

TLR4 gene variants modify endotoxin effects on asthma

Monika Werner, PhD,^a Rebekka Topp, PhD,^a Katrin Wimmer,^a Kai Richter, MD,^b
Wolfgang Bischof, MD, PhD,^c Matthias Wjst, MD,^a and Joachim Heinrich, PhD^a
Neuherberg, Grosshansdorf, and Erfurt, Germany

Background: Environmental exposure to endotoxin might have a crucial role in immune maturation and development of asthma. **Objective:** The aim of this study was to investigate whether the effect of endotoxin concentration in settled house dust on asthma is modified by the presence of variation in the *TLR4* gene. **Methods:** We performed a cross-sectional study within the German follow-up of the European Community Respiratory Health Survey. Multivariate logistic regression analysis and nonparametric effect estimates (S-Plus) were applied to examine the association between endotoxin exposure and diagnosed asthma, related clinical symptoms, and bronchial hyperreactivity (BHR) stratified for noncarriers and carriers of G299/I399 polymorphism in the *TLR4* gene. **Results:** In the noncarrier group ($n = 279$), the prevalence of asthma was significantly increased with elevated endotoxin levels in house dust with adjusted odds ratio 6.24 (95% CI, 1.33-29.17) in the second tertile, and 4.54 (95% CI, 0.94-21.96) in the third tertile compared with the lowest endotoxin tertile. The carriers of the polymorphisms ($n = 55$) showed a non-significant trend to have a lower risk of asthma (crude odds ratio, 0.67; 95% CI, 0.06-8.06 for the second tertile and 1.33; 95% CI, 0.17-10.58 for the third tertile). We found a similar association for wheeze and endotoxin exposure that was also attenuated in subjects with G299/I399 polymorphisms. **Conclusions:** The G299/I399 polymorphisms were associated with a modified response to endotoxin, but the functional relationship still needs clarification. (*J Allergy Clin Immunol* 2003;112:323-30.)

Key words: Endotoxin, *TLR4*, gene polymorphism, asthma, ECRHS

Several environmental factors have been described leading to the development of asthma and airway hyperreactivity. Although asthma is mostly considered to be an

Abbreviations used

BHR: Bronchial hyperreactivity
ECRHS: European Community Respiratory Health Survey
OR: Odds ratio
TLR4: *Drosophila* Toll receptor

atopic disease triggered by allergic sensitization and involving eosinophilic inflammation, in many cases non-allergenic exposures also induce asthmatic symptoms, and inflammatory mechanisms such as neutrophilic inflammation might play a prominent part in asthma pathogenesis. Airborne endotoxin, for example, is known to exacerbate existing asthma and possibly provoke new asthma.^{1,2} Furthermore, it has been reported that exposure to indoor endotoxin in early life is associated with increased risk of repeated wheeze.^{3,4} However, exposure to environmental endotoxin in infancy promotes the immune system toward a T_H1 response⁵ and away from a T_H2 response associated with atopy and asthma. Although the protective role of endotoxins for the development of asthma was considered controversial,^{6,7} recent reports showed consistently an inverse association between endotoxin exposure and the prevalence of hay fever, atopic asthma, and atopic sensitization in school-age children from the Alps area⁸ and of atopic sensitization in the German Indoor and Genetic Influences on Asthma and Allergies study.⁹

Endotoxin and its derivative LPS are soluble cell wall components of gram-negative bacteria. These potent inflammatory and immune-modulating substances are ubiquitous in the domestic^{10,11} and occupational environment.¹² Inhalation of LPS induces airway and systemic inflammatory responses characterized by blood leukocytosis and several asthmatic features such as neutrophilia, bronchial obstruction, and airway hyperreactivity.^{13,14} The responsiveness to inhaled LPS is dose dependent in both healthy subjects and patients with asthma.¹⁵ In atopic individuals with asthma environmental LPS and allergens might act in a synergistic manner.⁶ The bronchial reactivity is enhanced by concomitant inhalation of allergens and endotoxins. Moreover, in sensitized individuals with asthma, the challenge with allergen results in an increased concentration of LPS binding protein. Thus, they develop asthmatic symptoms at lower endotoxin concentrations.¹⁶ The concentration of endotoxin in house dust seems to be correlated with asthma severity.¹²

From the ^aInstitute of Epidemiology, GSF National Research Center for Environment and Health, Neuherberg; ^bPulmonary Research Institute at Grosshansdorf Hospital, Center for Pneumology and Thoracic Surgery, ^cInstitute of Occupational, Social and Environmental Medicine, Friedrich-Schiller-University of Jena, Erfurt.

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Reprint requests: Dr. Joachim Heinrich, PhD, GSF National Research Center for Environment and Health, Institute of Epidemiology, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany.

