

Original article

A polymorphism in *CD14* modifies the effect of farm milk consumption on allergic diseases and *CD14* gene expression

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Background: Consumption of farm milk in early life is associated with less asthma and allergies.

Objective: We hypothesized that genetic variation in the innate immunity receptor *CD14* might modify the association between farm milk consumption and asthma and atopy.

Methods: Questionnaire data, serum IgE levels, and genotypes for 4 single nucleotide polymorphisms in *CD14* were assessed in farmers' and nonfarmers' children from 2 European populations (Allergy and Endotoxin study, n = 576; Prevention of Allergy Risk factors for Sensitization in children related to Farming and Anthroposophic Lifestyle study, n = 1539). In a subsample (n = 222) *CD14* gene expression was measured in peripheral blood leukocytes. The effects of farm milk and *CD14* genotypes on asthma, allergies, and *CD14* expression and their interactions were investigated.

Results: We found a significant interaction between genetic variation in *CD14*–1721 and farm milk consumption.

Adjusted odds ratios for the association between farm milk and asthma varied between the genotypes: AA, 0.18 (95% CI, 0.07–0.47); AG, 0.47 (95% CI, 0.26–0.86); and GG, 0.98 (95% CI, 0.46–2.08). Similar patterns were observed for symptoms of allergic rhinoconjunctivitis and pollen

sensitization. *CD14*–1721 also modified the association between farm milk and *CD14* gene expression (adjusted geometric means ratios: AA, 1.61 (95% CI, 0.98–2.66); AG, 1.11 (95% CI, 0.71–1.72); and GG, 0.76 (95% CI, 0.39–1.48).

Conclusion: The protective effect of farm milk consumption on allergic diseases is stronger in children carrying the A allele in *CD14*–1721 than in children homozygous for the G allele. This might be mediated through farm milk–induced upregulated *CD14* gene expression.

Clinical implications: Our results support the hypothesis that the inverse association between farm milk consumption and allergic diseases is mediated by *CD14*-activated innate immune mechanisms. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergy, asthma, *CD14*, gene-environment interaction, gene expression, farming, epidemiology

Several studies in rural populations have demonstrated that living on a farm provides a protective environment for the development of allergic diseases.¹ In addition to farm animal contact² and microbial compounds,³ such as LPS,

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Abbreviations used

ALEX: Allergy and Endotoxin

OR: Odds ratio

PARSIFAL: Prevention of Allergy – Risk Factors for Sensitisation in Children Related to Farming and Anthroposophic Lifestyle

PBLC: Peripheral blood leukocyte

SNP: Single nucleotide polymorphism

more recently, farm milk consumption has been identified as another characteristic of a farming lifestyle that might confer protection against allergic diseases.^{2,4-7} The relevant compound or compounds responsible for the protective farm milk effect and the underlying biologic mechanisms are still unclear, although several suggestions have been put forward. Viable (lactobacilli⁶) and nonviable (LPS³) microbial components of farm milk interacting directly or indirectly (eg, through modification of the intestinal microflora) with the immune system have been proposed as possible causal factors. Alternatively, compounds other than microbes, such as high levels of omega-3 polyunsaturated fatty acids, in farm milk might play a role.⁸⁻¹⁰

CD14 is a key molecule of the innate immune system serving as a receptor and carrier for microbial ligands¹¹ and other lipid-containing molecules.¹²⁻¹⁴ As previously shown in genetic studies, a polymorphism in the *CD14* gene, *CD14/C-260T*, might interact with environmental factors in the development of asthma and atopic diseases.^{15,16} Although the mechanisms of gene-environment interactions are largely unknown, a polymorphism in the promoter region of a gene might exert its effect by modulating gene expression. Recently published reports support this possibility and suggest that variation in gene expression is of importance in the interplay among environment, genes, and disease.^{17,18}

In the present study we therefore aimed to investigate whether the negative association between allergic health outcomes and farm milk consumption is modified by polymorphisms in *CD14* and whether a genotype-specific protective effect is associated with increased expression of *CD14*.

METHODS

Population

Data from farmers' children and nonfarmers' children enrolled in 2 cross-sectional surveys, the Allergy and Endotoxin (ALEX) study and the Prevention of Allergy – Risk Factors for Sensitisation in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study, were analyzed in this study. Both surveys aimed to explore protective factors associated with farming and asthma and allergies in childhood. The methods used were similar and partly identical. Details of the selection process are described in Fig E1 in the Online Repository (available at www.jacionline.org). Ethical permission for each study was obtained from local authorities in each center.

In brief, the ALEX study² was a cross-sectional study conducted in 1999 with an overall participation rate of 75%. Of the families who gave consent for further investigations, all farmers' children, all

nonfarmers' children with contact to farming, and a random sample of nonexposed nonfarmers' children were invited for blood sampling. Complete questionnaire data and a blood sample were available for 812 children. Of these, consent for genetic analyses was not given for 120 Swiss children, and no adequate DNA sample was available for 8 children from Germany and Austria, leaving 684 children (258 farmers' and 426 nonfarmers' children) for genotyping.

In 2001, farmers' children and nonfarmers' children were recruited as part of the PARSIFAL study in the same countries but from different geographic areas and additionally in Sweden and The Netherlands.¹⁹ Parents of these children completed a self-administered questionnaire to assess asthma and allergies and exposures associated with farming. The overall participation rate was 70%. Participants were also asked for consent to further investigations, including a venous blood sample. Consent to genetic analyses and an adequate sample of DNA were available for 1907 children.

In case more than 1 child per family participated in the study, only the eldest sibling was included in the analyses to fulfill the assumption of independent observations. The final sample sizes for the ALEX and PARSIFAL studies were therefore reduced to 576 (209 farmers' and 367 nonfarmers' children) and 1478 (957 farmers' and 521 nonfarmers' children) children, respectively.

Phenotypes

In both study populations the same questions and thereby the same definition of phenotypes were used. Asthma was defined as a physician's diagnosis of asthma or recurrent asthmatic, spastic, or obstructive bronchitis. Children whose parents reported "wheezing or whistling in the chest in the last 12 months" were classified as having current wheeze. Current symptoms of allergic rhinoconjunctivitis were defined as a positive response to the following question: "In the last 12 months, has your child had problems with sneezing or a runny or blocked nose without a cold accompanied by itchy-watery eyes?"

The concentrations of specific IgE against common aeroallergens were measured with the ImmunoCAP assay (Phadia, Uppsala, Sweden; ImmunoCAP codes for the individual allergens are given in brackets below) in all sera and in both populations. In the ALEX study specific serum IgE levels against timothy grass pollen (g6), birch pollen (t3), house dust mite (*Dermatophagoides pteronyssinus*, d1), storage mite (*Lepidoglyphus destructor*, d71), cat dander (e1), and cow dander (e4)² were measured, and in the PARSIFAL study specific serum IgE levels against grass pollen mix (gx3; timothy grass, sweet vernal grass, rye grass, cultivated rye, and velvet grass), tree pollen mix (tx9; birch, grey alder, hazel, oak, and willow), house dust mite (*D pteronyssinus*, d1), storage mite (*L destructor*, d71), cat dander (e1), and horse dander (e3) were measured.⁴ Two different cut-off values (0.35 and 3.5 kU/L) were used for analyses.

