Online Repository at www.jacionline.org) separately for the exposed and unexposed individuals. The SNPs were coded for the interacting allele—that is, for a protective effect in the

TABLE II. Gene-by-environment interactions for significant SNPs from the GABRIEL asthma meta-analysis with farming as a environmental exposure

				Asthma			Atopic asthma			Nonatopic asthma		
Chr	Gene	SNP	Position	IAF	IOR	*P**	IAF	IOR	*P**	IAF	IOR	*P**
2	IL18R1	rs3771166	102352654	0.63	1.15	.46	0.63	1.26	.28	0.63	1.02	.94
6	HLA-DQ	rs9273349	32733847	0.59	1.11	.56	0.59	1.27	.23	0.41	1.07	.76
9	IL33	rs1342326	6180076	0.16	1.27	.29	0.16	1.37	.21	0.16	1.12	.69
9	IL33	rs928413	6203387	0.24	1.43	.08	0.24	1.45	.11	0.24	1.39	.19
15	SMAD3	rs744910	65233839	0.48	1.18	.38	0.48	1.03	.88	0.48	1.43	.15
17	GSDML	rs2305480	35315722	0.46	1.06	.74	0.54	1.01	.96	0.46	1.17	.53
17	GSDM1	rs3894194	35375519	0.55	1.21	.29	0.55	1.18	.42	0.55	1.28	.31

Chr, Chromosome; *IAF,* frequency of the potentially interacting allele.
*Uncorrected *P* value.

TABLE III. Gene-by-environment interactions in candidate SNPs for interactions with farming or related exposures

						IORs and *P* values		
Chr	rs number	Published as	Published interactions	Closest proxy	IAF	Exposure	Asthma	Atopy†
4	rs4696480	TLR2/-16934[7]	With farming for asthma diagnosis, current asthma symptoms, current hay fever symptoms, any specific IgE	rs13150331* rs7654018*	0.49	Farming	OR = 0.79; *P* = .268	OR = 0.70; *P* = .220
5	rs2569190	CD14/-260 = CD14/-159[8]	With endotoxin levels for total IgE, any specific IgE ≥3.5 kU/L; with contact to animals for total IgE, any specific IgE ≥3.5 kU/L	rs2569188 r^2 = 0.99	0.46	Farming	OR = 0.99; *P* = .953	OR = 0.70; *P* = .152
						Contact with cows	OR = 0.90; *P* = .575	OR = 0.92; *P* = .734
5	rs2915863	CD14/-1721[5]	With farm milk consumption for asthma	rs10463297 r^2 = 0.96	0.37	Farming	OR = 1.05; *P* = .804	**OR = 0.59; *P* = .041**
						Farm milk	OR = 0.96; *P* = .871	OR = 0.66; *P* = .154
7	rs2075817	NOD1/-21596[6]	With farming for pollen IgE, cat dander IgE, hay fever, atopic wheeze; with endotoxin levels for pollen IgE	rs2529447 r^2 = 1.00	0.27	Farming	OR = 0.71; *P* = .092	OR = 0.75; *P* = .261
						Contact with cows	**OR = 0.63; *P* = .045**	OR = 0.60; *P* = .067
9	rs10759932	TLR4/+4434[7]	With endotoxin levels for any specific IgE ≥3.5 kU/L	rs4837494 r^2 = 0.98	0.15	Farming	OR = 1.54; *P* = .088	OR = 0.67; *P* = .248
						Contact with cows	**OR = 1.96; *P* = .013**	OR = 0.72; *P* = .329

Chr, Chromosome; *IAF,* interacting allele frequency.
Significant interactions are in boldface.
*The SNP rs4696480 was imputed from the 2 SNPs rs13150331 and rs7654018 with an r^2 = 0.75.
†Atopy with specific IgE ≥3.5 kU/L.

exposed group and a risk effect in the unexposed group. In the unexposed group, the ORs were around unity representing full effect concentrations or greater unity indicating crossover interactions. Partial effect concentrations with inverse associations in the unexposed group were not detected. In the exposed group, none of the SNPs had an OR close to unity; hence effect concentrations in the unexposed group were absent. Some of the interacting SNPs had low minor allele frequencies (<0.1); more frequent SNPs with minor allele frequencies >0.3 were not found to interact.

