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Background: Perturbations in the gut microbiota have been linked to atopic diseases. However, the development of atopic diseases depends not only on environmental factors (like microbial stimulation) but also on genetic factors. It is likely that particularly gene-environmental interactions in early life determine the development of atopy.
ObjectiveWe examine the interaction between detection of fecal *Escherichia coli* and genetic variations in the *CD14* and Toll-like receptor 4 *(TLR4)* genes in relation to atopic manifestations.
Methods: Within the Child, Parent and Health: Lifestyle and Genetic Constitution (KOALA) Birth Cohort Study, fecal samples of 957 one-month-old infants were collected and quantitatively screened for *E coli*. Fourteen haplotype-tagging polymorphisms in the genes *TLR4* and *CD14* were genotyped in 681 of the 957 children. Atopic outcomes were parentally reported eczema in the first 2 years of life and clinically diagnosed eczema and allergic sensitization at age 2 years. Multiple logistic regression was used to evaluate a multiplicative model of interaction.
Results: Most of the single nucleotide polymorphisms (SNPs) showed no significant interaction with *E coli* exposure for both eczema and allergic sensitization. A borderline significant multiplicative interaction was found between *E coli* and the rs2569190 (CD14/-159) SNP regarding allergic sensitization. Furthermore, a statistically significant multiplicative interaction was found for the *TLR4* SNP rs10759932 (*P* for interaction = .001). *E coli* colonization was associated with a decreased risk of sensitization only in children with the rs10759932 TT genotype (adjusted odds ratio, 0.31; 95% CI, 0.14-0.68) and not in children with the minor C allele. This interaction remained statistically significant after controlling for multiple testing.

Conclusion: The current study is the first to address the potential effect-modifying role of genetic variations in the relationship between the intestinal microbiota and allergy development. (J Allergy Clin Immunol 2010;125:231-6.)

Key words: Atopy, Toll-like receptor 4, CD14, gene-environment interaction, Escherichia coli, intestinal microbiota

The gut microbiota is a key source of microbially driven immune regulation and oral tolerance induction in early life. Perturbations in the gut microbiota may therefore be involved in the pathogenesis of atopic diseases, and differences in gut microbiota between healthy and atopic children have indeed been shown in several cross-sectional epidemiologic studies.[1-5] In the prospective Child, Parent and Health: Lifestyle and Genetic Constitution (acronym KOALA in Dutch) Birth Cohort Study, we showed in addition that differences in the gut microbiota composition precede the development of childhood atopic manifestations (eczema, recurrent wheeze, and allergic sensitization).[6]

For example, colonization by *Escherichia coli*, a major gram-negative constituent of the infant microbiota, was shown to be associated with the development of eczema.

Microbes are recognized by the innate immune system using pattern recognition receptors (PRRs). CD14 is, together with Toll-like receptor (TLR)–4, involved in the recognition and signal transduction of bacterial endotoxin, a major component of the bacterial cell wall of gram-negative bacteria. Downstream effects of CD14/TLR receptor activation on antigen presenting cells include the release of cytokines, such as IL-10 and IL-12.[7]

Asthma and allergy are complex diseases—that is, both genetic and environmental factors contribute to disease development. Polymorphisms in the PRR encoding genes might alter immune responsiveness of the host to microbial agents, thereby modifying the development of immune-mediated diseases like asthma and atopy. So far, *TLR4* and *CD14* gene association studies with asthma and atopy are suggestive for a genetic component, but results have varied between studies.[8]

One of the most likely explanations for discrepancies between studies is differences in the level of microbial exposures interacting with the PRRs. For example, a single nucleotide polymorphism (SNP) in *TLR4* (Asp299Gly) was associated with a lower prevalence of bronchial responsiveness and allergy in 2 independent populations, but only in subjects heavily exposed to endotoxin.[9,10] Several studies have reported that the association between a functional SNP in the promoter region of *CD14* (-159C-T) and total serum IgE levels is modified by the level of exposure to microbial products/endotoxin.[11] Furthermore, Bieli et al[12] showed that 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.

Abbreviations used
AD: Atopic dermatitis
ALEX: Allergy and Endotoxin
FDR: False discovery rate
KOALA: Child, Parent and Health: Lifestyle and Genetic Constitution
OR: Odds ratio
PRR: Pattern recognition receptor
SNP: Single nucleotide polymorphism
TLR: Toll-like receptor
UK-WP: UK Working Party

So far, no gene-environment studies have been performed with respect to the most extensive source of microbial stimulation, the gut microbiota.

The aim of the current study is to examine the interaction between fecal *E coli* and SNPs in the *CD14* and *TLR4* genes in relation to the development of atopic manifestations.

METHODS
Subjects and study design

This study is conducted within the KOALA Birth Cohort Study, a prospective cohort in The Netherlands, described in detail elsewhere.[13] Briefly, pregnant women were recruited at 34 weeks of gestation. During pregnancy and early childhood, data on perinatal determinants of the child's health as well as on hygiene, infections, nutrition, child-rearing, other lifestyle characteristics, and atopic manifestations were collected for all members of the cohort by repeated questionnaires at 34 weeks of gestation and 3, 7, 12, and 24 months postpartum.

Participants recruited from January 2002 onward were asked to consent to sample the infant's feces at the age of 1 month postpartum (n = 1176). After exclusion of premature infants, infants who received antimicrobial agents during their first month of life, infants of whom an insufficient amount of feces (<1 g) was collected, infants of whom feces was not collected between the age of 3 and 6 weeks postpartum, and infants for whom the feces questionnaire was missing, the study cohort included 957 infants.[6]

The KOALA study was approved by the Ethical Committee of the University Hospital of Maastricht, and all parents signed informed consent for the study.