Exposure to farm milk in the first year of life

In both studies exposure to farm milk (cow's milk from self-production or directly purchased from a farm) in the first year of life was defined in children whose parents reported that the child began to drink farm milk at the age of 1 year or less ($n = 909$). Children not exposed to farm milk in the first year of life started either later ($n = 257$) or did not drink farm milk but rather any kind of shop-purchased cow's milk ($n = 847$). The latter 2 groups formed the group of children ($n = 1104$) who were not exposed to farm milk in the first year of life.

Non-milk-drinking children ($n = 8$) were excluded from the analyses to avoid potential primary milk avoidance because of allergy-related symptoms at younger ages.

Genotyping

DNA was extracted from peripheral blood leukocytes (PBLCs) by using standard techniques. *CD14* was screened for single nucleotide

polymorphisms (SNPs) in white subjects (<http://innateimmunity.net>). Haplotype tagging SNPs closely linked with known polymorphisms ($R^2 > 0.7$) and with a minor allele frequency of greater than 10% were chosen for this study by using the algorithm implemented in *ldSelect*.²⁰ This algorithm is based on linkage disequilibrium. Statistical power to detect unassayed, disease-associated polymorphisms depends on the correlation (R^2) between the unassayed site and the assayed site. These SNPs were as follows: *CD14*–4191 (rs574441), tagging 5 other SNPs; *CD14*–2839 (rs2569193) and *CD14*–260 (rs256190), each tagging 3 other SNPs; and *CD14*–1721 (rs2915863), a single SNP because of linkage disequilibrium with an R^2 value of 0.7 or less to other SNPs in the reference population (<http://innateimmunity.net>). Genotyping for both populations was done in the same laboratory by using identical methods. (Arizona Respiratory Center, Tucson, Ariz). Genomic DNA was assayed by using the 5' exonuclease reaction (Taqman; Applied Biosystems, Foster City, Calif). Primer and probe sequences used and further details of the genotyping method are available from the author on request.

Expression of *CD14*

For the Swiss branch of the PARSIFAL study, RNA samples of PBLs were collected from 316 children (95.3% of children who provided blood samples in Switzerland) to analyze gene expression of innate immunity receptors (see Fig E1 in the Online Repository at www.jacionline.org). After restriction to the eldest siblings, the final sample consisted of 222 children, of whom 132 were exposed to farm milk in the first year of life and 89 were not (1 missing).

Total RNA was isolated by using the QIAmp RNA Blood Mini Kit (Qiagen, Hilden, Germany) supplemented with RNase-free DNase (Qiagen). Quantitative real-time PCR (TaqMan, Applied Biosystems) was performed as described elsewhere.¹⁴ All PCR reactions were analyzed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Experiments assessing background signals were performed for every assay by running the reaction without templates ("no template control"). In none of these was any template control amplification observed, indicating that there was no contamination or unspecific fluorescence. The data are presented as normalized values (the amount of mRNA of the target molecule divided by the amount of mRNA of the endogenous control [18S rRNA]).

Statistical analyses

Genotype frequencies in the ALEX and PARSIFAL populations were calculated and compared with those of a population in Hardy-Weinberg equilibrium by using the χ^2 goodness-of-fit test with 1 *df*.

The prevalences of reported asthma, current wheezing, current rhinoconjunctivitis symptoms, and atopic sensitization (allergen specific serum IgE, ≥ 3.5 kU/L) were calculated in the genotypes of the 4 investigated *CD14* SNPs. Odds ratios (ORs) for the association between the phenotypes and being a farm child (a child who lived on a farm and whose family ran the farm), between phenotypes and contact with stable animals in the first year of life, and between phenotypes and farm milk consumption in the first year of life were estimated in the *CD14* genotypes and tested for homogeneity by using Mantel-Haenszel statistics.

The following factors were considered as potential confounders for the association between exposure and outcome and included in the multiple logistic and multiple linear regression models in case of a univariate association ($P < .150$) to the independent variable of interest: sex, age in years (5–6, 7–8, 9, 10–11, or 12–13 years), maternal education (low, medium, and high), number of elder siblings (none, 1, 2, 3, or more), passive smoking (0, 1–9, or >10 cigarettes per day), study center, being a farm child, and breast-feeding (exclusively ≥ 5 months and <5 months).

TABLE I. Frequencies of *CD14* genotypes in the 2 study populations

<i>CD14</i> polymorphism	Genotype	ALEX study (n = 576)	PARSIFAL study (n = 1478)
		n (%)	n (%)
<i>CD14</i> /C–4191T	CC	320 (55.6%)	816 (55.2%)
	CT	213 (37.0%)	521 (35.3%)
	TT	21 (3.6%)	85 (5.8%)
	Missing	22 (3.8%)	56 (3.8%)
<i>CD14</i> /C–2839T	CC	283 (49.1%)	768 (52.0%)
	CT	232 (40.3%)	537 (36.3%)
	TT	38 (6.6%)	111 (7.5%)
	Missing	23 (4.0%)	62 (4.2%)
<i>CD14</i> /C–1721T	AA	172 (29.9%)	494 (33.4%)
	AG	272 (47.2%)	639 (43.2%)
	GG	89 (15.5%)	254 (17.2%)
	Missing	43 (7.5%)	91 (6.2%)
<i>CD14</i> /C–260T	CC	133 (23.1%)	399 (27.0%)
	CT	279 (48.4%)	683 (46.2%)
	TT	121 (21.0%)	337 (22.8%)
	Missing	43 (7.5%)	59 (4.0%)

Associations are presented as ORs for binomial data and as exponentiated regression coefficients (geometric means ratios) for numeric data.

The coding of the genotype was done according to the best-fitting model on the basis of Akaike's information criterion²¹ to test for statistical interaction: ordinal (reflecting an additive genetic model), binomial (homozygous of one allele vs heterozygous and homozygous of the other allele, reflecting a dominant or a recessive genetic model, respectively), or categorical (no genetic model). The likelihood ratio test was performed to test the null hypothesis of no interaction. Two-sided *P* values of less than .05 were considered significant.

The robustness of our results was checked by repeating the analyses in the subsamples of farmers' and nonfarmers' children.

All analyses were carried out with STATA/SE 8.0 software (StataCorp, College Station, Tex) and R 2.4.0 (R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study population

Table I shows the genotype frequencies of the 4 investigated *CD14* polymorphisms. Genotype frequencies did not differ between the ALEX and PARSIFAL studies (Table I) or between early farm milk drinkers and non-farm milk drinkers, respectively (data not shown). In both populations, no significant deviation from Hardy-Weinberg equilibrium was observed (data not shown) for all 4 polymorphisms. Residual linkage disequilibrium was found between *CD14*–1721 and *CD14*–260 in both populations (see Tables E1 and E2 in the Online Repository at www.jacionline.org).