The assessment of 2.5 million imputed SNPs revealed only 2 additional SNPs significantly interacting with farming for asthma (see this article's Table E4 in the Online Repository at www.jacionline.org). Both SNPs were related to glutamate receptor, metabotropic 1 *(GRM1)*. For atopy, 1 additional SNP related to growth arrest specific 7 *(GAS7)* was found (Table E4).

A simulation study was performed to assess the extent of interaction detectable with the power of the present data set. The power to detect a significant crossover interaction for asthma with farming at a genome-wide level of significance in 500,000 SNPs was above 80% within the range of interacting allele frequencies 30% to 80% (Fig 4, *A*). Within the same range, there was >50% power for full effect concentrations. The power for partial effect concentrations was rather low (*below the blue lines* in Fig 4). These power estimates take into account the multiple comparisons in a genome-wide study. For selective hypothesis testing of candidate SNPs such as SNPs previously reported to be associated with asthma or to interact for asthma, our study had >95% power to detect full effect concentrations with common polymorphisms with interacting allele frequencies 20% to 90% at a significance level of 5% (Fig 4, *B*). The power to detect major partial effect concentrations was lower but still substantial.

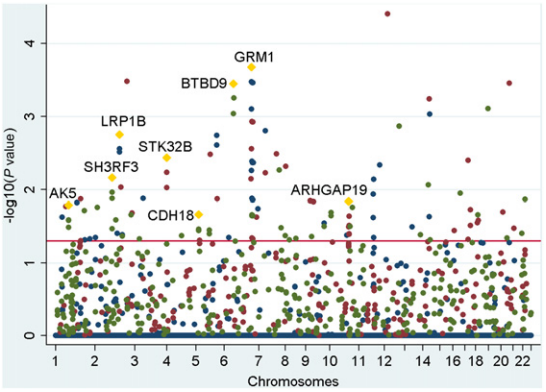

FIG 2. Genome-wide interaction analysis for asthma phenotypes. Bonferroni-corrected interaction *P* values are plotted against the genomic SNP position. The *red line* represents the effective significance level of .05. The *blue dots* represent the *P* values for interactions of farming and related exposures with asthma, the *red dots* with atopic asthma, and the *green dots* with nonatopic asthma. The *yellow diamonds* mark genes represented by at least 2 SNPs.

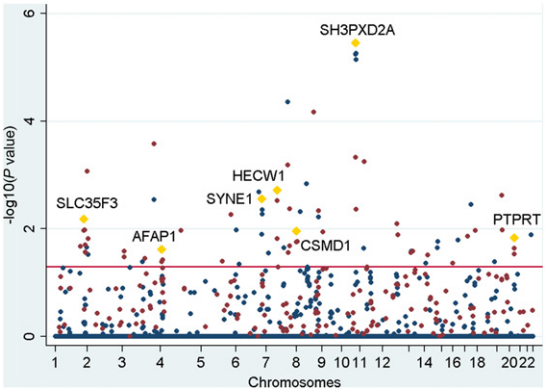

FIG 3. Genome-wide interaction analysis for atopy. Bonferroni-corrected interaction *P* values are plotted against the genomic position of the SNPs. The *red line* represents the effective significance level of .05. *Blue dots* represent *P* values for interactions of farming and related exposures with specific IgE ≥0.35 kU/L and *red dots* with specific IgE ≥3.5 kU/L. The *yellow diamonds* mark genes represented by at least 2 SNPs.

DISCUSSION

This is the first comprehensive analysis of gene-environment interactions for childhood asthma in relation to farming exposures. SNPs associated with asthma in a previous comprehensive meta-analysis of urban populations did not interact with farming. The genome-wide interaction study among the rural populations did not reveal any significant interactions with common SNPs (40% to 70%), for which our study had >50% power. Among rarer SNPs, we identified 15 genes with crossover interactions or effect concentrations in the exposed group for asthma or atopy in relation to farming, consumption of farm milk, and contact with cows and straw, which may deserve further investigation. Finally, we also tested previously published candidate SNPs that had shown interactions with farm-related exposures but could not fully confirm their effects.