Fecal microbiota composition

The collection and processing of fecal samples has been described in detail elsewhere.[6] Briefly, parents collected a fecal sample by placing a sanitary napkin in the diaper (to prevent absorption of the feces by the diaper) and collected the feces out of the napkin into the collection tube and sent it immediately to our laboratory by post. At the laboratory, fecal samples were 10-fold diluted in peptone-water (Oxoid CM0009, Hampshire, UK) containing 20% vol/vol glycerol (Merck, Damstadt, Germany) and stored at –20 °C until analysis. DNA was extracted by a combination of bead-beating and the QIAamp DNA Stool Mini Kit (Qiagen, Venlo, The Netherlands). Fecal samples were subjected to real-time PCRs for the enumeration of, among others, *E coli* and total bacterial counts. Samples were considered to be positive for *E coli* when threshold cycle values were below 35.

The log_{10} colony-forming units per gram were calculated for each stool sample from the threshold cycle values by using the constructed standard curves. Subsequently, samples were classified as yielding low or high *E coli* concentrations by using the median of detectable levels (9.35 log_{10} colony-forming units) as a cut-off.

Measurement of infant's specific IgE

Venous blood of the infants was collected during a home visit at 2 years postpartum. Infants' venous blood samples were analyzed for specific IgE against hen's eggs, cow's milk, peanuts, birch, grass pollen, cat, dog, and house dust mite by using a RAST as described earlier.[14] The detection limit for specific IgE was 0.10 IU/mL.

Definition of infant's atopic manifestations and sensitization

Infants were defined as having developed eczema in the first 2 years of life if their parents reported an itchy rash that was coming and going in the past months based on the 7-month, 12-month, and 24-month postpartum questionnaires, which were based on International Study of Asthma and Allergies in Childhood (ISAAC) questions. Subjects with only diaper rash, rash around the eyes, and/or scalp scaling were not considered to have eczema.

As a stricter eczema phenotype, we defined atopic dermatitis (AD) according to the United Kingdom Working Party (UK-WP) criteria[15] for all infants who were visited at home at age 2 years and examined by the trained research nurse. The probability of the presence of AD was derived from the presence of 4 clinical symptoms: (1) presence of itchy rash (coded as 0 = absent, 1 = present), (2) history of flexural dermatitis (0 = absent, 1 = present), (3) visible flexural dermatitis (0 = absent, 1 = present), and (4) onset before age 2 years (0 = absent, 1 = present). The UK-WP probability score of AD is then computed as follows:

$$\text{probability AD} = \text{odds (AD)} / [\text{odds (AD)} + 1]$$

where odds (AD) = exp[–4.36 + 1.84 (history of flexural dermatitis) + 3.46 (onset before age 2 years) + 2.09 (visible flexural dermatitis) +1.71 (presence of itchy rash)].[15] In this study, infants with a UK-WP probability score of AD >90% were regarded as infants with AD.

Infants were classified as having allergic sensitization when serum IgE levels were >0.3 IU/mL against 1 or more of the tested food or inhalant allergens.

DNA collection, processing, and genotyping

Parents were asked to collect buccal swabs as a source of DNA from their children.

Genomic DNA was extracted from these swabs from using standard methods.[16] DNA was amplified by using REPLI-g UltraFast technology (Qiagen). Genotyping was performed by Competitive Allele-Specific PCR using KASPar genotyping chemistry, under contract by K-Biosciences with extensive quality control as described previously.[17]

SNP selection

Haplotype tagging SNPs were selected from publicly available databases of the International HapMap Project, version 2005,[18] and from the Innate Immunity web site[19] depending on the largest number of SNPs with a minor allele frequency >0.1 available in each database. In addition, the biomedical literature was screened for SNPs with known functional impact or association with asthma or atopy.

Statistical analysis

Genotype distribution was tested for deviations ($P < .05$) from Hardy-Weinberg equilibrium using χ^2 analyses.

We used χ^2 tests to analyze whether *TLR4* and *CD14* SNPs were associated with parentally reported eczema, AD, and allergic sensitization (allergen specific IgE > 0.3 IU/mL) by using a codominant model. For SNPs with $P < .10$, the Akaike information criterion was evaluated to determine the best fitting genetic model (additive, dominant, or recessive).

Subsequently the prevalence of these atopic outcomes was calculated according to *E coli* colonization. χ^2 Tests were used to test for differences in the prevalence of the atopic outcomes according to *E coli* colonization.

Unadjusted and adjusted odds ratios (ORs) and 95% CIs for the association between *E coli* colonization and atopic outcomes according to the different genotypes were calculated by means of logistic regression. The adjusted

regression models included the following potential confounders: parental atopic history, defined as self-reported doctor's diagnosed eczema, hay fever, asthma, pet and/or house dust mite allergy (both parents nonatopic, at least 1 parent atopic); siblings' atopic history, defined as parentally reported doctor's diagnosed food allergy, eczema, hay fever, asthma, pet and/or house dust mite allergy (no siblings; ≥1 siblings, all none-atopic; or >1 siblings, at least 1 atopic); age at collection of fecal sample (age in days), infant's sex (boy, girl); pet exposure at home (no pet exposure; only cat exposure; only dog exposure; only other furry pet exposure; combination of cat, dog, and/or other furry pet exposure); and recruitment group (alternative, conventional).[13]

Multiple logistic regression was used for analyses, and thus, departure from a multiplicative model of interaction on the OR scale was investigated. The statistical significance of effect modification was tested by a likelihood ratio test for the improvement of goodness of model fit when including the interaction term of genotype and *E coli* colonization into the logistic regression models. Thus, the obtained *P* value for interaction estimates a departure from a multiplicative interaction model on the OR scale and indicates whether the effect (OR) of *E coli* colonization is altered by effects of *CD14* or *TLR4* genotype.

All analyses were performed by using SPSS 15.0 statistical software, and results were considered statistically significant when the *P* value was lower than .05. To control for multiple testing, we calculated the false discovery rate (FDR) for the tests for interaction according to Benjamini and Hochberg.[20]

RESULTS
Study population and genetic analysis

DNA from buccal swabs was available for genetic analyses in 681 of the 957 children (71.2%) from whom fecal samples had been collected at age 1 month. As shown in Table I, characteristics were comparable between subjects with and without genetic data. Furthermore, as shown previously,[6] infants who provided a fecal sample did not differ from those who did not provide a fecal sample.