Characteristics and the prevalence of asthma and allergies in the 2 main populations and in the PARSIFAL subpopulation are described in Table II. Because of different study designs in the ALEX and PARSIFAL surveys, which resulted in different proportions of farmers'

TABLE II. Description of the 2 study populations

Characteristic		ALEX study (n = 576)	PARSIFAL study (n = 1478)	PARSIFAL subpopulation (n = 222)
		n (%)	n (%)	n (%)
Farmer's child	Yes	209 (36.3%)	957 (64.7%)	124 (55.9%)
Farm milk consumption in the first year of life	Yes	288 (50.0%)	621 (42.0%)	132 (59.5%)
	Missing	0 (0.0%)	18 (1.2%)	1 (0.5%)
Sex	Girl	270 (46.9%)	713 (48.2%)	110 (49.5%)
Age groups	5-6 y	0 (0.0%)	126 (8.5%)	26 (11.7%)
	7-8 y	174 (30.2%)	399 (27.0%)	50 (22.5%)
	9 y	115 (20.0%)	259 (17.5%)	20 (9.0%)
	10-11 y	231 (40.1%)	507 (34.3%)	60 (27.0%)
	12-13 y	56 (9.7%)	187 (12.7%)	66 (29.7%)
Asthma	Yes	48 (8.3%)	125 (8.5%)	15 (6.8%)
	Missing	0 (0.0%)	23 (1.6%)	2 (0.9%)
Current wheezing	Yes	59 (10.2%)	108 (7.3%)	16 (7.2%)
	Missing	9 (1.6%)	14 (0.9%)	3 (1.4%)
Current symptoms of allergic rhinoconjunctivitis	Yes	58 (10.1%)	90 (6.1%)	10 (4.5%)
	Missing	11 (1.9%)	16 (1.1%)	1 (0.5%)
Specific serum IgE ≥ 3.5 kU/L				
Any allergen tested	Yes	125 (21.7%)	193 (13.1%)	35 (15.8%)
	Missing	0 (0.0%)	61 (4.1%)	0 (%)
Pollen	Yes	89 (15.5%)	101 (6.8%)	21 (9.5%)
	Missing	0 (0.0%)	60 (4.1%)	0 (0%)
House dust mite	Yes	59 (10.2%)	120 (8.1%)	17 (7.7%)
	Missing	0 (0.0%)	4 (0.3%)	0 (0%)

children, the prevalence of asthma, allergic rhinoconjunctivitis, and specific serum IgE to aeroallergens differed between the study populations.

Effects of *CD14*–*1721* genotypes and farm milk consumption on allergic diseases

The *CD14*–*1721* SNP is characterized by a substitution of adenine by guanine in the promoter of the *CD14* gene. There was no significant association between *CD14*–*1721* genotypes and allergic diseases (data not shown). Children who consumed farm milk in the first year of life were less likely to have asthma, symptoms of allergic rhinoconjunctivitis, and atopic sensitization (allergen specific serum IgE, ≥ 3.5 kU/L; crude ORs [95% CIs] for asthma, rhinoconjunctivitis symptoms, and atopic sensitization were as follows: 0.36 [95% CI, 0.25–0.51], 0.55 [95% CI, 0.38–0.78], and 0.60 [95% CI, 0.47–0.77], respectively), thereby confirming previously reported results.^{2,4}

By using Mantel-Haenszel test statistics for homogeneity of ORs across strata, the protective effect of farm milk consumption differed between the genotypes of *CD14*–*1721* in the ALEX and PARSIFAL populations (data not shown; prevalences of allergic health outcomes stratified by genotypes and main exposures are shown in Tables E3–E5 in the Online Repository at www.jacionline.org). This heterogeneity between genotypes remained after adjusting for potential confounding in multiple regression analyses (Table III).

Moreover, the effect patterns were similar in both populations for asthma, current wheeze, symptoms of allergic rhinoconjunctivitis, and pollen sensitization. Overall, in the majority of the health outcomes, children

homozygous for GG in *CD14*–*1721* did not show an inverse association with farm milk consumption. For asthma, current wheeze, and symptoms of allergic rhinoconjunctivitis, the inverse association with farm milk was strongest in the AA genotype, intermediate in the AG genotype, and absent in the GG genotype. No effect modification by *CD14*–*1721* genotypes was observed for the association between farm milk consumption and sensitization to any allergen (≥ 3.5 IU/mL). However, for specific IgE against pollen (≥ 3.5 IU/mL), a strong protective farm milk effect was seen in the *CD14*–*1721* AA and AG genotypes but not in the GG genotype (Table III). Similar associations were observed when a cut-off value of 0.35 kU/L was used to define sensitization, even though trends were more pronounced in the PARSIFAL population.

When the combined population was stratified into farmers' and nonfarmers' children, the gene-environment interaction between *CD14*–*1721* and farm milk consumption in the first year of life was also seen, although the formal test for interaction was mostly not significant (Table IV).

When both study populations were combined, interactions between *CD14*–*1721* genotypes and farm milk consumption were significant for asthma ($P = .003$), current symptoms of allergic rhinoconjunctivitis ($P = .04$), and specific IgE against pollen of 3.5 kU/mL or greater ($P = .027$).

To evaluate the effect of timing of farm milk consumption, we categorized early and current farm milk consumption into 4 groups (early no, current no; early yes, current no; early no, current yes; early yes, current

TABLE III. Adjusted ORs for the association between farm milk consumption in the first year of life and allergic diseases in *CD14/–1721* genotypes in 2 independent rural populations

Response	<i>CD14/–1721</i>	ALEX study (n = 533)			PARSIFAL study (n = 1387)		
		n*	aOR (95% CI)†	P value‡	n*	aOR (95% CI)†	P value‡
Asthma	AA	172	0.29 (0.07–1.14)	.331 ^d	488	0.11 (0.03–0.49)	.004 ^a
	AG	272	0.38 (0.12–1.16)		628	0.54 (0.26–1.11)	
	GG	89	0.75 (0.18–3.1)		251	1.14 (0.46–2.86)	
Current wheezing	AA	169	0.53 (0.17–1.67)	.150 ^d	488	0.69 (0.3–1.62)	.441 ^a
	AG	270	0.80 (0.31–2.1)		634	0.90 (0.43–1.9)	
	GG	88	1.87 (0.53–6.6)		251	1.12 (0.4–3.19)	
Current symptoms of allergic rhinoconjunctivitis	AA	167	0.50 (0.14–1.76)	.242 ^d	491	0.42 (0.15–1.22)	.074 ^a
	AG	269	0.51 (0.19–1.36)		629	0.72 (0.32–1.6)	
	GG	87	1.21 (0.33–4.39)		251	1.57 (0.54–4.63)	
Specific serum IgE ≥3.5 kU/L Any allergen tested	AA	172	0.23 (0.09–0.57)	.119 ^f	479	0.86 (0.46–1.63)	.848 ^f
	AG	272	0.52 (0.27–1.02)		612	0.69 (0.38–1.27)	
	GG	89	0.50 (0.18–1.40)		241	1.14 (0.48–2.71)	
Pollen	AA	172	0.15 (0.04–0.56)	.107 ^a	480	0.58 (0.25–1.36)	.049 ^d
	AG	272	0.28 (0.12–0.66)		612	0.39 (0.15–0.97)	
	GG	89	0.56 (0.18–1.78)		241	1.73 (0.54–5.56)	
House dust mite	AA	172	0.34 (0.11–1.03)	.207 ^a	494	0.83 (0.37–1.84)	.523 ^a
	AG	272	0.69 (0.28–1.69)		637	0.93 (0.45–1.90)	
	GG	89	0.97 (0.23–4.08)		253	1.29 (0.44–3.82)	

Reference group: no farm milk consumption in the first year.

aOR, Adjusted OR.