Environmental exposures

The protective effect of farming on asthma and atopy has been shown in numerous studies consistently across many parts of the world, and the beneficial effects of childhood farm exposure are likely to last until adulthood.[4] Recent investigations of the environmental exposures in the rural population included in the current analyses have shown that the protective influence of farm exposure on asthma can be attributed to few distinct elements: consumption of raw farm milk and the concomitant exposure to cows and straw (Illi S et al, November 2010, unpublished data). We note with interest that almost the same farm-related exposures emerged from the interaction analysis in the GWAS sample (Tables E2 and E3 in this article's Online Repository at www.jacionline.org).

Interactions with asthma-associated SNPs

As a first approach to identify G*E interactions, we tested the most significant SNPs of a previous genome-wide meta-analysis on asthma.[9] Although the current GWAS data set was part of that meta-analysis, it did not influence the combined estimate to a major extent, thereby rendering the G*E interaction analysis almost completely independent from the selection of the meta-analysis SNPs. The observation that no significant interactions were found may partly be explained by the fact that the meta-analysis was mainly based on nonfarming populations. Therefore, only effect concentrations in the unexposed group or crossover interactions might have been detected, but not effect concentrations in the exposed group. Furthermore, the effects of SNPs detected by the meta-analysis might be so strong that modification by an environmental exposure might be negligible.

Interactions with previously reported SNPs

As illustrated by Table III, 5 SNPs have been published to interact with farm-related exposures. For the *TLR2* SNP, the earlier described[7] interactions for asthma (IOR, 0.29) and atopy (IOR, 0.26) did not emerge as statistically significant, although our results are in the same direction (Table III). For the *TLR4* SNP, we detected a protective effect of the major allele "A" for asthma in the children exposed to cows or farming in general, whereas the original publication described a protective effect of the minor allele for atopy in children exposed to high endotoxin levels.[7] Also for *CD14/-260*, the original interaction was with endotoxin levels, which in our data set could not be tested because endotoxin levels were measured in only a minority of subjects. The SNP *CD14/-1721* is of special interest because it is located in the promoter region and has been shown to be involved in *CD14* expression.[5] In contrast with the original publication, we found a protective interaction for the minor but not the major allele.[5] Furthermore, the previously reported interactions of the *NOD1* SNP for atopy[6] were not significant in our data set. However, for asthma, we found a significant protective effect concentration. A partial protective effect concentration of similar size can also be found in the publication by Eder et al,[6] although it did not reach statistical significance.

Genome-wide interaction analysis

To maximize power while correcting for multiple comparisons in our genome-wide analysis, we applied a 2-step method, for