Of the 9 genotyped *TLR4* SNPs, the rs10759931 SNP deviated from Hardy-Weinberg equilibrium and was not considered for further analysis. None of the 5 genotyped *CD14* SNPs deviated from Hardy-Weinberg equilibrium (Table II). Fig 1, *A,* shows the position of the genotyped SNPs within the *TLR4* and *CD14* genes. Fig 1, *B,* shows the linkage disequilibrium between these SNPs as presented by D' and r^2. D' is presented as a number if it deviates from 100. D' is 100 when no recombination has occurred between 2 SNPs. r^2 is represented by color, changing from dark gray when $r^2 = 1$ (the minor alleles at 2 SNP positions are always present on the same haplotype) to white when $r^2 = 0$ (the minor alleles are always on separate haplotypes).

Genetic association of *TLR4* and *CD14* and atopy

This article's Table E1 in the Online Repository at www. jacionline.org shows the prevalence of atopic outcomes according to the *TLR4* and *CD14* genotypes. One *TLR4* SNP (rs2770150) was borderline significantly associated with AD according to the UK-WP criteria. Furthermore, a borderline significant association were found between parentally reported eczema and 3 *CD14* SNPs (rs2569190, rs2569191, rs2915863), with the prevalence of eczema always lowest in the minor allele homozygotes.

None of the *TLR4* and *CD14* SNPs was associated with allergic sensitization.

Gene-by-environment interaction

When considering the prevalence of the atopic outcomes according to intestinal *E coli* colonization at age 1 month

TABLE I. Characteristics of the study population (n = 957)*

	Genetic data (n = 681)	No genetic data (n = 276)
Age at collection of feces (d), mean (SD)	31.5 (3.23)	32.1 (3.39)
Parental history of atopic manifestations, n/N (%)		
At least 1 parent with atopy	373/681 (54.8)	142/276 (51.4)
Sibling history of atopic manifestations, n/N (%)		
No siblings	269/681 (39.5)	116/276 (42.0)
≥1 siblings, all nonatopic	294/681 (43.2)	111/276 (40.2)
≥1 siblings, at least 1 atopic	118/681 (17.3)	48/276 (17.4)
Sex of infant, boys, n/N (%)	351/681 (51.5)	134/276 (48.6)
Pets at home, n/N (%)		
No pets	365/681 (53.6)	145/276 (52.5)
Cat	97/681 (14.4)	57/276 (20.7)
Dog	151/681 (22.2)	49/276 (17.8)
Other furry pet	22/681 (3.2)	9/276 (3.2)
Combination of cat, dog, and/or other furry pet	45/681 (6.6)	16/276 (5.8)
Infants atopic outcome at age 2 y, n/N (%)		
Eczema	219/678 (32.3)	85/259 (32.8)
Atopic dermatitis (UK-WP)†	82/513 (16.0)	16/94 (17.0)
Sensitization†	140/499 (28.1)	25/84 (29.8)

*Overall numbers are not always 957 because of missing data.
†Only available for those infants visited at home (n = 607).

postpartum, infants colonized with this bacterium were at increased risk of developing eczema in the subsequent 2 years (see this article's Table E2 in the Online Repository at www. jacionline.org). This increased risk was highest for infants with high fecal *E coli* concentrations (as described previously in the complete cohort).[6]

This article's Tables E3 to E5 in the Online Repository at www.jacionline.org show the associations between *E coli* colonization at age 1 month postpartum and parentally reported eczema, clinically diagnosed AD (UK-WP criteria), and allergic sensitization, respectively, stratified for the *TLR4* and *CD14* SNPs. Almost none of the SNPs showed significant multiplicative interaction with *E coli* exposure for either eczema (parental report or UK-WP criteria) or allergic sensitization.

A statistically significant multiplicative interaction was found for the *TLR4* SNP rs10759932 (*P* for interaction = .001) and *E coli* exposure in association with allergic sensitization. After controlling for multiple testing by FDR, the interaction remained statistically significant.

E coli colonization was associated with a strongly decreased risk of sensitization in children homozygous for the T allele (n = 357; adjusted OR, 0.31; 95% CI: 0.14-0.68). By contrast, heterozygotic children were at increased risk of sensitization, although not statistically significantly, when colonized by *E coli* (n = 108; adjusted OR, 6.68; 95% CI: 0.75-64.96; note: for C homozygotes, the OR was not calculated because the number of subjects was only 5). In addition, a secondary analysis was performed to examine the risk of allergic sensitization according to the concentration of fecal *E coli* stratified for the *TLR4* rs10759932 genotype. T homozygotes

TABLE II. Selected *TLR4* and *CD14* SNPs and minor allele frequencies in the study population

Gene	Rs number	Alleles*	Synonym	Minor allele frequency	*P* value† for Hardy-Weinberg equilibrium
TLR4	rs2770150	T > C	c.-3612T/C	0.26	.97
TLR4	rs10759931	G > A	c.- 2604 G/A	0.43	<.001
TLR4	rs6478317	A > G	c.-2570A/G	0.35	.19
TLR4	rs10759932	T > C	c.-1607 T/C	0.13	.86
TLR4	rs1927911	C > T	c.93 + 3211C/T	0.26	.68
TLR4	rs11536878	C > A	c.260 + 546C/A	0.12	.43
TLR4	rs4986790	A > G	Asp299Gly	0.07	.84
TLR4	rs4986791	C > T	Thr399Ile	0.08	>.999
TLR4	rs11536889	G > C	c.3648 + 78G/C	0.15	.83
CD14	rs2915863	T > C	-1619 T/C	0.41	.70
CD14	rs2569191	T > C	-1145 T/C	0.48	>.999
CD14	rs5744455	C > T	-550 C/T	0.23	.20
CD14	rs2569190	C > T	-159 C/T	0.48	.71
CD14	rs2563298	C > A	3'UTR	0.26	.52

*Minor alleles first.