*Sums of genotype group sizes and study population differ because of missing values in the respective phenotype variable.

†ORs are adjusted for being a farm child, age, study center, sex, environmental tobacco smoke, maternal education, and breast-feeding (exclusively ≥5 months).

‡Likelihood ratio test for interaction. P values are given for the best-fitting model, as represented by superscript letters: a, additive; d, dominant; r, recessive.

yes). Only children who drank farm milk in the first year of life (independently of current consumption) showed heterogeneity in frequencies of allergic health outcomes when stratified by *CD14/–1721* genotypes (see Table E6 in the Online Repository at www.jacionline.org).

The genotypes of *CD14/–1721* modified neither the association between being a farm child and allergic phenotypes (asthma, current wheeze, symptoms of allergic rhinoconjunctivitis, and atopic sensitization) nor the association between stable animal contact in the first year of life and allergic phenotypes (see Tables E4 and E5 in this article's Online Repository at www.jacionline.org).

Effects of *CD14/–1721* genotypes and farm milk consumption on gene expression of *CD14*

The subpopulation (n = 222) in which gene expression of *CD14* was measured consisted of 132 children who did drink farm milk in the first year of life and 89 who did not (no information about first-year farm milk consumption was available for 1 child). Previously published results showed that farmers' children expressed higher amounts of *CD14* on PBLs than nonfarmers' children (data not shown).^{17,18} In the univariate analysis PBLs of early farm milk drinkers appeared to express more *CD14* than those of non-farm milk drinkers. However, when

adjusted for being a farm child, this association was no longer significant (adjusted geometric means: no early farm milk consumption, 0.35 [95% CI, 0.29–0.43]; early farm milk consumption, 0.41 [95% CI, 0.36–0.47]; *P* = .258), thus making a direct farm milk effect unlikely.

Moreover, mean gene expression was similar in *CD14/–1721* genotypes (geometric means: AA, 0.39 [95% CI, 0.32–0.47]; AG, 0.37 [95% CI, 0.32–0.43]; GG, 0.42 [95% CI, 0.33–0.54]; *P* = .666).

However, stratification by genotype of *CD14/–1721* revealed heterogeneity of the farm milk effect on *CD14* gene expression (see Fig 1, including ORs for asthma presented in Table III to emphasize the similarity of the effect pattern). Children with a history of drinking farm milk in the first year of life and having either the AA or the AG genotype in *CD14/–1721* expressed 61% and 11%, respectively, more *CD14* compared with children not drinking farm milk in the first year of life. In contrast, children with the GG genotype expressed 24% less *CD14* if they had consumed farm milk in the first year of life than if they had not.

Results of the *CD14/–4191*, *CD14/–2839*, and *CD14/–260* polymorphisms

There was no significant association between the *CD14/–4191*, *CD14/–2839*, and *CD14/–260* polymorphisms

TABLE IV. Adjusted ORs for the association between farm milk consumption in the first year of life and allergic diseases in *CD14*–1721 genotypes in nonfarmers' and in farmers' children

Response	<i>CD14</i> –1721	Nonfarmers' children (n = 839)			Farmers' children (n = 1081)		
		n*	aOR (95% CI)†	P value‡	n*	aOR (95% CI)†	P value‡
Asthma	AA	258	0.14 (0.05-0.45)	.001 ^a	402	0.18 (0.02-1.39)	.288 ^a
	AG	430	0.57 (0.25-1.31)		470	0.27 (0.09-0.81)	
	GG	145	1.72 (0.50-5.96)		195	0.58 (0.15-2.20)	
Current wheezing	AA	255	0.99 (0.39-2.51)	.347 ^d	402	0.11 (0.01-0.85)	.029 ^a
	AG	431	0.89 (0.35-2.30)		473	0.53 (0.22-1.29)	
	GG	145	1.87 (0.48-7.33)		194	1.01 (0.31-3.24)	
Current symptoms of allergic rhinoconjunctivitis	AA	252	0.34 (0.12-1.0)	.026 ^a	406	0.60 (0.16-2.22)	.165 ^d
	AG	427	1.16 (0.41-3.32)		471	0.38 (0.15-0.96)	
	GG	144	1.76 (0.45-6.93)		194	1.2 (0.37-3.88)	
Specific serum IgE ≥3.5 kU/L Any allergen tested	AA	258	0.58 (0.28-1.19)	.283 ^a	393	0.45 (0.18-1.10)	.277 ^d
	AG	431	0.83 (0.40-1.73)		453	0.34 (0.17-0.69)	
	GG	144	1.13 (0.36-3.5)		186	0.69 (0.26-1.85)	
Pollen	AA	258	0.25 (0.1-0.67)	.025 ^a	394	0.37 (0.12-1.13)	.038 ^d
	AG	431	0.64 (0.2-2.02)		453	0.18 (0.07-0.49)	
	GG	144	1.65 (0.3-8.93)		186	0.96 (0.33-2.78)	
House dust mite	AA	259	0.89 (0.33-2.38)	.796 ^r	407	0.60 (0.19-1.85)	.82 ^r
	AG	433	0.62 (0.29-1.35)		476	0.62 (0.24-1.58)	
	GG	146	1.38 (0.35-5.50)		196	0.93 (0.23-3.69)	

Reference group: no farm milk consumption in the first year.

*Sums of genotype group sizes and study population differ because of missing values in the respective phenotype variable.

†ORs are adjusted for study, age, study center, sex, environmental tobacco smoke, maternal education, and breast-feeding (excluding >5 months).

‡Likelihood ratio test for interaction. *P* values are given for the best-fitting model, as represented by superscript letters: *a*, additive; *d*, dominant;

r, recessive.

and asthma or allergies or *CD14* gene expression. There was also no clear pattern of interaction between any of the polymorphisms and farm milk consumption on the phenotypes under study, except for symptoms of allergic rhinoconjunctivitis in the last 12 months in *CD14*–4191 (see Tables E7-E10 in the Online Repository at www.jacionline.org).

