

Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis

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Background: Cross-sectional studies have suggested that prenatal farm exposures might protect against allergic disease and increase the expression of receptors of the innate immune system. However, epidemiologic evidence supporting the association with atopic dermatitis remains inconsistent.

Objective: To study the association between prenatal farm-related exposures and atopic dermatitis in a prospective study. We further analyzed the association between the expression of innate immune genes at birth and atopic dermatitis.

Methods: A total of 1063 children who participated in a birth cohort study, Protection against Allergy-Study in Rural Environments, were included in this study. Doctor diagnosis of atopic dermatitis was reported by the parents from 1 to 2 years of age by questionnaire. Gene expression of Toll-like receptors (TLRs) and CD14 was assessed in cord blood leukocytes by quantitative PCR.

Results: Maternal contact with farm animals and cats during pregnancy had a significantly protective effect on atopic dermatitis in the first 2 years of life. The risk of atopic dermatitis was reduced by more than half among children with mothers having contact with 3 or more farm animal species during pregnancy compared with children with mothers without contact (adjusted odds ratio, 0.43; 95% CI, 0.19-0.97). Elevated expression of TLR5 and TLR9 in cord blood was associated with decreased doctor diagnosis of atopic dermatitis. A significant interaction between polymorphism in *TLR2* and prenatal cat exposure was observed in atopic dermatitis.

Conclusion: Maternal contact with farm animals and cats during pregnancy has a protective effect on the development of atopic dermatitis in early life, which is associated with a lower expression of innate immune receptors at birth. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Prenatal, farm animal, Toll-like receptors, atopic dermatitis, gene-environment interaction

Atopic dermatitis is a chronic, inflammatory, pruritic skin disease, often occurring in early infancy, that affects up to 20% of the children in industrialized countries.¹ Asthma develops in approximately 30% of children with atopic dermatitis, and allergic rhinitis in 35%.² According to the International Study of Asthma and Allergies in Childhood (ISAAC), the prevalence of symptoms of atopic dermatitis in children 6 or 7 years of age during a 1-year period varied from less than 2% in Iran and China to approximately 20% in Australia, England, and Scandinavia.¹ The etiology of atopic dermatitis is complex and involves an interaction between genetic and environmental factors and the immune system. The hygiene hypothesis has contributed to the understanding of allergic diseases, suggesting that lack of microbial exposure might be a risk factor. A protective effect of microbial exposure on asthma has been described.³⁻⁵ However, epidemiologic evidence supporting these associations with atopic dermatitis remains inconsistent.⁶⁻⁸

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Abbreviations used

EFRAIM: Mechanism of Early Protective Exposures on Allergy Development
 GEE: General estimating equation
 GMR: Geometric mean ratio
 ISAAC: International Study of Asthma and Allergies in Childhood
 OR: Odds ratio
 PASTURE: Protection against Allergy-Study in Rural Environments
 SNP: Single nucleotide polymorphism
 TLR: Toll-like receptor

In addition, recent cross-sectional studies have shown that the protective effect of farm exposures on allergic diseases could already be effective during pregnancy.^{9,10}

Although it has been suggested that an altered stimulation of the innate immune system might influence the development of allergic disease, the underlying immunologic mechanisms of these protective effects of farm exposures remain unclear. Activation of the innate immune system is mediated by pattern recognition receptors, such as Toll-like receptors (TLRs), which are present on immune cells and recognize pathogen-associated molecular patterns. Previous studies have suggested that gene expression of these receptors is upregulated by exposure to environmental factors rich in microbial compounds.^{9,11} Moreover, several findings indicate that environmental factors associated with high levels of microbial components may interact with existing polymorphisms in *TLRs* on the development of allergic disease, and direct associations between single nucleotide polymorphisms (SNPs) in *TLRs* and allergies have been shown.¹²⁻¹⁵ Recently an association between a *TLR9* promoter polymorphism and atopic eczema has been reported.¹⁶ However the direct correlation between the presence of allergic diseases in children and TLRs or CD14 expression was not made. Defining whether there is a difference in gene expression would facilitate a better understanding of the determinants of atopic dermatitis.

In this study, we longitudinally analyzed the effect of prenatal environmental exposures related to the hygiene hypothesis on the development of atopic dermatitis in the first 2 years of life within a prospective birth cohort, the Protection against Allergy-Study in Rural Environments (PASTURE)/Mechanism of Early Protective Exposures on Allergy Development (EFRAIM) study.¹⁷ Because gene expression of innate immunity receptors reflects both genetic and environmental influences, we further examined whether TLRs and CD14 expression in cord blood samples from these children was associated with the development of atopic dermatitis. In addition, we investigated whether the interaction between polymorphisms in *TLRs* and prenatal exposures has an impact on gene expression and on atopic dermatitis.