†As determined by χ^2 test.
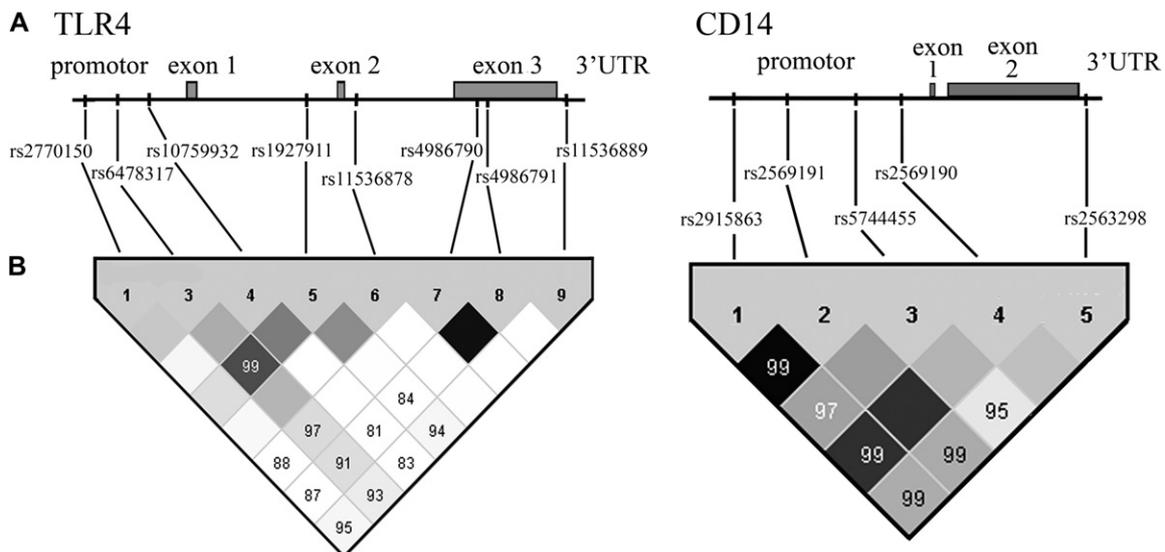
FIG 1. Position and linkage disequilibrium of the SNPs within *TLR4* and *CD14*. **A,** *TLR4* is located on chromosome 9 (9q32) 119,506,431. *CD14* is located on chromosome 5 (5q31) 139991505. **B,** Pairwise linkage disequilibrium plot according to Haploview3.32[29] for *TLR4* and *CD14* SNPs. D' is presented as a number if it deviates from 100. The measure of r^2 is represented by color, changing from *dark gray* when $r^2 = 1$ to *white* when $r^2 = 0$. *UTR*, Untranslated region.

colonized with low and high concentrations had a comparable decreased risk for developing allergic sensitization as compared with uncolonized children (adjusted OR [95% CI], respectively, 0.29 [0.13-0.67] and 0.33 [0.15-0.76]). Heterozygotes colonized with low or high concentrations were at a similar risk for developing allergic sensitization (adjusted OR [95% CI], respectively, 6.6 [0.69-63.5] and 7.8 [0.76-80.7]). *P* for interaction was .005.

Fig 2 shows that the effect of this SNP was in the same direction when analyzing eczema (both parentally reported and clinically diagnosed AD according to UK-WP criteria).

Furthermore, a borderline significant multiplicative interaction was found between *E coli* and the rs2569190 (*CD14*/-159) SNP regarding allergic sensitization (Table E5). *E coli* colonization was associated with an increased risk of sensitization in CC homozygotes, whereas it was associated with a decreased risk

of sensitization in heterozygotes and even more in TT homozygotes. However, this interaction did not withstand correction for multiple testing by FDR.

DISCUSSION

Within the KOALA Birth Cohort Study, we have examined the interaction between intestinal *E coli* colonization at 1 month of age and *TLR4*/*CD14* haplotype tagging and functional SNPs in association with subsequent development of eczema and allergic sensitization. To our knowledge, this is the first prospective study investigating the interaction between intestinal microbes and candidate genes in association with allergy development.

When considering the host and microbial factors separately, none of the *TLR4* SNPs, nor *E coli* colonization, were associated with
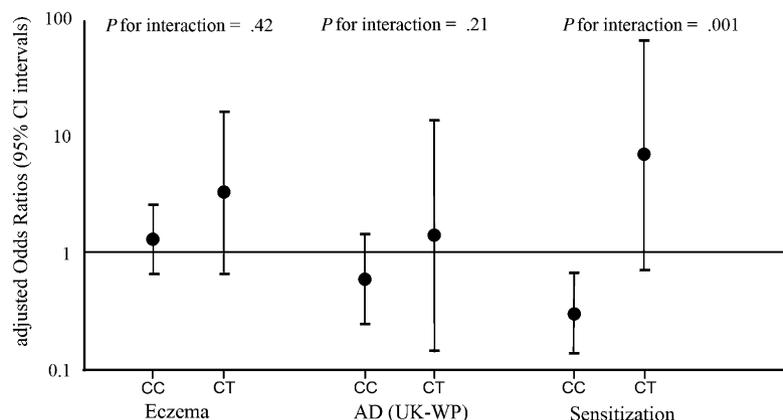
FIG 2. Association between *E coli* and atopic outcomes stratified for *TLR4* SNP rs10759932. The figure shows the adjusted ORs for the development of eczema, AD, and sensitization in infants colonized with *E coli* compared with uncolonized infants (reference category, OR = 1) stratified according to genotype.

allergic sensitization. However, CC homozygotes for the *TLR4* rs1927911 SNP had a significantly decreased risk to become sensitized if they were colonized with *E coli* in early infancy.

Furthermore, a significant multiplicative interaction was found for *E coli* colonization and the *TLR4* SNP rs10759932 with respect to allergic sensitization. *E coli* colonization was associated with a strongly decreased risk of sensitization among T homozygotes, whereas it was associated with an increased risk of sensitization among heterozygotic children (cross-over interaction).