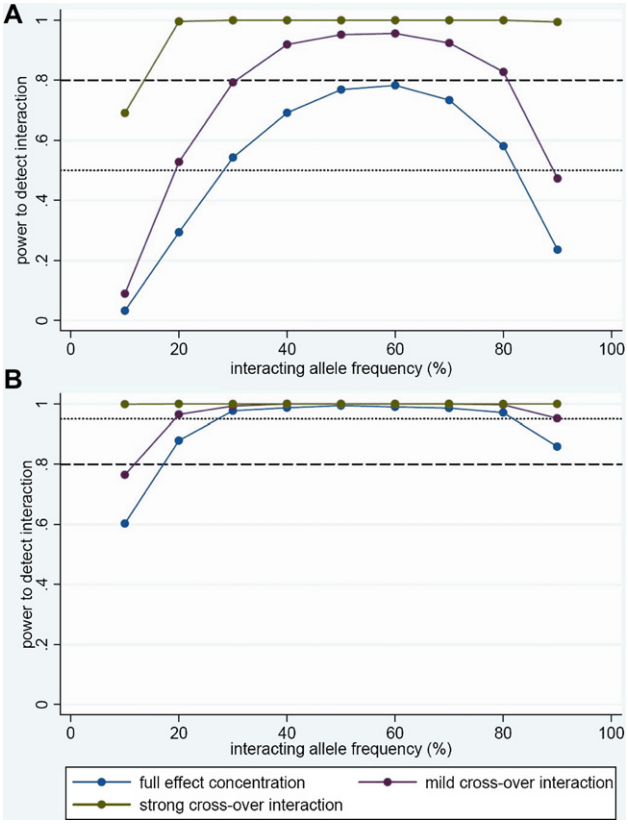

FIG 4. Power calculation for the genome-wide interaction analysis for asthma with farming as environmental exposure. The power to detect full effect concentrations *(blue lines)*, mild crossover interactions *(red lines)*, and strong crossover interactions *(green lines)* is given for interacting allele frequencies ranging from 0.1 to 0.9 for genome-wide significance ($\alpha = 0.05$ corrected for 500,000 SNPs; **A**) and single hypotheses ($\alpha = 0.05$; **B**). The *dotted lines* indicate 50% and 95% power in *A and B*, respectively.

which a gain in power is achieved by the reduction of the number of tests performed at step 2, allowing for a less stringent correction for multiple testing. All interactions detected by this method were of effect concentration in the exposed group or of the crossover type, and many of them were for moderately rare SNPs. The failure to detect interactions for SNPs with interacting allele frequencies in the range 30% to 70%, for which our study was adequately powered, suggests that common polymorphisms are unlikely to moderate the protective influence of the farming environment on childhood asthma and atopy in Central Europe. For SNPs with allele frequencies outside this range, we had only adequate power to detect crossover interactions. These are interesting insofar as the SNPs involved may not be detected by a classic GWAS when not considering environmental exposures. Here lies a potential strength of G*E interaction analyses because they provide the opportunity to unravel genetic effects masked by environmental exposures. Because the power for partial effect concentration was insufficient, we might have missed such interactions, which may sum up to substantial effects. However, much larger sample sizes would be required than our GWAS data set provided.

The genes identified in the genome-wide interaction analysis have previously not been implicated in the pathogenesis of asthma and atopy. A cluster of 4 highly correlated ($r^2 > 0.99$) genotyped SNPs (Fig 2) and 2 imputed SNPs related to the *GRM1* gene were found to interact for asthma with farming, contact with cows and straw, or contact with straw alone (Table E2). *GRM1* is involved not only in synaptic transmission of neuronal signals but also in immunologic synapses, where it plays a role in T-cell activation and production of T_H1 and proinflammatory cytokines.[13,14] Despite biological plausibility, the discovered interactions need to be interpreted with caution because the SNPs are highly correlated and rare. Ideally, we would seek replication of the interaction effects in an independent sample, but because of the unusual nature of the farming exposure prevailing in the study regions, such independent replication may prove difficult. In the unexposed urban population of the GABRIEL meta-analysis, associations with the *GRM1* SNPs could not be replicated because the interactions detected in our population were effect concentrations in the exposed individuals with no associations in the unexposed individuals.

We have performed a very comprehensive gene-environment interaction analysis for childhood asthma with farming as environmental exposure. The study was well powered to detect significant gene-environment interactions for common genetic polymorphisms. Nonetheless, we did not discover novel genes with common polymorphisms specifically interacting with the farming environment. For rarer polymorphisms the power was limited, and therefore our findings relating to *GRM1* and the other identified genes must be interpreted with caution. If replicated in other rural populations, *GRM1* may be of interest in light of its biological plausibility.

Overall, these findings may indicate that the strong protective effect of a farming environment is a result of neither the genetic make-up of the farming population *per se* nor common genetic polymorphisms interacting with these particular exposures. This implies that environmental exposures or the lack thereof can determine the new onset of childhood asthma. Conversely, in an unexposed population, genes such as those identified in the GWAS meta-analysis are causal factors for childhood asthma. In this scenario, both genetic and environmental components may independently contribute to distinct mechanisms underlying this condition.