METHODS**Study design**

The PASTURE/EFRAIM study is a prospective birth cohort study involving children from rural areas in 5 European countries (Austria, Finland, France, Germany, and Switzerland), designed to evaluate risk factors and preventive factors for atopic diseases. The design of this cohort has been described in detail elsewhere.¹⁷ Briefly, pregnant women were recruited during the third trimester of pregnancy and divided into 2 groups. Women who lived or worked on family-run farms where any kind of livestock was kept

were assigned to the farm group. The reference group was composed of women from the same rural areas not living on a farm. In total, 1133 children were included in this birth cohort. The questionnaires developed within the PASTURE study group used questions on various exposures from ISAAC,¹⁸ the Allergy and Endotoxin (ALEX) study,³ and the Prevention of Allergy Risk factors for Sensitization in children related to Farming and Anthroposophic Lifestyle (PARSIFAL) study.⁵ Questionnaires were administered in interviews or self-administered to the mothers within the third trimester of pregnancy and when the children were 2, 12, 18, and 24 months of age. The study was approved by the local research ethics committees in each country, and written informed consent was obtained from all parents.

Study population

Children from the PASTURE/EFRAIM birth cohort with data available on atopic dermatitis at least once between 1 and 2 years of age and on farming status ($n = 1063$) were included. Among these children, 905 have data available on mRNA analysis in cord blood samples and between 961 and 987 on polymorphisms in *TLRs*, depending on the SNP.

Definitions

Children were labeled as having doctor's diagnosis of atopic dermatitis when the parents reported at least once in the questionnaire at the age of 12, 18, or 24 months that the child had been diagnosed with atopic dermatitis by a doctor. Farmer children were defined as children whose parents answered positively to the question, "Does your child live on a farm?" and whose family ran the farm. Maternal farm-related and pet exposures during pregnancy were obtained from the self-reported questionnaires at the third trimester of pregnancy. Prenatal exposures to barn or stable were defined as an exposure of at least a quarter of an hour per week. Contact with farm animal species was assumed if the mother reported contact at least several times per month in 1 of the pregnancy trimesters. Consumption of farm milk was defined as a consumption of at least on average 10 mL farm milk per day. Data on potential confounders, such as smoking during pregnancy, sex, mode of delivery, birth weight, gestational age, maternal education, and breast-feeding, were obtained from the self-reported questionnaires at the third trimester of pregnancy, at 2 months, and 1 year of age. Maternal atopy was defined as ever having asthma or hay fever. This information was self-reported.

Expression of Toll-like receptors and CD14

Blood samples were collected from the umbilical cord at birth. For the assessment of mRNA, the blood was collected in a PAXgene Blood RNA tube containing an RNA-stabilizing solution (PreAnalytiX/Qiagen, Hilden, Germany) and then frozen to -80°C within 24 hours.¹⁹ In a central laboratory (Zurich, Switzerland), the RNA was isolated by using the PAXgene 96 Blood RNA Kit (PreAnalytiX/Qiagen) supplemented with RNase-free DNase (Qiagen). The mRNA was reverse-transcribed into cDNA by using the TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, Calif). Quantitative real-time PCR was performed on the 7900HT Fast Real-Time PCR System by using the Micro fluidic card TaqMan Array system of Applied Biosystems. The data presented are normalized values for the endogenous controls (18S ribosomal RNA and β -2-microglobulin) using the comparative (delta delta cycle threshold, $\Delta\Delta\text{Ct}$) method according to the manufacturer's instructions (Applied Biosystems). TLR3 expression was excluded from the analyses because the expression level was less than the detection limit on most of the cord blood samples.

Genotyping

Genotyping was performed by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as described previously.²⁰ Derived genotype frequencies were compared with the expected allelic population equilibrium based on the Hardy-Weinberg equilibrium test to control for technical genotyping errors. cDNA was amplified in duplicate by using an iCycler (Bio-Rad, Hercules, Calif), with 18s as a reference gene.

TABLE I. Characteristics of children

Characteristics	Total study population N = 1063 n (%)	Farmer n = 508 n (%)	Nonfarmer n = 555 n (%)
Girl	517 (48.7)	249 (49.0)	268 (48.5)
Center			
Austria	209 (19.6)	99 (19.5)	110 (19.8)
Switzerland	226 (21.3)	101 (19.9)	125 (22.5)
France	191 (18.0)	91 (17.9)	100 (18.0)
Germany	233 (21.9)	109 (21.5)	124 (22.4)
Finland	204 (19.2)	108 (21.3)	96 (17.3)
Mother with atopy history*	310 (29.2)	118 (23.3)	192 (34.6)
Mode of delivery: cesarean section	185 (17.6)	86 (17.0)	99 (18.0)
Birth weight			
<2500 g	16 (1.5)	9 (1.8)	7 (1.3)
2500-4500 g	1019 (96.0)	484 (95.3)	535 (96.7)
>4500 g	26 (2.5)	15 (2.9)	11 (2.0)
No. of siblings*			
0	383 (36.0)	129 (25.4)	254 (45.8)
1-2	567 (53.4)	291 (57.3)	276 (49.7)
≥3	113 (10.6)	88 (17.3)	25 (4.5)
Mother smoking during pregnancy* (at least in 1 trimester)	140 (13.2)	43 (8.5)	97 (17.5)
Prenatal farm exposures			
Work on a farm during pregnancy*	406 (38.3)	364 (71.8)	42 (7.6)
Farm milk during pregnancy*	445 (42.0)	372 (73.5)	73 (13.2)
Farm milk during pregnancy: unboiled*	358 (33.8)	296 (58.5)	62 (11.2)
Work in stable during pregnancy* (at least 15 min/wk in 1 of the trimesters)	541 (53.2)	443 (89.3)	98 (18.8)
Work in barn during pregnancy* (at least 15 min/wk in 1 of the trimesters)	402 (39.6)	342 (69.1)	60 (11.5)
Contact with farm animals during pregnancy* (horse, cow, pig, or poultry, at least several times per month)			
3-4 species	120 (11.7)	96 (20.0)	24 (4.4)
1-2 species	508 (49.7)	345 (71.9)	163 (30.1)
0 species	394 (38.6)	39 (8.1)	355 (65.5)
Contact with any pets during pregnancy*	666 (62.7)	419 (82.5)	247 (44.5)
Contact with cats during pregnancy*	472 (44.4)	349 (68.8)	123 (22.2)
Contact with dogs during pregnancy*	344 (32.5)	241 (47.7)	103 (18.6)