This association between *E coli* and allergic sensitization would never have been detected if the genotype had not been taken into account, because the positive association in the heterozygotes and the negative association in the homozygotes evens out to no association when both groups are combined. This demonstrates the importance of gene-environment interactions, with effects of genes and/or microbial factors masked when they are not being studied together.

To our knowledge, these SNPs (rs10759932 and rs1927911) have not been linked to atopic sensitization or symptoms previously but have been linked to several other diseases like prostate cancer, chorionic plate inflammation, and myocardial infarction.[21-24]

Because of the strong regional correlation between SNPs across *TLR4*, rs10759932 may serve as a proxy for the predisposing variant, or the polymorphism itself may have functional consequences on *TLR4* activity.[22]

Among the most extensively studied *TLR4* SNPs in association with allergic diseases are Asp299Gly and Thr399Ile (respectively, rs4986790 and rs4986791), 2 common cosegregating missense mutations. Arbour et al[25] reported that these SNPs are associated with airway hyporesponsiveness and that the *TLR4* Asp299Gly variant interrupted TLR4-mediated LPS-signaling.

Within the Allergy and Endotoxin (ALEX) study, genetic variation in *TLR4* Asp299Gly (rs4986790) was found to be associated with increased risk of sensitization to aeroallergens in children heavily exposed to endotoxin.[9] This indicates that *TLR4* SNP may be mainly associated with sensitization with inhalation allergens and respiratory allergies. Because the natural development of sensitization starts with sensitization to food allergens in infancy, which decreases over time, whereas specific IgE to indoor allergens increases in later childhood,[26] associations may be found only in later childhood. This might explain the lack of association with this particular *TLR4* SNP in the current study.

We found no significant departure from multiplicativity regarding *CD14* polymorphisms and *E coli* colonization, except for a borderline significant interaction between *CD14* –159C/T and *E coli* in association with allergic sensitization. Both the ALEX study[27] and the Manchester Asthma and Allergy Study (MAAS)[11] found support for the endotoxin switch theory as proposed by Vercelli,[28] where the C allele of this *CD14/-159* SNP confers risk at low endotoxin exposures whereas the T allele confers risk at high exposures of endotoxin. Our data do not support a protective effect of high endotoxin exposure on sensitization in C homozygotes.

One possible explanation is that the influences of *CD14*-environment interactions on atopy are age-specific, as recently demonstrated by Bottema et al.[17] In that study, partly based on the same population as the current study, interactions between *CD14* polymorphisms and pet exposure influenced total and specific IgE levels at 4 and 8 years, but not at 1 and 2 years. Therefore, the influence of *CD14*-environment interactions may be stronger in midchildhood compared with infancy. This is in agreement with the fact that both the ALEX and the Manchester Asthma and Allergy Study determined allergic sensitization in midchildhood (5-13 years), whereas specific IgE was determined at age 2 years in the current study.

An accurate definition of the disease outcome in genetic studies is important, because misclassification of individuals reduces the power of genetic studies to a great extent. This might be an explanation for the fact that significant interactions were limited to allergic sensitization, a more objective disease outcome than eczema and therefore less prone to misclassification.

To minimize the chance that *E coli* colonization is merely an epiphenomenon of an underlying immunologic disease, we have determined colonization very early in life when allergic diseases had not manifested yet. Furthermore, statistical analyses were adjusted for family history of atopy to control for the possibility that a familial predisposition to allergies is associated with a predisposition to become colonized with *E coli*.

Although this is the largest cohort study to date in which fecal samples have been analyzed, the high proportion of *E coli* colonization, and thus the low proportion of unexposed infants, negatively influences the power of the current study. Nevertheless, we did find a multiplicative interaction between *E coli* and *TLR4* rs10759932 in association with allergic sensitization that

persisted after controlling for FDR. These findings support the hypothesis that the association between the intestinal microbiota and allergic diseases is mediated by genetic variations in pattern recognition receptors.

Further research on the immune-modulating effect of intestinal microbes and the modifying role of host genetics may lead us to attractive new routes to modulate the gut microbiota to treat or prevent allergic diseases in susceptible individuals.

Clinical implications: Our results support the hypothesis that the association between fecal *E coli* and allergic sensitization is mediated by genetic variations in *TLR4*.

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TABLE E1. Prevalence (%) of atopic outcomes according to *CD14* and *TLR4* genotype