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A broad interindividual variability in LPS sensitivity has been reported.^{17,18} The first identified human homologue of the *Drosophila* Toll receptor (*TLR4*) mediates the response to bacterial LPS by means of induction of the NF κ B signaling pathway.¹⁹ The findings of Arbour et al²⁰ showed that 2 common cosegregating missense mutations in the extracellular domain of the human *TLR4* receptor are associated with differences in the airway response to inhaled endotoxins between individuals. Healthy subjects with the sequence variants D299G and the cosegregating T399I were hyporesponsive to inhaled endotoxin. Heterozygous genotypes seemed to be sufficient to reveal a hyporesponsive phenotype, even though subjects homozygous for D299G showed the lowest response to endotoxin. Carriers of this allele were recently found to have a lower risk of atherosclerosis and less inflammatory blood markers.²¹

Because asthma is an inflammatory disorder caused by an interaction of various genetic and environmental factors, the aim of this study was to investigate whether the effect of endotoxins on respiratory health might be modified by variations within genes contributing to the endotoxin signaling pathway. In particular, we measured endotoxin concentration in settled house dust and determined *TLR4* polymorphisms D299G and T399I in the follow-up study of the European Community Respiratory Health Survey (ECRHS). We tested the hypothesis that variations in the *TLR4* gene might protect against the development of asthma and respiratory hyperreactivity.

METHODS

Study population

This study included individuals participating in the follow-up of the ECRHS from the German centers in Hamburg and Erfurt. The first stage of the ECRHS was performed from 1990 to 1992 in 22 countries.²² Study areas had pre-existing administrative boundaries, a total population of at least 150,000 people, and up-to-date sampling frames that could be used. Of the examined subjects of the first surveys, 731 subjects in the age range of 20 to 44 years were selected in Erfurt and a random sample of 900 in Hamburg. Excluding subjects who could not be traced ($N = 275$), had refused further participation ($N = 12$), had moved out of the area ($N = 110$), and died ($N = 14$), a total of 1220 subjects were eligible for a follow-up. From this sample 287 subjects from Erfurt and 197 subjects from Hamburg were re-examined in the follow-up study starting in 2000 for an extensive interviewer-administered questionnaire and an extended examination composed of spirometric measurements, methacholine or bronchodilator inhalation tests, and determination of total and specific IgE. Furthermore, endotoxin concentrations in the living room floor dust were available from 176 subjects from Erfurt and 197 subjects from Hamburg (373 of 484 agreed with the home visit—77%). *TLR4* polymorphisms could be determined in 334 of these subjects (88%), because 26 subjects refused DNA extraction, and 13 subjects could not be genotyped. The study population consisted of 334 subjects with complete data on *TLR4* polymorphisms, endotoxin house dust, and major respiratory outcomes (54.2% from Erfurt, 50.0% females, 35.3% between 30 and 39 years, and 64.7% between 40 and 54 years). The study protocol was approved by the local ethics committees.

Lung function

Pulmonary function testing was made up of baseline spirometry and determination of flow-volume curves (Masterlab, Jaeger, Wuertzburg, Germany). All measurements, as well as methacholine challenges, were performed strictly adhering to the ECRHS protocol and quality standards.²² After inhalation of saline as a diluent, increasing doses of methacholine were administered until a 20% fall in postdiluent FEV₁ was observed or the maximum cumulative dose of 2 mg was reached. According to baseline characteristics of the subjects, challenges were started using a short protocol, with 4-fold increments, or a long protocol, with 2-fold increments. The method has been described in detail elsewhere.²³ Methacholine challenges were performed only if baseline FEV₁ was greater than 1.5 L and 70% of the predicted value.²⁴ Furthermore, challenges were not performed if the subject met specific exclusion criteria such as the subject had had a heart attack in the last 3 months, any heart disease with concomitant medication, epilepsy with current medication, taking β -blockers for any reason, or if she was pregnant or breast-feeding. The dose necessary for a 20% fall of FEV_{1,0} is given as PD₂₀, and probands with a PD₂₀ $\leq$ 2 mg were considered bronchial hyperreactive (BHR) in this analysis.^{23,25}

Allergic sensitization

Allergic sensitization was assessed by measurement of serum-specific IgE to house dust mite, grass, cat, and *Cladosporium* using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Moreover, total IgE was measured by the same method. Subjects were considered allergic sensitized if at least 1 of the 5 tested specific IgE showed levels $\geq$ 3.5 kU/L ($\geq$ CAP class 3) as defined in other recent studies.^{8,9}

DNA preparation and genotyping

DNA was extracted from blood samples using a commercially available kit (Puregene, Gentra Inc., Minneapolis, Minn). All individuals were genotyped for the A to G transition at nucleotide 896 responsible for the D299G amino acid change of the *TLR4* protein and for the C to T at nucleotide 1196 responsible for the T399I amino acid change. Genotyping was based on MALDI-TOF MS analysis of allele-specific primer extension products generated from previously amplified DNA sequences (MassARRAY, SEQUENOM Inc., San Diego, Calif). Primers were obtained from MWG Biotech AG (Ebersberg, Germany). Assay details are available from the authors on request.