* $P < .05$, based on χ^2 test between farmer and nonfarmer.

Polymorphisms in *TLRs* were selected as previously described.²¹ These SNPs were as follows: *TLR1/C-2299T* (rs5743594), *TLR1/T-2192C* (rs5743595), *TLR1/A742G* (rs4833095), *TLR2/T596C* (rs3804099), *TLR2/T-16934A* (rs4696480), *TLR4/C8851T*, *TLR4/A8551G*, *TLR4/T-1607C*, *TLR5/T1845C* (rs5744174), *TLR5/C1173T* (rs5744168), *TLR5/A1774G* (rs2072493), *TLR6/T-2079A* (rs5743789), *TLR7/A1796T* (rs179008), *TLR7/C12318T*, *TLR8/C10907A*, *TLR8/A-4824G* (rs3761624), *TLR9/T-2622C* (rs5743836), and *TLR9/T-2871C* (rs187084).

Statistical analysis

Data analysis was conducted by using SAS software version 9.2 (SAS Institute, Inc, Cary, NC).

General estimating equations (GEEs) were used to investigate the longitudinal effects of prenatal farm exposures, gene expression of *TLRs* and *CD14* in cord blood, and genotypes of SNPs in *TLRs* on atopic dermatitis from age 1 to 2 years, taking into account the correlation between repeated measures in the same individual. The associations between the different SNPs in *TLR* and atopic dermatitis were corrected for multiple testing by using the false discovery rate (Benjamini procedure; $P \leq .05$ is significant).²² To test for effect modification, we calculated terms for interactions between farm exposures and *TLR* expression and the different centers in the GEE model, and they were not significant. For gene expression, geometric mean ratios (GMRs) with 95% CIs were calculated by exponentiation of the regression coefficients and their 95% CIs. Estimates of cumulative incidence of atopic dermatitis in the first 2 years of age were calculated by the discrete time survival analysis among 3 groups of children categorized by the tertile of gene expression. The discrete time hazard is the conditional probability that a child will develop

an atopic dermatitis onset in a period given that atopic dermatitis was not reported in an earlier period. Linear regression was performed to analyze the association between prenatal exposure and mRNA gene expression in cord blood, and when heterogeneity between study centers was significant, the model was adjusted for center as a random effect estimate, and as a fixed effect estimate when there was no heterogeneity. To test for gene-environment interactions, interaction terms were included in the models. Adjustment was made for the following potential confounders: farming status, maternal atopy, sex, number of older siblings, smoking during pregnancy, and study center. Birth weight, mode of delivery, maternal education, exposure to stable in the first year of life, breast-feeding, and consumption of farm milk in the first year of life were added to the model but did not change the results, so they were not kept in the final model. A P value below .05 was considered statistically significant.

RESULTS

General characteristics

Among the 1063 children included in this study, there were 508 farmers and 555 nonfarmers. The proportion of children having a doctor diagnosis of atopic dermatitis at least once between 1 and 2 years of age among the total study population was 17.8%. The characteristics of the study population showed no differences of the distribution of farmer and nonfarmer children among the 5 centers (Table I). Among children in the nonfarmer group, the frequency of mothers with allergy and those smoking during

pregnancy were higher compared with the children in the farmer group. Among farmer children, the number of siblings was higher compared with nonfarmer children. For the other characteristics, mode of delivery and birth weight, there were no differences depending on the farming status. We observed that a small number of mothers ($n = 42$) from the nonfarmer group were working on a farm (from neighbors or relatives) or were in contact with a farm animal during pregnancy. The maternal contact with pets was higher among the farmer than the nonfarmer group.

There was no difference with respect to farming status between the total study population and the subgroup of children with data available on gene expression of TLRs and CD14 at birth (data not shown).