		Eczema Percent (cases/total)	AD* Percent (cases/total)	Sensitization Percent (cases/total)
TLR4 c.-3612	TT	34.7 (127/366)	19.1 (52/272)	28.1 (74/263)
(rs2770150)	CT	28.9 (74/256)	11.9 (24/202)	26.9 (53/197)
	CC	34.9 (15/43)	12.9 (4/31)	32.3 (10/31)
		P = .30	***P* = .09**	*P* = .82
TLR4 c.-2570	AA	32.2 (87/270)	16.1 (33/205)	29.5 (59/200)
(rs6478317)	GA	32.8 (105/320)	16.5 (40/242)	26.0 (61/235)
	GG	29.3 (22/75)	10.5 (6/57)	32.7 (18/55)
		P = .85	*P* = .52	*P* = .52
TLR4 c.-1607	TT	32.2 (155/481)	16.6 (61/368)	28.4 (102/359)
(rs10759932)	CT	32.0 (47/147)	14.4 (16/111)	29.6 (32/108)
	CC†	40.0 (4/10)	20.0 (1/5)	20.0 (1/5)
		P = .87	*P* = .59	*P* = .81
TLR4 c.93 + 3211	CC	33.4 (119/356)	17.0 (46/270)	30.3 (79/261)
(rs1927911)	CT	30.2 (78/258)	13.5 (27/200)	25.0 (49/196)
	TT	35.7 (15/42)	17.2 (5/29)	34.5 (10/29)
		P = .63	*P* = .56	*P* = .35
TLR4 c.260 + 546	CC	32.1 (159/495)	16.0 (59/369)	28.3 (101/357)
(rs11536878)	CA	31.7 (46/145)	17.7 (20/113)	27.9 (31/111)
	AA†	28.6 (2/7)	0 (0/6)	33.3 (2/6)
		P = .93	*P* = .68	*P* = .94
TLR4 Asp299Gly	AA	32.6 (187/573)	16.3 (71/435)	28.4 (121/426)
(rs4986790)	GA	30.2 (26/86)	15.4 (10/65)	29.5 (18/61)
	GG†	50.0 (2/4)	0 (0/3)	0 (0/2)
		P = .66	*P* = .84	*P* = .86
TLR4 Thr399lle	CC	32.7 (183/560)	16.0 (68/425)	28.8 (120/416)
(rs4986791)	CT	30.0 (27/90)	13.8 (9/65)	27.9 (17/61)
	TT†	50.0 (2/4)	0 (0/3)	0 (0/2)
		P = .61	*P* = .66	*P* = .87
TLR4 c.3648 +78	GG	33.2 (159/479)	16.6 (60/362)	30.0 (105/350)
(rs11536889)	CG	28.4 (48/169)	14.1 (18/128)	22.2 (28/126)
	CC†	38.5 (5/13)	27.3 (3/11)	27.3 (3/11)
		P = .25	*P* = .50	*P* = .10
CD14/-1619	TT	32.4 (72/222)	16.1 (27/168)	28.7 (47/164)
(rs2915863)	CT	35.5 (114/321)	17.8 (43/241)	28.4 (66/232)
	CC	23.1 (25/108)	8.5 (7/82)	28.4 (23/81)
		***P* = .06**	*P* = .13	*P* = .99
CD14/-1145	TT	30.4 (55/181)	15.0 (21/140)	28.5 (39/137)
(rs2569191)	CT	36.0 (117/325)	18.4 (44/239)	28.6 (66/231)
	CC	25.8 (40/155)	10.7 (13/122)	26.9 (32/119)
		***P* = .07**	*P* = .15	*P* = .94
CD14/-550	CC	31.0 (116/374)	13.9 (40/287)	27.4 (77/281)
(rs5744455)	CT	35.8 (86/240)	20.8 (36/173)	29.8 (50/168)
	TT	25.0 (8/32)	10.3 (3/29)	30.8 (8/26)
		P = .30	*P* = .10	*P* = .83
CD14/-159	CC	30.3 (54/178)	13.9 (19/137)	26.9 (36/134)
(rs2569190)	CT	36.5 (120/329)	18.9 (46/243)	29.8 (70/235)
	TT	25.7 (39/152)	10.8 (13/120)	26.5 (31/117)
		***P* = .05**	*P* = .11	*P* = .75
CD14 3'ÚTR	CC	32.3 (119/368)	15.9 (44/277)	29.7 (79/266)
(rs2563298)	CA	31.4 (76/242)	15.9 (30/189)	25.8 (48/186)
	AA	36.5 (19/52)	17.1 (6/35)	31.4 (11/35)
		P = .77	*P* = .98	*P* = .61

*AD according to UK-WP criteria.
†Minor allele homozygotes excluded from χ^2 test analyses because of limited number of subjects.
Boldface indicates statistically significant associations (alpha = .1)

TABLE E2. Prevalence (%) of atopic outcomes according to *E* colonization

		Eczema Percent (cases/total)	AD* Percent (cases/total)	Sensitization Percent (cases/total)
E coli	No	22.2 (24/108)	15.9 (10/63)	30.6 (19/62)
	Yes	33.8 (279/826)	16.2 (88/542)	28.1 (146/519)
		P = **.01**	*P* = .94	*P* = .68
E coli	No	22.2 (24/108)	15.9 (10/63)	30.6 (19/62)
	Low†	31.5 (131/416)	15.4 (44/286)	27.1 (75/277)
	High‡	36.1 (148/410)	17.2 (44/256)	29.3 (71/242)
		P = **.02**	*P* = .85	*P* = .78

*AD according to UK-WP criteria.
†Low concentration of *E coli* (<9.35 $\log_{10}$ colony-forming units/g feces).
‡High concentration of *E coli* (≥9.35 $\log_{10}$ colony-forming units/g feces).
Boldface indicates statistically significant association (alpha = .05)

TABLE E3. Parentally reported eczema: ORs for the association with *E coli* colonization in the first month of life, stratified for *CD14* and *TLR4* genotypes