DISCUSSION

The results of our analyses suggest that a polymorphism in the *CD14* gene (*CD14*–1721) modifies the previously described inverse association of early farm milk consumption on allergic diseases in children. This gene–environment interaction was seen in 2 independent rural populations and was independent of being a farm child. In addition, *CD14*–1721 genotypes significantly modified the association between farm milk consumption and *CD14* expression in peripheral blood cells. These results suggest a biologic mechanism underlying the gene–environment interaction found for genetic variation in the *CD14* gene and farm milk consumption in early life on the development of asthma and allergies in this study.

CD14 is a pattern-recognition receptor of the innate immune system for a wide spectrum of microbial compounds,¹¹ such as LPS,²² lipoteichoic acid,²³ and

peptidoglycan,²⁴ but also for nonmicrobial compounds, such as phospholipids.¹²⁻¹⁴ This diversity of potential *CD14*-binding ligands and the fact that the allergy-protective compounds of farm milk have not yet been identified allow speculation about ways in which farm milk consumption might mediate the protective effect through *CD14*.

Farm milk is known to contain various bacterial species,²⁵ and there are 2 plausible and not mutually exclusive ways as to how the microbial composition of the farm milk might interact with the immune system through *CD14*. First, farm milk itself might contain microbial compounds that are able to bind to *CD14* after penetrating the intestinal mucosa.²⁶ Second, strains of probiotic bacteria contained in farm milk might have a balancing effect on the intestinal microflora, and their degradation products might interact with *CD14* after absorption.²⁷ Because *CD14* is known to be a coreceptor of Toll-like receptors 4²⁸ and 2,²⁹ complexes of *CD14* and their ligands might bind to one of these pattern-recognition receptors on the cell surface of antigen-presenting cells and hence activate the immune system, resulting in subsequent production of immunomodulatory chemokines and cytokines. Yet first analyses of milk samples of an ongoing birth cohort study in rural areas of Europe indicate that endotoxin levels are similar in milk consumed by farmers' and nonfarmers' families (Gehring, submitted) and thus challenge the role

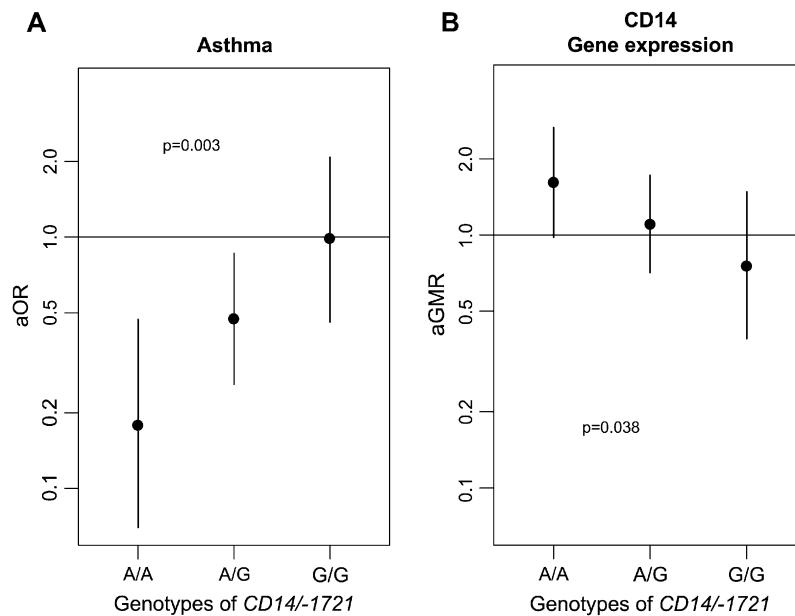


FIG 1. The effect of farm milk consumption on asthma (**A**; ALEX and PARSIFAL studies combined, $n = 1920$) and *CD14* gene expression (**B**; Swiss PARSIFAL subsample, $n = 222$) by genotypes of *CD14/-1721*. ORs and geometric means ratios are adjusted for being a farm child, age, and sex. ORs for asthma are additionally adjusted for study center, environmental tobacco smoke, maternal education, and breast-feeding (exclusively ≥ 5 months). P values are based on the test for interaction, assuming an additive effect of the A allele. aOR, Adjusted OR; aGMR, adjusted geometric means ratio.

of gram-negative microorganisms for the protective farm milk effect.

Alternatively, nonmicrobial molecules contained in the farm milk might be responsible for the protection against allergic diseases. There is some evidence that omega-3 dietary fatty acids, which have been found at higher levels in the milk of exclusively grass-fed cows than in the milk of cows fed with conserved grass,³⁰ are potentially beneficial to patients with allergic diseases.⁸⁻¹⁰ Thus animal feeding and also milk processing (fat standardization, pasteurization, and homogenization)³¹ might influence the milk's fat content or fatty acid profile. One might therefore speculate that farm milk differs from shop-purchased milk (which represents a mixture of milk produced in different regions and different types of farms) regarding its fat amount or composition. Because it is known that CD14 also acts as a receptor and carrier for phospholipids,¹²⁻¹⁴ fatty acids offer another potential mechanism as to how farm milk consumption might mediate protection against allergic diseases through CD14. However, because the immunologic relevant component has not been identified, the mechanisms underlying the observed association between farm milk consumption and allergic diseases remain speculative.

Variations in genotypes of *CD14/-1721* not only interacted with the effect of farm milk consumption on clinical outcomes but also on gene expression of *CD14*, suggesting that differential gene expression of *CD14* might mediate the farm milk effect on allergic diseases. Because *CD14/-1721* is located in the promoter region of the

CD14 gene, it appears plausible that a biologically relevant effect of this polymorphism would be mediated by changes in the transcriptional activity of the *CD14* gene. *CD14* gene expression was measured in a subsample of the Swiss PARSIFAL children to confirm the biologic relevance of the *CD14/-1721* polymorphism. However, neither farm milk consumption nor one of the *CD14/-1721* genotypes was directly associated with gene expression of *CD14*. An association between the *CD14/-1721* polymorphism and *CD14* gene expression became evident only on exposure to farm milk consumption. Thus the gene-environment interactions underlying the different health outcomes become evident at the level of gene expression, indicating that assessment of gene expression might be helpful when evaluating the biologic relevance of polymorphisms in promoter regions. One might speculate that exposure to farm milk activates antigen-presenting cells and induces expression of *CD14*. This might lead to an increased susceptibility to further stimulation by farm milk-associated agents.

To date, no functional data for *CD14/-1721* have been published. We can therefore not exclude the possibility that polymorphisms in linkage disequilibrium with *CD14/-1721* underlie our findings. For example, a well-characterized functional promoter polymorphism in *CD14* (*CD14/-260*)³² has been found in different populations to modify the effect of microbial exposures on the development of atopy.^{33,34} *CD14/-260* is in high linkage disequilibrium with *CD14/-1721* in our population ($R^2 > 0.7$). Therefore, not surprisingly, several ORs for

the associations between farm milk and the phenotypes studied were similar in both SNPs (Table III and Table E9 in the Online Repository at www.jacionline.org). The ORs were, however, less heterogeneous across the genotypes of *CD14*−260 than across *CD14*−1721 in both populations. Functional studies are needed to further evaluate the mechanisms underlying our observations and to elucidate the role of *CD14*−1721 in the development of asthma- and allergy-related phenotypes in the context of environmental exposures.