Association between prenatal farm exposures and atopic dermatitis

The cumulative prevalence of atopic dermatitis was lower in farmer children than nonfarmer children between 1 and 2 years of age, even though it was not significant (Fig 1, A). Among children with mothers working on a farm during pregnancy, this cumulative prevalence was significantly lower compared with children with mothers not working on a farm (14.4% and 20.0% at 2 years of age, respectively; Fig 1, B). The longitudinal analyses accounting for repeated measurements (GEE) showed that prenatal farm animal contact was associated with a lower risk of developing atopic dermatitis in the first 2 years of life (Table II). The consumption of farm milk or unboiled farm milk during pregnancy was not associated with atopic dermatitis. Contact during pregnancy with horses, cows, pigs, and poultry, for which we observed a negative association with atopic dermatitis, was included to calculate a score of the number of species the mother had contact with during pregnancy. The risk of developing atopic dermatitis was reduced by a factor of 0.7 among children with mothers having contact with 1 to 2 species and by a factor of 0.4 with prenatal contact with 3 to 4 species compared with the reference group with no prenatal contact with farm animals. For each additional farm animal species the mother had contact with, we observed a reduction of 20% in the development of atopic dermatitis (adjusted odds ratio [OR], 0.80; 95% CI, 0.65-0.99; Fig 2). Moreover, we found a significant negative association between prenatal contact with cats and the presence of atopic dermatitis in the first 2 years of life (adjusted OR, 0.68; 95% CI, 0.46-1.00).

There was no significant association between the different SNPs in *TLRs* and atopic dermatitis after correction for multiple testing (data not shown). The protective effect of prenatal contact with farm animals and cats might differ between the genotypes of SNPs in *TLRs* (see this article's Table E1 in the Online Repository at www.jacionline.org). The analyses for a gene-environment interaction showed a significant ($P = .02$) effect modification of 1 SNP in *TLR2* (*TLR2/T-16934A*) on the association between prenatal exposure to cats and the development of atopic dermatitis early in life and a marginally significant effect modification ($P = .09$) for prenatal exposure to farm animals. We observed a strong protective effect of the prenatal animal (farm animals and cats) exposures only among children with genotype AA in *TLR2/T-16934A* (Fig 3). We did not observe a significant gene-environment interaction for the other SNPs in *TLRs*. However, the significant protective effect of contact with farm animals and cats during pregnancy on atopic dermatitis was more

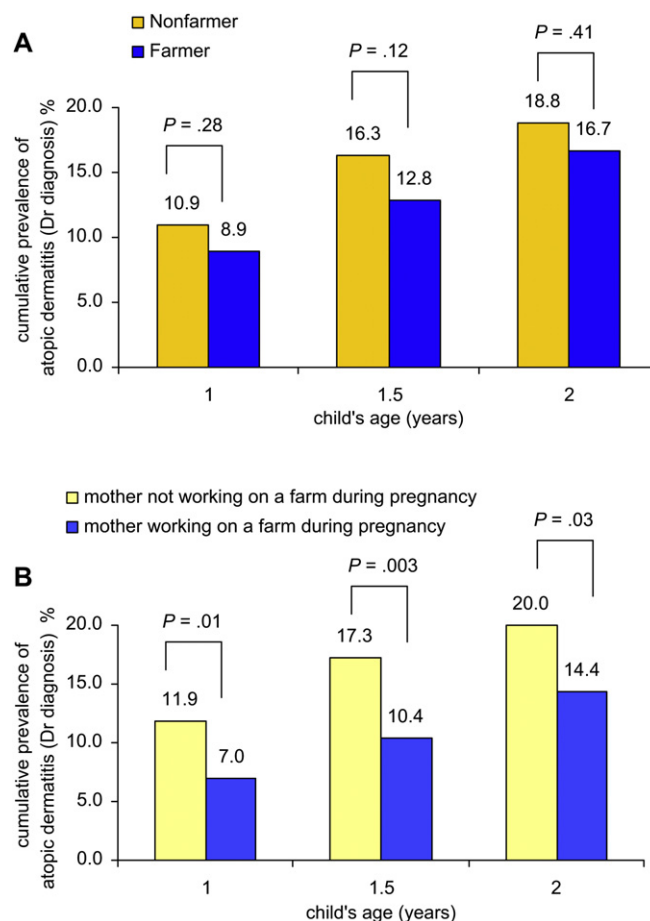


FIG 1. Cumulative prevalence of atopic dermatitis between farmers and nonfarmers (A) and between mothers working and not working on a farm during pregnancy (B). P value based on the χ^2 test. Dr, Doctor.

pronounced among certain genotypes. Because we found differences between the distribution of genotypes between Finland and the other centers, we repeated the same analyses excluding the Finnish population, and we observed strongly comparable results (data not shown).