Key messages

- **A genome-wide interaction analysis revealed several novel interaction candidate genes for asthma and atopy in a farming environment.**
- **In turn, the top SNPs 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.**

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TABLE E1. Gene symbols

Gene symbol	Explanation
AFAP1	Actin filament associated protein 1
AK5	Adenylate kinase 5
ARHGAP19	Rho GTPase activating protein 19
BTBD9	BTB (POZ) domain containing 9
CD14	CD14 antigen
CDH18	Cadherin 18, type 2
CSMD1	CUB and Sushi multiple domains 1
GAS7	Growth arrest specific 7
GRM1	Glutamate receptor, metabotropic 1
GSDM1	Gasdermin 1
GSDML	Gasdermin L
HECW1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
HLA-DQ	MHC-II, DQ
IL18R1	IL-18 receptor 1
IL33	IL-33
LRP1B	Low-density lipoprotein receptor–related protein 1B
NOD1	Nucleotide-binding oligomerization domain containing 1
PTPRT	Protein tyrosine phosphatase, receptor type T
SH3PXD2A	SH3 and PX domains 2A
SH3RF3	SH3 domain containing ring finger 3
SLC35F3	Solute carrier family 35, member F3
SMAD3	SMAD family member 3
STK32B	Serine/threonine kinase 32B
SYNE1	Spectrin repeat containing, nuclear envelope 1
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4

TABLE E2. Genome-wide interaction analysis for asthma phenotypes

Chr	Gene	Function	SNP	Position	Allele	IAF	Outcome	Exposure	OR_{Exp}	OR_{Unexp}	IOR	P*
1	AK5	Intron	rs1874817	77686105	A	0.76	Nonatopic a	Raw farm milk	0.27	1.01	0.27	.0259
1	AK5	Intron	rs12044354	77690935	A	0.77	Nonatopic a	Raw farm milk	0.27	1.01	0.27	.0261
1	AK5	Intron	rs3113637	77699732	G	0.76	Nonatopic a	Raw farm milk	0.27	1	0.27	.0326
2	SH3RF3	Intron	rs6752599	109140121	T	0.19	Nonatopic a	Contact with hay	0.4	1.48	0.27	.0341
2	SH3RF3	Intron	rs1430307	109174151	T	0.18	Nonatopic a	Contact with hay	0.35	1.42	0.24	.0109
2	SH3RF3	Intron	rs6542803	109176127	A	0.19	Nonatopic a	Contact with hay	0.35	1.4	0.25	.0133
2	LRP1B	Intron	rs12052880	141872650	G	0.71	Asthma	Contact with straw	0.36	1.11	0.32	.0028
2	LRP1B	Intron	rs16846007	141874976	T	0.77	Asthma	Contact with straw	0.35	1.03	0.34	.0031
4	STK32B	Intron	rs17677074	5242853	A	0.88	Atopic asthma	Raw farm milk	0.17	1.06	0.16	.0058
4	STK32B	Intron	rs17734127	5266184	A	0.88	Atopic asthma	Raw farm milk	0.17	1.01	0.17	.0094
5	CDH18	Intron	rs1486844	19539303	C	0.17	Nonatopic a	Raw farm milk	0.17	1.2	0.14	.0346
5	CDH18	Intron	rs1000727	19555993	A	0.19	Nonatopic a	Raw farm milk	0.18	1.13	0.16	.0363