TLR4/CD14 SNP	Genotype	No.	OR (95% CI)*	ORadj (95% CI)†	*P* value for interaction§	FDR-controlled *P* value‖
TLR4 c.-3612	TT	365	1.56 (0.73-3.32)	1.70 (0.77-3.74)	.82	1.0
(rs2770150)	CT	254	1.31 (0.53-3.20)	1.36 (0.54-3.40)		
	CC	43	2.33 (0.23-23.00)	1.64 (0.10-28.05)		
TLR4 c.-2570	AA	268	1.20 (0.51-2.85)	1.05 (0.43-2.60)	.85	1.0
(rs6478317)	GA	319	1.67 (0.76-3.67)	1.72 (0.75-3.92)		
	GG	75	1.71 (0.18-16.27)	1.35 (0.12-15.57)		
TLR4 c.-1607	TT	478	1.33 (0.70-2.53)	1.35 (0.69-2.62)	.42	.96
(rs10759932)	CT	147	2.19 (0.59-8.10)	3.31 (0.67-16.38)		
	CC‡	10	—	—		
TLR4 c.93 + 3211	CC	354	1.45 (0.68-3.10)	1.50 (0.69-3.26)	.55	.97
(rs1927911)	CT	257	1.72 (0.71-4.16)	2.15 (0.84-5.53)		
	TT‡	42	—	—		
TLR4 c.260 + 546	CC	493	1.47 (0.78-2.79)	1.49 (0.78-2.88)	.83	1.0
(rs11536878)	CA	144	1.46 (0.45-4.82)	2.02 (0.53-7.67)		
	AA‡	7	—	—		
TLR4 Asp299Gly	AA	570	1.48 (0.82-2.69)	1.64 (0.89-3.02)	.88	.98
(rs4986790)	GA	86	1.58 (0.31-8.20)	1.05 (0.17-6.60)		
	GG‡	4	—	—		
TLR4 Thr399lle	CC	557	1.56 (0.73-3.32)	1.63 (0.88-3.02)	.86	.99
(rs4986791)	CT	90	1.31 (0.53-3.20)	0.99 (0.16-6.27)		
	TT‡	4	2.33 (0.23-23.00)	—		
TLR4 c.3648 + 78	GG	477	1.34 (0.72-2.52)	1.30 (0.68-2.47)	.40	1.0
(rs11536889)	CG	169	2.12 (0.59-7.70)	2.55 (0.63-10.32)		
	CC‡	13	—	—		
CD14/-1619	TT&CT	540	1.65 (0.89-3.04)	1.79 (0.95-3.35)	.34	1.0
(rs2915863)	CC	108	0.89 (0.22-3.58)	0.71 (0.15-3.42)		
CD14/-1145	TT&CT	503	1.67 (0.88-3.14)	1.89 (0.98-3.65)	.41	1.0
(rs2569191)	CC	155	1.15 (0.35-3.75)	1.20 (0.34-4.29)		
CD14/-550	CC	373	1.69 (0.78-3.67)	1.74 (0.79-3.85)	.92	.97
(rs5744455)	CT	238	1.77 (0.72-4.36)	2.32 (0.86-6.27)		
	TT	32	0.64 (0.05-8.12)	0.53 (0.004-74.2)		
CD14/-159	CC&CT	504	**1.94 (1.01-3.70)**	**2.14 (1.10-4.16)**	.26	1.0
(rs2569190)	TT	152	1.04 (0.31-3.44)	1.11 (0.31-4.04)		
CD14 3'ÚTR	CC	366	1.79 (0.83-3.89)	1.82 (0.81-4.06)	.51	.99
(rs2563298)	CA	242	1.20 (0.48-3.00)	1.33 (0.52-3.44)		
	AA	51	5.0 (0.57-44.6)	4.46 (0.35-56.26)		

*Unadjusted ORs (95% CIs) for parentally reported eczema in *E coli*–colonized infants compared with uncolonized infants stratified for genotype.
†Adjusted ORs (95% CIs); adjusted for parental and sibling's atopic history, recruitment group, sex, age at collection of feces, and pets.
‡Minor allele homozygotes excluded from analysis because of limited numbers.
§Likelihood ratio test for interaction (based on comparison of multivariate models with and without interaction term).
‖*P* value controlled for FDR according to the Benjamini-Hochberg approach (based on a total of 39 tests for interaction).
Boldface indicates statistically significant associations (alpha = .05)

TABLE E4. AD (according to UK-WP criteria): ORs for the association with *E coli* colonization in the first month of life, stratified for *CD14* and *TLR4* genotypes

TLR4/CD14 SNP	Genotype	No.	OR (95% CI)*	ORadj (95% CI)†	*P* value for interaction§	FDR-controlled *P* value‖
TLR4 c.-3612	TT	263	1.07 (0.39-2.98)	0.91 (0.31-2.67)	.51	1.0
(rs2770150)	CT&CC	228	0.60 (0.19-1.91)	0.60 (0.17-2.06)		
TLR4 c.-2570	AA	200	0.67 (0.21-2.17)	0.62 (0.17-2.27)	.74	1.0
(rs6478317)	GA	235	1.06 (0.34-3.27)	0.92 (0.27-3.09)		
	GG	55	0.44 (0.04-4.79)	0.14 (0.01-3.28)		
TLR4 c.-1607	TT	359	0.61 (0.26-1.43)	0.61 (0.25-1.48)	.21	1.0
(rs10759932)	CT	108	2.25 (0.27-18.61)	1.43 (0.15-13.90)		
	CC‡	29	—	—		
TLR4 c.93 + 3211	CC	261	1.05 (0.34-3.24)	0.94 (0.28-3.13)	.70	1.0
(rs1927911)	CT	196	0.78 (0.25-2.48)	0.73 (0.21-2.58)		
	TT‡	29	—	—		
TLR4 c.260 + 546	CC	357	1.00 (0.40-2.53)	0.87 (0.33-2.29)	.63	1.0
(rs11536878)	CA	111	0.55 (0.13-2.30)	0.63 (0.13-3.11)		
	AA‡	6	—	—		
TLR4 Asp299Gly	AA	424	0.84 (0.37-1.90)	0.84 (0.36-1.96)	.99	.99
(rs4986790)	GA	61	1.04 (0.11-9.86)	0.79 (0.05-13.07)		
	GG‡	3	—	—		
TLR4 Thr399Ile	CC	416	0.79 (0.35-1.80)	0.79 (0.33-1.87)	.93	.95
(rs4986791)	CT	61	0.89 (0.09-8.57)	0.60 (0.04-9.35)		
	TT‡	3	—	—		
TLR4 c.3648 + 78	GG	350	0.73 (0.30-1.78)	0.62 (0.24-1.56)	.41	1.0
(rs11536889)	CG	126	1.10 (0.22-5.31)	1.41 (0.22-8.81)		
	CC‡	11	—	—		
CD14/-1619	TT	166	0.83 (0.22-3.12)	0.95 (0.21-4.25)	.52	.97
(rs2915863)	CT	241	0.97 (0.31-3.02)	0.92 (0.28-3.10)		
	CC	82	0.30 (0.05-1.80)	0.17 (0.01-2.31)		
CD14/-1145	TT	134	1.07 (0.26-17.14)	1.51 (0.25-9.26)	.26	1.0
(rs2569191)	CT	232	1.09 (0.35-3.38)	1.10 (0.33-3.68)		
	CC	122	0.37 (0.089-1.57)	0.24 (0.04-1.54)		
CD14/-550	CC	286	1.02 (0.33-3.10)	0.88 (0.28-2.80)	.91	.99
(rs5744455)	CT	172	0.85 (0.26-2.77)	0.83 (0.22-3.09)		
	TT‡	29	—	—		
CD14/-159	CC	136	2.05 (0.25-16.80)	2.73 (0.27-27.20)	.12	.94
(rs2569190)	CT	242	1.19 (0.39-3.68)	1.34 (0.40-4.44)		
	TT	120	0.34 (0.08-1.46)	0.22 (0.03-1.51)		
CD14 3'ÚTR	CC	276	0.58 (0.23-1.45)	0.53 (0.20-1.40)	.27	.96
(rs2563298)	CA	189	1.35 (0.29-6.28)	1.78 (0.36-8.77)		
	AA‡	34	—	—		

*Unadjusted ORs (95% CIs) for AD in *E coli*–colonized infants compared with uncolonized infants, stratified for genotype.