Association between gene expression and atopic dermatitis

We observed a negative association between the gene expression of TLRs and CD14 assessed in cord blood and the development of atopic dermatitis in the first 2 years of life (Fig 4). Gene expression of TLR5 and TLR9 in cord blood was significantly reduced among children with atopic dermatitis compared with children with no dermatitis (adjusted GMR, 0.66; 95% CI, 0.48-0.89; and adjusted GMR, 0.73; 95% CI, 0.56-0.96, respectively). The same trend was observed for TLRs 1, 2, 4, 6, 7, and 8 and CD14, even though it was not significant. None of the potential confounding factors (maternal atopy, sex, siblings, maternal smoking during pregnancy, study center, or farming status) had a major influence on the results. Survival analyses showed that children with a level of TLR5 or TLR9 expression in the upper tertile had a risk reduced by around 50% of developing atopic dermatitis compared with children with an expression in the lower

TABLE II. Association between prenatal exposures and the development of atopic dermatitis in the first 2 years of life

Prenatal exposures	Crude ORs	95% CI	Multivariate adjusted ORs*	95% CI
Farming status	0.83	0.59-1.17	0.85	0.59-1.21
Work on a farm during pregnancy	0.65	0.45-0.94	0.83	0.55-1.25
Farm milk during pregnancy	0.72	0.50-1.02	0.86	0.56-1.33
Farm milk during pregnancy: unboiled	0.72	0.50-1.04	0.93	0.62-1.41
Work or stay in stable during pregnancy	0.76	0.54-1.08	0.84	0.47-1.51
Work or stay in barn during pregnancy	0.85	0.60-1.23	1.09	0.71-1.66
Contact with farm animal during pregnancy				
Horse	0.65	0.39-1.07	0.64	0.39-1.06
Cow	0.74	0.53-1.04	0.79	0.49-1.27
Pig	0.43	0.21-0.89	0.54	0.26-1.11
Sheep	1.01	0.61-1.65	1.15	0.68-1.95
Hare	0.84	0.53-1.32	0.96	0.61-1.53
Poultry	0.66	0.43-1.03	0.76	0.47-1.21
Horse, cow, pig, or poultry				
3-4 species	0.40	0.19-0.82	0.43	0.19-0.97
1-2 species	0.71	0.49-1.01	0.71	0.45-1.15
0 species, reference	1.00		1.00	
Contact with any pets during pregnancy	0.63	0.45-0.89	0.65	0.44-0.96
Contact with cat during pregnancy	0.58	0.41-0.82	0.68	0.46-1.00
Contact with dog during pregnancy	0.78	0.54-1.13	0.71	0.48-1.07

*Adjusted for center, atopic mother, sex, smoking during pregnancy, and farming status (not adjusted for farming status for, as exposures: farming status and working on a farm during pregnancy). Interaction test with center: not significant for all of the exposure variables. Boldface values: $P < .05$

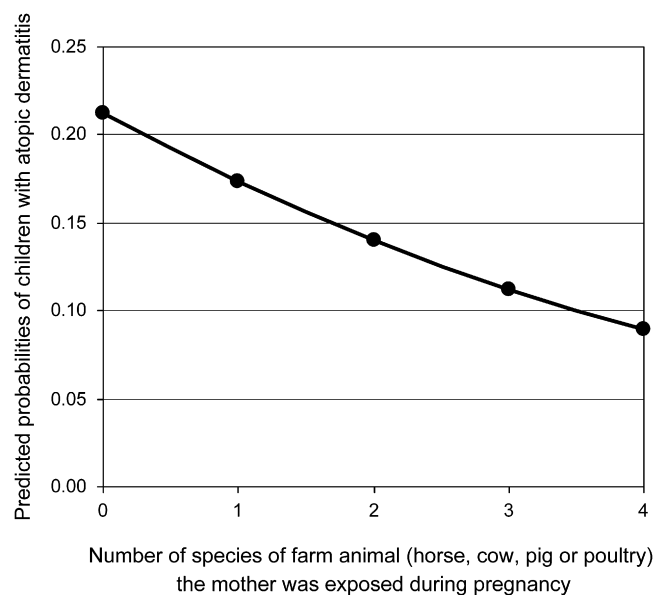


FIG 2. Association between prenatal farm animal exposures and atopic dermatitis. OR, 0.80 (95% CI, 0.65-0.99) for atopic dermatitis with each additional farm animal species, $P = .04$, adjusted for center, atopic mother, sex, smoking during pregnancy, and farming status (GEE analysis). Test of interaction between center and prenatal exposure to farm animal was non-significant; $P = .81$.

tertile (TLR5: adjusted OR, 0.56; 95% CI, 0.37-0.83; TLR9: adjusted OR, 0.53; 95% CI, 0.36-0.80; Fig 5).

Association between prenatal exposures and gene expression

There were no significant associations between contact with farm animals or pets during pregnancy and gene expression of

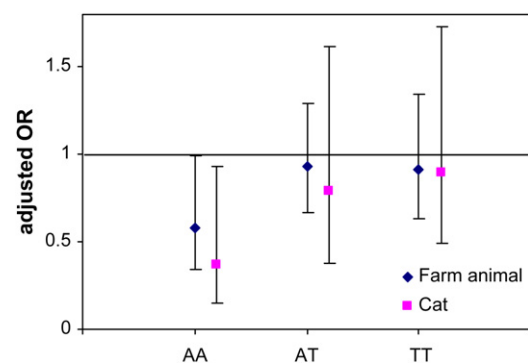


FIG 3. The effect of prenatal contact with farm animals and cat on atopic dermatitis by genotypes of *TLR2_min16934*. Reference group: no prenatal contact with animals. OR adjusted for study center, mother with atopy, sex, smoking during pregnancy, and farming status. $P = .09$ for interaction term between *TLR2_min16934* and prenatal farm animal contact. $P = .02$ for interaction term between *TLR2_min16934* and prenatal cat contact.