6	BTBD9	Intron	rs12200371	38554612	G	0.1	Nonatopic a	Contact with hay	0.24	1.85	0.13	.0009
6	BTBD9	Intron	rs9470887	38558410	T	0.1	Nonatopic a	Contact with hay	0.23	1.96	0.12	.0006
6	GRM1	Intron	rs4551188	146529860	A	0.91	Atopic asthma	Cows and straw	0.11	1.04	0.1	.0028
6	GRM1	Intron	rs4551188	146529860	A	0.91	Asthma	Contact with straw	0.23	1.22	0.19	.0055
6	GRM1	Intron	rs4551188	146529860	A	0.91	Asthma	Cows and straw	0.13	1.19	0.11	.0008
6	GRM1	Intron	rs2143324	146541958	T	0.91	Asthma	Child lives on farm	0.3	1.11	0.27	.0132
6	GRM1	Intron	rs2143324	146541958	T	0.91	Asthma	Cows and straw	0.1	1.17	0.09	.0003
6	GRM1	Intron	rs2143324	146541958	T	0.91	Atopic asthma	Contact with straw	0.19	1.03	0.18	.0457
6	GRM1	Intron	rs2143324	146541958	T	0.91	Asthma	Contact with straw	0.21	1.2	0.18	.0041
6	GRM1	Intron	rs2143324	146541958	T	0.91	Atopic asthma	Cows and straw	0.09	1.02	0.08	.0012
6	GRM1	Intron	rs2143324	146541958	T	0.91	Nonatopic a	Cows and straw	0.14	1.53	0.09	.022
6	GRM1	Intron	rs2179509	146542278	T	0.91	Atopic asthma	Contact with straw	0.19	1.02	0.18	.047
6	GRM1	Intron	rs2179509	146542278	T	0.91	Asthma	Child lives on farm	0.3	1.11	0.27	.0138
6	GRM1	Intron	rs2179509	146542278	T	0.91	Nonatopic a	Cows and straw	0.14	1.52	0.09	.0226
6	GRM1	Intron	rs2179509	146542278	T	0.91	Asthma	Contact with straw	0.21	1.19	0.18	.0043
6	GRM1	Intron	rs2179509	146542278	T	0.91	Atopic asthma	Cows and straw	0.09	1.02	0.08	.0012
6	GRM1	Intron	rs2179509	146542278	T	0.91	Asthma	Cows and straw	0.1	1.17	0.09	.0003
6	GRM1	Intron	rs9497474	146545933	C	0.91	Atopic asthma	Contact with straw	0.19	1.02	0.18	.047
6	GRM1	Intron	rs9497474	146545933	C	0.91	Atopic asthma	Cows and straw	0.09	1.02	0.08	.0012
6	GRM1	Intron	rs9497474	146545933	C	0.91	Asthma	Contact with straw	0.21	1.19	0.18	.0043
6	GRM1	Intron	rs9497474	146545933	C	0.91	Asthma	Child lives on farm	0.3	1.11	0.27	.0138
6	GRM1	Intron	rs9497474	146545933	C	0.91	Nonatopic a	Cows and straw	0.14	1.52	0.09	.0226
6	GRM1	Intron	rs9497474	146545933	C	0.91	Asthma	Cows and straw	0.1	1.17	0.09	.0003
10	ARHGAP19	Near-gene-3	rs953097	98971904	T	0.39	Atopic asthma	Contact with straw	0.4	1.01	0.39	.0322
10	ARHGAP19	Intron	rs871988	98993365	A	0.69	Atopic asthma	Contact with straw	0.4	0.94	0.43	.0402
10	ARHGAP19	Coding-synonymous	rs13439	98996073	A	0.31	Atopic asthma	Contact with straw	0.38	1.04	0.36	.0407
10	ARHGAP19	Intron	rs3740522	99009134	C	0.39	Atopic asthma	Contact with straw	0.39	1.02	0.38	.023
10	ARHGAP19	Intron	rs793520	99022365	A	0.31	Atopic asthma	Contact with straw	0.37	1.01	0.36	.0308