†Adjusted ORs (95% CIs); adjusted for parental and sibling's atopic history, recruitment group, sex, age at collection of feces, and pets.

‡Minor allele homozygotes excluded from analysis because of limited numbers.

§Likelihood ratio test for interaction (based on comparison of multivariate models with and without interaction term).

‖*P* value controlled for FDR according to the Benjamini-Hochberg approach (based on a total of 39 tests for interaction).

TABLE E5. Allergic sensitization: ORs for the association with *E coli* colonization in the first month of life, stratified for *CD14* and *TLR4* genotypes

TLR4/CD14 SNP	Genotype	No.	OR (95% CI)*	ORadj (95% CI)†	*P* value for interaction§	FDR-controlled *P* value‖
TLR4 c.-3612	TT	262	0.76 (0.33-1.79)	0.78 (0.33-1.87)	.85	1.0
(rs2770150)	CT	196	0.60 (0.22-1.62)	0.55 (0.19-1.61)		
	CC‡	31	—	—		
TLR4 c.-2570	AA	199	0.49 (0.18-1.31)	0.41 (0.13-1.23)	.43	.93
(rs6478317)	GA	234	0.72 (0.30-1.77)	0.64 (0.25-1.64)		
	GG	55	2.06 (0.21-19.91)	2.23 (0.21-24.00)		
TLR4 c.-1607	TT	357	**0.38 (0.19-0.79)**	**0.31 (0.14-0.68)**	**.001**	**.04**
(rs10759932)	CT	108	5.81 (0.73-46.74)	6.98 (0.75-64.96)		
	CC‡	5	—	—		
TLR4 c.93 + 3211	CC	260	0.48 (0.20-1.12)	**0.37 (0.15-0.95)**	.14	.91
(rs1927911)	CT	195	1.01 (0.38-2.70)	1.11 (0.39-3.11)		
	TT‡	29	—	—		
TLR4 c.260 + 546	CC	356	0.93 (0.44-1.96)	0.85 (0.39-1.85)	.34	1.0
(rs11536878)	CA	110	0.43 (0.12-1.52)	0.33 (0.08-1.36)		
	AA‡	6	—	—		
TLR4 Asp299Gly	AA	424	0.69 (0.35-1.35)	0.64 (0.32-1.29)	.80	1.0
(rs4986790)	GA	61	1.05 (0.18-6.00)	1.05 (0.12-8.94)		
	GG‡	2	—	—		
TLR4 Thr399lle	CC	414	0.68 (0.35-1.33)	0.63 (0.31-1.26)	.86	1.0
(rs4986791)	CT	61	0.96 (0.17-5.50)	0.95 (0.11-8.45)		
	TT‡	2	—	—		
TLR4 c.3648 + 78	GG	348	0.64 (0.30-1.33)	0.57 (0.26-1.23)	.74	1.0
(rs11536889)	CG	126	0.76 (0.22-2.60)	0.69 (0.19-2.58)		
	CC‡	11	—	—		
CD14/-1619	TT	162	1.25 (0.38-4.1)	1.15 (0.33-4.01)	.11	1.0
(rs2915863)	CT	232	0.67 (0.27-1.68)	0.60 (0.23-1.57)		
	CC	81	0.34 (0.09-1.31)	0.35 (0.08-1.63)		
CD14/-1145	TT	136	1.54 (0.40-5.83)	1.33 (0.32-5.53)	.12	1.0
(rs2569191)	CT	230	0.73 (0.29-1.81)	0.59 (0.23-1.55)		
	CC	119	0.44 (0.14-1.38)	0.44 (0.13-1.50)		
CD14/-550	CC	108	0.69 (0.31-1.56)	0.65 (0.28-1.51)	.76	1.0
(rs5744455)	CT	167	0.76 (0.27-2.19)	0.71 (0.23-2.16)		
	TT	26	0.88 (0.07-11.32)	2.86 (0.07-117.8)		
CD14/-159	CC	133	2.17 (0.46-10.33)	1.82 (0.36-9.26)	**.05**	.97
(rs2569190)	CT	234	0.84 (0.34-2.06)	0.72 (0.28-1.84)		
	TT	117	0.37 (0.11-1.20)	0.36 (0.01-1.29)		
CD14 3'ÚTR	CC	265	0.60 (0.27-1.31)	0.56 (0.25-1.25)	.22	1.0
(rs2563298)	CA	186	1.05 (0.32-3.42)	1.06 (0.32-3.53)		
	AA	34	2.11 (0.21-21.45)	0.88 (0.05-18.44)		

*Unadjusted ORs (95% CIs) for allergic sensitization in *E coli*–colonized infants compared with uncolonized infants, stratified for genotype.

†Adjusted ORs (95% CIs); adjusted for parental and sibling's atopic history, recruitment group, sex, age at collection of feces, and pets.

‡Minor allele homozygotes excluded from analysis because of limited numbers.

§Likelihood ratio test for interaction (based on comparison of multivariate models with and without interaction term).

‖*P* value controlled for FDR according to the Benjamini-Hochberg approach (based on a total of 39 tests for interaction).

Boldface indicates statistically significant associations (alpha = .05)