TLRs and CD14 measured at birth after adjustment for farming status, maternal atopy, sex, and study center (data not shown). However, we observed a slight trend of an enhanced gene expression of TLR5 and TLR9 with the increasing number of species of farm animals the mother was exposed to during pregnancy, even though it was not significant (GMR for TLR5 expression, 1.25; 95% CI, 0.92-1.70; GMR for TLR9 expression, 1.13; 95% CI, 0.80-1.59; ratio of prenatal contact with 4 species compared with no contact). The different SNPs in *TLRs* did not reveal a gene-environment effect on the gene expression of these receptors assessed at birth. When TLR5 or TLR9 expressions were included in the model of the association between exposure and atopic dermatitis, we observed a reduction of the direct effect of exposure on atopic dermatitis. This indicated that an effect of exposure on atopic dermatitis might be mediated by TLR5 or TLR9 expressions.

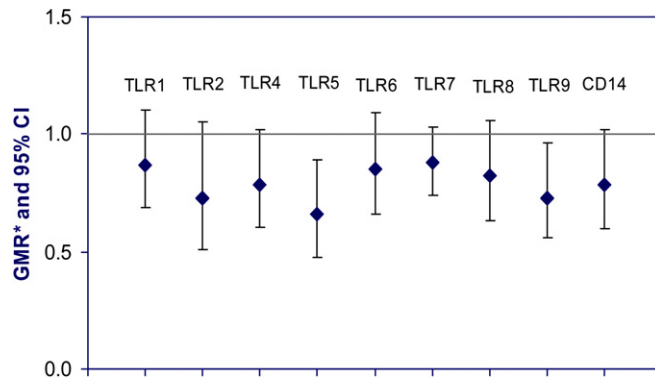


FIG 4. Association between the development of atopic dermatitis in the first 2 years of life and the gene expression of TLRs and CD14 at birth. *GMR*, Ratio of the TLRs geometric mean in children with atopic dermatitis compared with children without atopic dermatitis. *Adjusted for farmer, mother with atopy, sex, older siblings, smoking during pregnancy, and study center.

DISCUSSION

This study shows that children with mothers having contact with farm animals and cats during pregnancy have a reduced risk of developing atopic dermatitis in the first 2 years of life. The interaction between polymorphisms in *TLRs* and environment on atopic dermatitis reveals a slight effect with a SNP in *TLR2*. We also show that children with higher gene expression of *TLR5* and *TLR9* at birth have a decreased risk of atopic dermatitis compared with children with lower expression of these receptors. These results suggest a role of the innate immune system in mediating the protective effect of prenatal exposures on the development of atopic dermatitis in children, along the line of recent data obtained in the mouse model.²³

Several epidemiologic studies have shown that environmental factors rich in microbial compounds such as the farming environment have a protective effect on allergic disease.^{3,24} Nevertheless, in the previous studies on allergic disease and living on a farm, the association with atopic dermatitis remains unclear.^{4,25-27} In our study, we observed a protective effect on atopic dermatitis in children when the mother was working on a farm during pregnancy and especially when she was exposed to an increasing number of different farm animal species. These results from longitudinal analyses support the previous cross-sectional findings of a protective effect of prenatal exposure to a farm environment on atopic sensitization and on atopic dermatitis, asthma, and hay fever.^{9,10}

Moreover, our findings support that gene-environment interactions play a role in the development of atopic dermatitis in children and suggest a biological underlying mechanism for this relationship. Because TLRs are acting as the first line of defense against microbial compounds, the level of gene expression of these receptors might be a marker of the influence of the genes and environment on the development of atopic dermatitis.

As previously suggested in studies showing that the expression of innate immunity receptors was increased in children exposed to environments rich in microbial components, these results show that the innate immune system might modulate the development of allergic diseases.^{11,28} Moreover, in a cross-sectional study, Ege et al⁹ observed a positive association between maternal contact with farm animals during pregnancy and the gene expression of receptors of the innate immune system in children 5 to 13 years of age. Similarly, 1 previous study reported a decreased *TLR2*