Recent studies suggest that pollen and house dust mite allergy represent 2 different phenotypes with respect to their genetic and environmental determinants.⁴ Therefore this finding might represent thus far unappreciated peculiarities in the biology of allergen/immune system interactions, but experimental studies are needed for further investigation of potential mechanisms.³⁵

We are aware that the sample size, particularly that of the ALEX population, was low to study gene-environment interactions. Therefore several formal tests for interaction might not have reached statistical significance. We do, however, believe that similar trends in the majority of the health outcome measures in the 2 independent study populations strongly suggest that the results have not only emerged because of chance.

Although our study included objective measurements, such as gene expression measurement, genotyping, and measurement of allergen-specific IgE, the limitations of the cross-sectional and observational design in elucidating biologic mechanisms are well recognized. Nevertheless, our results strongly suggest that biologic effects underlie the observed inverse association between farm milk consumption and allergic diseases, thus offering attractive avenues for future preventive measures.

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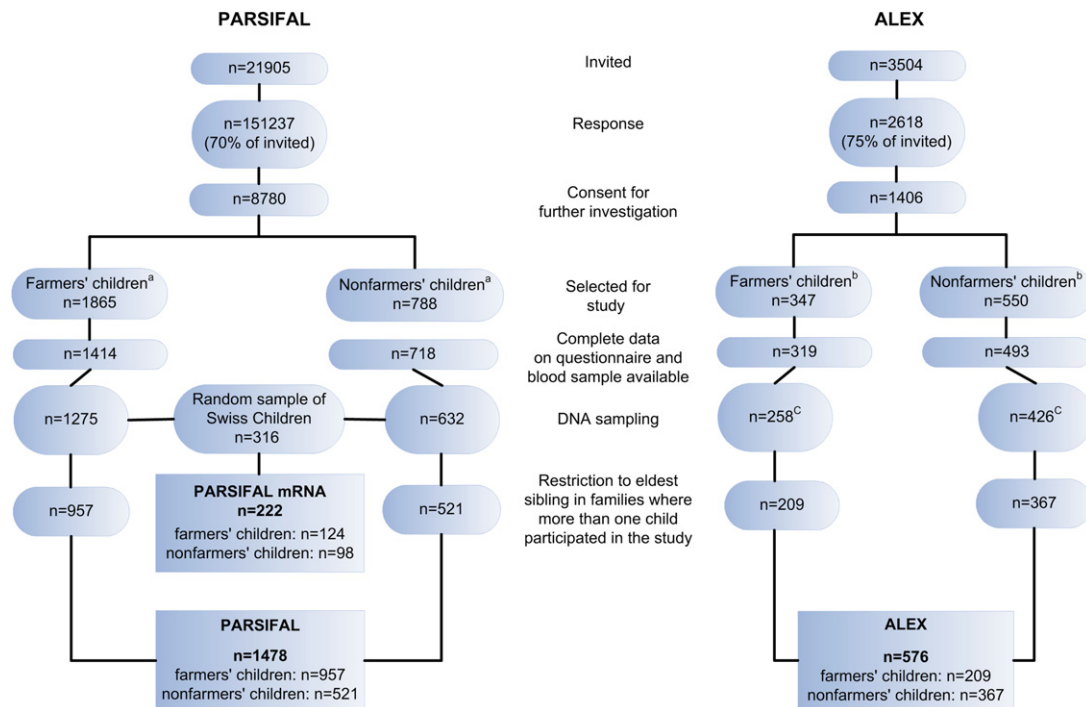


FIG E1. Selection of the 2 study populations. ^aRestriction to farmers' and nonfarmers' children (1450 children from Steiner schools and their 751 reference children excluded). ^bAll farmers' children, all nonfarmers' children with contact to farming, and a random sample of nonexposed nonfarmers' children were selected. ^cNo genetic testing was done for the Swiss children (n = 120).

TABLE E1. Linkage disequilibrium (R^2) between *CD14* SNPs in the ALEX population (n = 576)

	<i>CD14/C–2839T</i>	<i>CD14/A–1721G</i>	<i>CD14/C–260T</i>
<i>CD14/C–4191T</i>	0.115	0.218	0.278
<i>CD14/C–2839T</i>	—	0.282	0.369
<i>CD14/A–1721G</i>	—	—	0.764

The R^2 value is calculated with R (R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, Vienna, Austria; package “genetics,” function “LD”).

TABLE E2. Linkage disequilibrium (R^2) between *CD14* SNPs in the PARSIFAL population (n = 1539)

	<i>CD14/C–2839T</i>	<i>CD14/A–1721G</i>	<i>CD14/C–260T</i>
<i>CD14/C–4191T</i>	0.117	0.226	0.294
<i>CD14/C–2839T</i>	—	0.258	0.335
<i>CD14/A–1721G</i>	—	—	0.769

The R^2 value is calculated with R (R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, Vienna, Austria; package “genetics,” function “LD”).

TABLE E3. Prevalences of allergic health outcomes stratified by *CD14*–*1721* genotypes and farm milk consumption in the first year of life

Phenotype	<i>CD14</i> – <i>1721</i>	Farm milk consumption in the first year of life	
		No	Yes
		n (%)	n (%)
Asthma	A/A	45/374 (12.1)	6/287 (2.1)
	A/G	58/507 (11.5)	18/395 (4.6)
	G/G	20/180 (11.3)	15/160 (9.4)
Current wheezing	A/A	39/374 (10.5)	14/287 (5.0)
	A/G	45/507 (8.9)	22/395 (5.6)
	G/G	18/180 (10.1)	17/160 (10.8)
Current symptoms of allergic rhinoconjunctivitis	A/A	31/374 (8.4)	9/287 (3.2)
	A/G	46/507 (9.2)	19/395 (4.9)
	G/G	16/180 (9.1)	14/160 (8.8)
Specific serum IgE $\geq$ 3.5 kU/L Any allergen tested	A/A	72/374 (19.7)	31/287 (11.0)
	A/G	89/507 (18.1)	46/395 (12.0)
	G/G	31/180 (18.0)	23/160 (14.8)
Pollen	A/A	50/374 (13.7)	13/287 (4.6)
	A/G	64/507 (13.0)	18/395 (4.7)
	G/G	18/180 (10.5)	14/160 (9.0)
House dust mite	A/A	37/374 (9.9)	19/287 (6.6)
	A/G	43/507 (8.5)	31/395 (7.9)
	G/G	14/180 (7.8)	15/160 (9.4)

TABLE E4. Prevalences of allergic health outcomes stratified by *CD14/– 1721* genotypes and being a farm child