Only SNPs related to genes are listed.

a, Asthma; *Chr,* chromosome; *IAF,* frequency of the interacting allele; OR_{Exp}, OR in the exposed group; OR_{Unexp}, OR in the unexposed group.

*Bonferroni-corrected *P* value for the interaction.

TABLE E3. Genome-wide interaction analysis for atopy (IgE to any allergen)

Chr	Gene	SNP†	Position	Allele	IAF	Cutoff	Exposure	OR_{Exp}	OR_{Unexp}	IOR	*P**
1	SLC35F3	rs9435541	232231502	A	0.05	3.5 kU/L	Cows and straw	0.09	1.09	0.08	.0108
1	SLC35F3	rs2199074	232232752	C	0.05	3.5 kU/L	Cows and straw	0.09	1.09	0.08	.0105
1	SLC35F3	rs4564211	232233430	G	0.05	3.5 kU/L	Cows and straw	0.09	1.09	0.08	.0105
1	SLC35F3	rs4612688	232233560	T	0.05	3.5 kU/L	Cows and straw	0.09	1.09	0.08	.0105
1	SLC35F3	rs12079513	232233825	C	0.05	3.5 kU/L	Cows and straw	0.1	1.09	0.09	.02
1	SLC35F3	rs6676512	232234440	A	0.05	3.5 kU/L	Cows and straw	0.1	1.03	0.09	.0274
1	SLC35F3	rs1458587	232237510	A	0.05	3.5 kU/L	Cows and straw	0.1	1.03	0.09	.0276
4	AFAP1	rs4997103	7821960	G	0.91	3.5 kU/L	Raw farm milk	0.18	1.2	0.15	.0409
4	AFAP1	rs11723068	7848335	G	0.91	3.5 kU/L	Raw farm milk	0.18	1.2	0.15	.0377
6	SYNE1	rs2141153	152935302	A	0.19	0.35 kU/L	Child lives on a farm	0.24	0.91	0.26	.0126
6	SYNE1	rs17779152	152944479	A	0.2	0.35 kU/L	Child lives on a farm	0.24	0.96	0.25	.0054
6	SYNE1	rs17701297	152952632	A	0.2	0.35 kU/L	Child lives on a farm	0.24	0.97	0.24	.0044
7	HECW1	rs2330745	43161980	C	0.05	3.5 kU/L	Cows and straw	0.1	0.9	0.11	.0154
7	HECW1	rs3757564	43169187	C	0.07	3.5 kU/L	Cows and straw	0.1	0.81	0.13	.003
8	CSMD1	rs10107025	3210581	C	0.08	3.5 kU/L	Raw farm milk	0.1	0.81	0.12	.0175
8	CSMD1	rs1161531	3248890	T	0.1	3.5 kU/L	Raw farm milk	0.1	0.82	0.12	.0173
10	SH3PXD2A	rs880549	105396119	C	0.69	3.5 kU/L	Cows and straw	0.26	1.06	0.25	.0005
10	SH3PXD2A	rs7914478	105397780	C	0.69	3.5 kU/L	Cows and straw	0.26	1.06	0.25	.0005
10	SH3PXD2A	rs2145309	105440115	G	0.69	3.5 kU/L	Cows and straw	0.29	1.02	0.28	.0043
20	PTPRT	rs6124418	40192864	T	0.94	3.5 kU/L	Raw farm milk	0.07	1.39	0.05	.0231
20	PTPRT	rs6124419	40196282	G	0.94	3.5 kU/L	Raw farm milk	0.06	1.4	0.04	.0298

Chr, chromosome; *IAF,* frequency of the interacting allele; OR_{Exp}, OR in the exposed group; OR_{Unexp}, OR in the unexposed group.
Only SNPs related to genes are listed. Atopy was defined as specific IgE to rye grass, timothy, birch, mugwort, grass mix gx3, mite, cat, or food mix fx5 at 2 different cutoff levels (≥0.35 kU/L and ≥3.5 kU/L).
*Bonferroni-corrected *P* value for the interaction.
†All SNPs are located in introns.

TABLE E4. Genome-wide interaction analysis for asthma and atopy (IgE to any allergen) with farming by using imputed SNPs

Outcome	Chr	Gene	SNP	rsq	IAF	OR_{Exp}	P_{Exp}	OR_{Unexp}	P_{Unexp}	IOR	*P*
Asthma	6	GRM1	rs2143324	1	0.08	3.30	.034	0.90	1.00	3.92	.047
	6	GRM1	rs2179509	1	0.08	3.30	.034	0.90	1.00	3.91	.049
Atopy	17	GAS7	rs4791382	0.41	0.49	4.54	.003	0.84	1.00	5.32	.004

Chr, Chromosome; *IAF,* frequency of the interacting allele; OR_{Exp}, OR in the exposed group; OR_{Unexp}, OR in the unexposed group; *P, P* value for the interaction; P_{Exp}, *P* value for association in the exposed; P_{Unexp}, *P* value for association in the unexposed; *rsq,* r-square-hat (quality of imputation).
Atopy was defined as specific IgE to rye grass, timothy, birch, mugwort, grass mix gx3, mite, cat, or food mix fx5 ≥3.5 kU/L. All *P* values are Bonferroni-corrected.