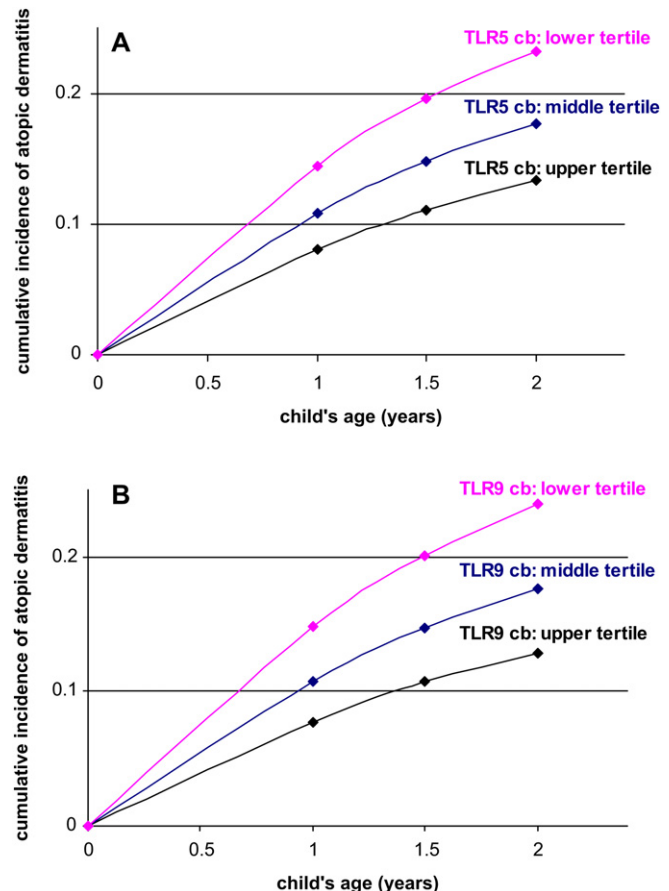


FIG 5. Cumulative incidence of atopic dermatitis, stratified between tertiles of gene expression of *TLR5* (A) and *TLR9* (B) at birth. A, *TLR5*. The cumulative incidences of atopic dermatitis by age 2 years were significantly different between the tertiles of *TLR5*: $P = .003$. B, *TLR9*. The cumulative incidences of atopic dermatitis by age 2 years were significantly different between the tertiles of *TLR9*: $P < .001$. *cb*, Cord blood.

and *TLR4* expression in cord blood in relation to maternal allergy but did not examine allergic outcomes.²⁹ In our study, the negative association between the level of gene expression assessed in cord blood samples and atopic dermatitis was not influenced by adjusting for maternal atopy.

The strength of this study is the prospective design, which avoids recall bias and gives the opportunity to study the effect of timing of exposures and the influence of innate immunity at birth on the development of atopic dermatitis.

The development of atopic dermatitis occurs usually in the first year of life.³⁰ For this reason, we defined children having the disease when diagnosed in the 2 first years of life. Because a definition of atopic dermatitis based on symptoms could lead to an overestimation of the prevalence of the disease, we used doctor diagnosis of atopic dermatitis. Moreover, we repeated the analysis with a definition based on symptoms as the outcome and observed a similar tendency of an association with the gene expression of TLRs at birth (data not shown).

Toll-like receptors, part of innate immunity, are the first line of defense against microbial compounds, and are therefore biomarkers of enhanced exposure to microbial substances. *TLR5* and *TLR9* are both receptors for bacterial components. *TLR5* recognizes bacterial flagellin,³¹ found on nearly all motile

bacteria, and TLR9 recognizes oligodeoxynucleotide containing unmethylated CpG motifs present in bacterial DNA.³² It was shown that TLR5 activation and TLR9 induce maturation of antigen-presenting cells to a T_H1-biased immune response.^{33,34}

Several SNPs in genes encoding for TLR have been shown to be associated with allergic diseases, especially with asthma.¹²⁻¹⁴ In 2 studies, associations between SNPs in *TLR2* and *TLR9* genes and atopic dermatitis have been reported.^{16,35} However, previous studies on the gene expression of these receptors related to the development of allergic disease had mainly shown an association between the level of expression and exposure to environmental factors rich in microbial substances but not with allergic diseases. A previous study demonstrated that a polymorphism in *TLR2* was associated with the frequency of asthma and allergies among farmer children.¹² Our results showed a slight effect of gene-environment interaction on atopic dermatitis, with the same SNP in *TLR2*.

Maternal contact with farm animals and cats during pregnancy and a higher expression of the receptors of innate immunity at birth have a protective effect on the development of atopic dermatitis in the first 2 years of life.

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Clinical implications: Prenatal exposures influence the development of atopic dermatitis in children.

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TABLE E1. Associations between prenatal contact with animals and the development of atopic dermatitis in the first 2 years of age, stratified by genotype