Phenotype	<i>CD14/– 1721</i>	Farmers' child	
		No	Yes
		n (%)	n (%)
Asthma	A/A	25/259 (9.7)	27/407 (6.7)
	A/G	46/433 (10.7)	30/478 (6.4)
	G/G	21/147 (14.5)	15/196 (7.7)
Current wheezing	A/A	29/259 (11.4)	25/407 (6.2)
	A/G	42/433 (9.7)	25/478 (5.3)
	G/G	21/147 (14.5)	15/196 (7.7)
Current symptoms of allergic rhinoconjunctivitis	A/A	23/259 (9.1)	17/407 (4.2)
	A/G	45/433 (10.5)	20/478 (4.2)
	G/G	17/147 (11.8)	14/196 (7.2)
Specific serum IgE $\geq$ 3.5 kU/L			
Any allergen tested	A/A	62/259 (24.0)	43/407 (10.9)
	A/G	91/433 (21.1)	46/478 (10.2)
	G/G	36/147 (25.0)	19/196 (10.2)
Pollen	A/A	41/259 (15.9)	23/407 (5.8)
	A/G	64/433 (14.8)	18/478 (4.0)
	G/G	22/147 (15.3)	10/196 (5.4)
House dust mite	A/A	34/259 (13.1)	24/407 (5.9)
	A/G	40/433 (9.2)	36/478 (7.6)
	G/G	17/147 (11.6)	13/196 (6.6)

TABLE E5. Prevalences of allergic health outcomes stratified by *CD14/– 1721* genotypes and stable animal contact in the first year of life

Phenotype	<i>CD14/– 1721</i>	Stable animal contact in the first year of life	
		No	Yes
		n (%)	n (%)
Asthma	A/A	25/262 (9.6)	15/264 (5.7)
	A/G	33/390 (8.5)	21/341 (6.3)
	G/G	17/148 (11.6)	7/129 (5.5)
Current wheezing	A/A	25/262 (9.8)	18/264 (6.8)
	A/G	30/390 (7.8)	19/341 (5.6)
	G/G	20/148 (13.7)	8/129 (6.2)
Current symptoms of allergic rhinoconjunctivitis	A/A	19/262 (7.4)	12/264 (4.6)
	A/G	37/390 (9.6)	10/341 (3.0)
	G/G	19/148 (13.1)	7/129 (5.5)
Specific serum IgE $\geq$ 3.5 kU/L Any allergen tested	A/A	53/262 (20.5)	23/264 (9.0)
	A/G	73/390 (19.0)	29/341 (8.9)
	G/G	28/148 (19.3)	11/129 (8.9)
Pollen	A/A	33/262 (12.7)	10/264 (3.9)
	A/G	47/390 (12.2)	13/341 (4.0)
	G/G	20/148 (13.8)	2/129 (1.6)
House dust mite	A/A	26/262 (9.9)	14/264 (5.3)
	A/G	35/390 (9.0)	19/341 (5.6)
	G/G	12/148 (8.1)	10/129 (7.8)

TABLE E6. Prevalence of allergic health outcomes by *CD14*/– *1721* genotypes and timing of farm milk consumption

Phenotype	<i>CD14</i> /– <i>1721</i>	Timing of farm milk consumption			
		First no, current no	First yes, current no	First no, current yes	First yes, current yes
		n (%)	n (%)	n (%)	n (%)
Asthma	A/A	32/289 (11.1)	0/44 (0.0)	11/80 (13.8)	6/240 (2.5)
	A/G	49/378 (13.1)	4/63 (6.5)	9/114 (8.0)	14/328 (4.3)
	G/G	16/130 (12.4)	5/27 (18.5)	4/47 (8.9)	10/130 (7.7)
Current wheezing	A/A	30/289 (10.5)	3/44 (7.0)	7/80 (9.0)	11/240 (4.7)
	A/G	34/378 (9.0)	3/63 (4.8)	9/114 (8.0)	19/328 (5.9)
	G/G	16/130 (12.4)	7/27 (26.9)	2/47 (4.3)	10/130 (7.8)
Current symptoms of allergic rhinoconjunctivitis	A/A	24/289 (8.5)	1/44 (2.3)	6/80 (7.5)	8/240 (3.3)
	A/G	34/378 (9.1)	5/63 (7.9)	9/114 (8.0)	14/328 (4.4)
	G/G	12/130 (9.4)	5/27 (18.5)	4/47 (8.7)	7/130 (5.4)
Specific serum IgE $\geq$ 3.5 kU/L Any allergen tested	A/A	57/289 (20.1)	5/44 (11.9)	11/80 (14.1)	26/240 (11.0)
	A/G	67/378 (18.2)	8/63 (13.3)	20/114 (18.2)	38/328 (11.9)
	G/G	25/130 (20.2)	8/27 (30.8)	6/47 (13.0)	15/130 (11.7)
Pollen	A/A	40/289 (14.1)	2/44 (4.8)	7/80 (9.0)	11/240 (4.6)
	A/G	48/378 (13.0)	3/63 (5.0)	14/114 (12.7)	15/328 (4.7)
	G/G	16/130 (12.9)	8/27 (30.8)	2/47 (4.3)	6/130 (4.7)
House dust mite	A/A	29/289 (10.0)	6/44 (13.6)	6/80 (7.5)	13/240 (5.4)
	A/G	32/378 (8.5)	7/63 (11.1)	10/114 (8.8)	24/328 (7.3)
	G/G	10/130 (7.8)	2/27 (7.4)	4/47 (8.5)	13/130 (10.0)

TABLE E7. Adjusted ORs for the association between farm milk consumption in the first year of life and allergic diseases in *CD14/-4191* genotypes in 2 independent rural populations

Phenotype	<i>CD14/-4191</i>	ALEX study (n = 554)			PARSIFAL study (n = 1422)		
		n*	aOR (95% CI)†	P value‡	n*	aOR (95% CI)†	P value‡
Asthma	CC	320	0.29 (0.12-0.74)		810	0.77 (0.43-1.38)	.017
	CT	213	0.33 (0.11-1.01)		509	0.21 (0.07-0.61)	
	TT	21	No cases		83	No cases	
Current wheezing	CC	316	0.95 (0.46-1.96)		811	0.95 (0.50-1.79)	
	CT	209	0.53 (0.19-1.50)		513	0.71 (0.31-1.63)	
	TT	20	No cases		84	0.82 (0.18-3.84)	
Current symptoms of allergic rhinoconjunctivitis	CC	317	0.87 (0.42-1.81)	.014	810	1.04 (0.54-2.00)	.082
	CT	208	0.14 (0.03-0.63)		511	0.40 (0.14-1.11)	
	TT	19	No cases		85	No cases	
Specific serum IgE ≥3.5 kU/L Any allergen tested	CC	320	0.49 (0.27-0.88)		781	1.31 (0.80-2.14)	.095 ^f
	CT	213	0.31 (0.15-0.66)		500	0.65 (0.34-1.23)	
	TT	21	0.13 (0.01-1.06)		84	1.28 (0.32-5.12)	
Pollen	CC	320	0.32 (0.16-0.65)		781	0.97 (0.50-1.88)	.086 ^f
	CT	213	0.22 (0.09-0.54)		501	0.40 (0.16-0.99)	
	TT	21	0.11 (0.01-1.37)		84	0.66 (0.05-7.95)	
House dust mite	CC	320	1.41 (0.65-3.07)	.006 ^f	813	1.49 (0.80-2.78)	
	CT	213	0.28 (0.09-0.83)		520	0.76 (0.36-1.58)	
	TT	21	0.16 (0.01-2.23)		85	2.01 (0.30-13.41)	

Reference group: children without farm milk consumption in the first year of life.

aOR, Adjusted OR.

*Sums of genotype group sizes and study population differ because of missing values in the respective phenotype variable.

†ORs are adjusted for being a farm child, age, sex, and study center.

‡Likelihood ratio test for interaction. P values are given for the best-fitting model: r, recessive.

TABLE E8. Adjusted ORs for the association between farm milk consumption in the first year of life and allergic diseases in *CD14/-2839* genotypes in 2 independent rural populations

Phenotype	<i>CD14/-2839</i>	ALEX study (n = 553)			PARSIFAL study (n = 1416)		
		n*	aOR (95% CI)†	P value‡	n*	aOR (95% CI)†	P value‡
Asthma	CC	283	0.33 (0.13-0.85)		755	0.40 (0.2-0.77)	
	CT	232	0.38 (0.13-1.10)		530	0.68 (0.33-1.39)	
	TT	38	No cases		111	No cases	
Current wheezing	CC	278	0.85 (0.40-1.83)		760	0.67 (0.35-1.26)	.045 ^d
	CT	229	0.89 (0.33-2.38)		532	1.41 (0.67-2.98)	
	TT	37	0.28 (0.03-2.76)		110	0 (0-Inf)	
Current symptoms of allergic rhinoconjunctivitis	CC	275	0.55 (0.24-1.25)		758	0.64 (0.31-1.32)	
	CT	231	0.73 (0.28-1.90)		531	0.65 (0.28-1.52)	
	TT	37	0.35 (0.03-3.63)		111	2.78 (0.43-18.0)	
Specific serum IgE ≥3.5 kU/L Any allergen tested	CC	283	0.42 (0.23-0.77)		737	0.94 (0.56-1.57)	
	CT	232	0.35 (0.17-0.72)		513	1.10 (0.61-1.99)	
	TT	38	0.29 (0.05-1.70)		108	0.95 (0.28-3.23)	
Pollen	CC	283	0.31 (0.15-0.62)		737	0.8 (0.39-1.64)	
	CT	232	0.22 (0.09-0.55)		514	0.49 (0.21-1.14)	
	TT	38	No cases		108	0.85 (0.19-3.82)	
House dust mite	CC	283	0.75 (0.33-1.72)		766	0.98 (0.53-1.83)	
	CT	232	0.69 (0.29-1.68)		535	1.62 (0.79-3.33)	
	TT	38	1.01 (0.12-8.51)		111	0.61 (0.11-3.32)	

Reference group: children without farm milk consumption in the first year of life.

*Sums of genotype group sizes and study population differ because of missing values in the respective phenotype variable.

†ORs are adjusted for being a farm child, age, sex, and study center.

‡Likelihood ratio test for interaction with $P \leq .1$. *P* value is given for the best-fitting interaction model: *d*, dominant.

TABLE E9. Adjusted ORs for the association between farm milk consumption in the first year of life and allergic diseases in *CD14/-260* genotypes in 2 independent rural populations

Phenotype	<i>CD14/-260</i>	ALEX study (n = 533)			PARSIFAL study (n = 1419)		
		n*	aOR (95% CI)†	P value‡	n*	aOR (95% CI)†	P value‡
Asthma	CC	133	0.45 (0.12-1.70)		393	0.09 (0.01-0.67)	.018 ^r
	CT	279	0.24 (0.07-0.75)		672	0.51 (0.27-0.98)	
	TT	121	0.37 (0.10-1.32)		333	0.87 (0.38-1.95)	
Current wheezing	CC	131	0.65 (0.20-2.18)		393	0.77 (0.30-1.99)	
	CT	276	0.71 (0.28-1.79)		678	0.82 (0.42-1.61)	
	TT	119	1.64 (0.54-4.94)		334	0.9 (0.37-2.19)	
Current symptoms of allergic rhinoconjunctivitis	CC	129	0.69 (0.15-3.10)		396	0.66 (0.22-1.97)	
	CT	275	0.48 (0.20-1.16)		674	0.56 (0.26-1.20)	
	TT	118	1.01 (0.32-3.21)		333	1.11 (0.44-2.78)	
Specific serum IgE ≥3.5 kU/L Any allergen tested	CC	133	0.30 (0.12-0.79)		386	1.00 (0.51-1.99)	
	CT	279	0.37 (0.20-0.7)		656	0.84 (0.48-1.45)	
	TT	121	0.60 (0.25-1.45)		321	1.55 (0.77-3.14)	
Pollen	CC	133	0.12 (0.02-0.56)		387	0.53 (0.21-1.37)	.033 ^d
	CT	279	0.22 (0.10-0.49)		656	0.51 (0.24-1.11)	
	TT	121	0.53 (0.18-1.50)		321	1.73 (0.65-4.6)	
House dust mite	CC	133	0.41 (0.13-1.28)	0.087 ^a	398	1.16 (0.5-2.71)	
	CT	279	0.75 (0.31-1.8)		682	0.99 (0.51-1.91)	
	TT	121	1.47 (0.45-4.85)		336	1.61 (0.67-3.88)	

Reference group: children without farm milk consumption in the first year of life.

*Sums of genotype group sizes and study population differ because of missing values in the respective phenotype variable.

†ORs are adjusted for being a farm child, age, sex, and study center.

‡Likelihood ratio test for interaction with $P \leq .1$. *P* values are given for the best-fitting interaction model: *d*, dominant; *r*, recessive; *a*, additive.

TABLE E10. Effect modification of *CD14* polymorphisms on the association between farm milk consumption in the first year of life and gene expression of *CD14* (n = 222)

Polymorphism	Genotype	n	PARSIFAL Switzerland	
			aGMR (95% CI)*	P value†
<i>CD14/C-4191T</i>	CC	121	1.29 (0.89-1.86)	.077 ^d
	CT	82	1.57 (1.01-2.46)	
	TT	17	3.30 (1.25-8.76)	
<i>CD14/C-2839T</i>	CC	118	1.40 (0.97-2.01)	.073 ^e
	CT	86	1.66 (1.07-2.59)	
	TT	15	1.75 (0.61-5.05)	
<i>CD14/C-260T</i>	CC	64	2.10 (1.26-3.49)	
	CT	105	1.37 (0.92-2.03)	
	TT	48	1.03 (0.58-1.82)	

aGMR, Adjusted geometric means ratio.

*Geometric means ratios are given for farm milk consumption in the first year of life. Reference group: no farm milk consumption in the first year of life.

Geometric means ratios are adjusted for being a farm child, age, and sex.

†Likelihood ratio test for interaction with $P \leq .1$. P values are given for the best-fitting interaction model: *d*, dominant; *r*, recessive; *a*, additive.