Gene	SNP	Genotype	Frequency % (N)	Contact with farm animals: horse, cow, pig, poultry		Contact with cats	
				Atopic dermatitis		Atopic dermatitis	
				OR*	95% CI	OR*	95% CI
<i>TLR1</i>	tlr1_cmin2299t (rs5743594)	CC	69.8 (683)	0.84	0.64-1.08	0.61	0.36-1.04
		CT	27.6 (270)	0.91	0.59-1.39	0.94	0.45-1.97
		TT	2.6 (25)	n/a		n/a	
	tlr1_tmin2192c (rs5743595)	TT	63.8 (629)	0.75	0.55-1.03	0.55	0.34-0.91
		TC	30.8 (304)	0.74	0.47-1.16	1.28	0.60-2.73
		CC	5.4 (53)	n/a		n/a	
	tlr1_a742g (rs4833095)	AA	53.9 (531)	0.69	0.49-0.97	0.50	0.29-0.86
		AG	38.0 (375)	0.81	0.55-1.20	1.1	0.51-2.35
		GG	8.1 (80)	n/a		n/a	
<i>TLR2</i>	tlr2_t596c (rs3804099)	TT	31.5 (303)	0.85	0.60-1.22	0.65	0.35-1.19
		TC	50.1 (481)	0.70	0.50-0.99	0.67	0.36-1.27
		CC	18.4 (177)	1.05	0.70-1.59	0.61	0.20-1.84
	tlr2_tmin16934a† (rs4696480)	AA	28.2 (276)	0.58	0.34-0.99	0.37	0.15-0.93
		AT	45.9 (449)	0.93	0.67-1.29	0.79	0.38-1.62
		TT	25.9 (253)	0.91	0.63-1.34	0.9	0.49-1.73
	tlr4_c8851t	CC	86.9 (855)	0.81	0.64-1.02	0.68	0.43-1.10
		CT	12.6 (124)	0.86	0.52-1.43	0.62	0.26-1.48
		TT	0.5 (5)	n/a		n/a	
<i>TLR4</i>	tlr4_a8551g	AA	88.2 (863)	0.77	0.62-0.98	0.68	0.42-1.08
		AG	11.3 (111)	1.00	0.56-1.80	0.78	0.32-1.93
		GG	0.5 (5)	n/a		n/a	
	tlr4_tmin1607c	TT	72.9 (707)	0.87	0.67-1.12	0.60	0.37-1.00
		TC	24.2 (235)	0.77	0.50-1.20	0.95	0.43-2.12
		CC	2.9 (28)	n/a		n/a	
	tlr5_a1774g (rs2072493)	AA	71.5 (691)	0.82	0.63-1.07	0.74	0.45-1.21
		AG	26.6 (257)	0.77	0.55-1.09	0.57	0.23-1.40
		GG	1.9 (18)	n/a		n/a	
<i>TLR5</i>	tlr5_c1173t (rs5744168)	CC	88.7 (874)	0.76	0.59-0.96	0.66	0.41-1.06
		CT	10.8 (106)	1.05	0.62-1.78	0.82	0.23-2.84
		TT	0.5 (5)	n/a		n/a	
	tlr5_t1845c (rs5744174)	TT	32.7 (318)	0.75	0.40-1.41	0.92	0.37-2.25
		TC	50.0 (486)	0.78	0.59-1.05	0.51	0.30-0.88
		CC	17.3 (168)	0.82	0.53-1.27	0.85	0.31-2.29
	tlr6_tmin2079a (rs5743789)	TT	63.9 (621)	0.74	0.54-1.02	0.54	0.33-0.88
		TA	30.3 (294)	0.9	0.60-1.33	1.41	0.59-3.38
		AA	5.8 (56)	n/a		n/a	
<i>TLR7</i>	tlr7_a17961t (rs179008)	AA	69.1 (679)	0.86	0.65-1.13	0.78	0.47-1.29
		AT	15.8 (155)	0.76	0.49-1.16	0.64	0.24-1.73
		TT	15.1 (148)	0.66	0.35-1.24	0.5	0.16-1.53
	tlr7_c12318t	CC	85.4 (836)	0.8	0.63-1.02	0.76	0.48-1.20
		CT	8.4 (82)	0.93	0.52-1.66	0.70	0.18-2.70
		TT	6.2 (61)	n/a		n/a	
	tlr8_c10907a	CC	53.7 (527)	0.92	0.68-1.25	0.72	0.39-1.32
		CA	24.1 (236)	0.63	0.43-0.94	0.58	0.22-1.22
		AA	22.2 (218)	0.8	0.47-1.35	0.80	0.36-1.80
<i>TLR8</i>	tlr8_amin4824g (rs3761624)	AA	65.1 (643)	0.8	0.58-1.11	0.67	0.38-1.18
		AG	20.2 (199)	0.91	0.63-1.31	0.47	0.21-1.04
		GG	14.7 (145)	n/a		n/a	
	tlr9_tmin2622c (rs5743836)	TT	76.4 (750)	0.83	0.66-1.04	0.55	0.35-0.87
		TC	22.3 (219)	0.75	0.42-1.35	0.93	0.35-2.49
		CC	1.3 (13)	n/a		n/a	
	tlr9_tmin2871c rs187084	TT	30.8 (300)	1.08	0.75-1.54	1.03	0.50-2.13
		TC	50.8 (495)	0.65	0.44-0.94	0.61	0.35-1.07
		CC	18.4 (180)	0.8	0.57-1.12	0.38	0.14-1.09

n/a, Not applicable.

Boldface values: $P < .05$.

Reference group: no prenatal contact with animals.

*Adjusted for study center, mother with atopy, sex, smoking during pregnancy, and farming status.

†Interaction test with $P < .1$ (P values for interaction term between tlr2_tmin16934a and farm animal contact, .09; and between tlr2_tmin16934a and cats contact, .02).