Endotoxin measurement in house dust

For endotoxin measurements, dust samples were taken from the living room floor as described in reference 26. Endotoxin content in house dust was determined using a chromogenic kinetic Limulus amoebocyte lysate test.¹⁰ Concentrations are given in endotoxin units per square meter of sampled floor.

Statistical analysis

Logistic regression analysis was applied to examine the association between endotoxin exposure and clinical outcomes stratified by *TLR4* polymorphisms. Specifically, we compared effect estimates resulting from the separate analysis of individuals showing the *TLR4* variant and individuals without this variant. For both subpopulations, we estimated the effects of log-transformed endotoxin levels categorized in tertiles on the outcomes listed in Table III. The results are presented as odds ratios (ORs) and 95% CIs. In multivariate analysis, we controlled for the influence of potential confounders and other risk factors such as age, sex, center, smoking, and season of dust sampling. Statistical significance was set at a conventional 5% level. All computations were performed using

TABLE I. Description of the study population

	<i>TLR4</i> D299/T399*		<i>TLR4</i> G299/I399	
	N	Percent	N	Percent
Physician-diagnosed diseases				
Asthma (ever)	279	8.2	55	9.1
Hay fever (ever)	279	21.9	55	27.3
Atopic dermatitis (ever)	279	44.4	55	43.6
Clinical symptoms				
Wheeze (last 12 mo)	279	18.6	55	12.7
Runny/stuffed nose (ever)	279	35.8	55	40.0
Itchy skin (last 12 mo)	279	12.2	55	18.2
BHR†	247	18.2	48	16.7
Allergic sensitization				
RAST ≥3.5 kU/L‡	249	15.3	49	10.2
Total IgE >100 kU/L	260	26.5	52	28.9

*One subject without endotoxin value.

†Bronchial hyperreactivity defined as FEV₁ fall of at least 20% after a cumulative dose of 2 mg methacholine.

‡At least 1 of 5 specific IgE against birch or grass pollen, mite or cat allergens, and *Cladosporium* ≥ 3.5 kU/L.

SAS for windows V8 (SAS Institute, Cary, NC). Nonparametric smoothing techniques were applied to determine estimated prevalences of the studied outcomes in relation to the log-transformed endotoxin levels. We used a smoothing span of 0.8 for all plots. Calculations were made using the S-plus software (Genbank accession number: U93091).

RESULTS

In this study, we examined whether the effects of endotoxin exposure on respiratory health is modified by the recently described polymorphisms in the *TLR4* gene. Table I represents the characteristics of the study population stratified by *TLR4* polymorphisms, including diagnosed respiratory diseases and clinical symptoms and allergic sensitization. Endotoxin concentrations measured in house dust are categorized according to general characteristics, as well as specific respiratory and atopic outcomes of the study population and displayed in Table II.

We genotyped 334 individuals for the polymorphisms D299G and the T399I in the extracellular domain of *TLR4*. The allele frequency of the nucleotide A896G substitution was 8.3%, of the cosegregating nucleotide C1196T substitution it was 8.6% in our study population from 2 German centers. Because Kiechl et al²¹ also observed subjects with an isolated D299G polymorphism in the Italian population, cosegregation seems to result from population history. Both *TLR4* mutations were in accordance with the Hardy-Weinberg equilibrium. Although subjects from the study centers differed only slightly in allele distribution, we found no differences concerning sex or age. We could not find any association between *TLR4* variants and disease outcomes (Table I). Considering that only 2 individuals were homozygous for the mutant alleles, further statistical analysis refers to carriers of the gene variant.

Table III displays crude prevalence data of respiratory and atopic outcomes stratified by *TLR4* status and endotoxin exposure. As shown in Table IV, we examined whether any association exists between endotoxin expo-

sure and respiratory health with respect to *TLR4* gene polymorphisms by univariate and multivariate analysis. We found that moderately elevated endotoxin concentrations seem to be a risk factor for asthma; however, it is striking that individuals with the G299/I399 polymorphism have a lower risk in contrast to individuals with the D299/T399 wild-type (univariate OR, 0.67; 95% CI, 0.06-8.06 versus OR, 5.66; 95% CI, 1.23-26.04 within the second tertile of endotoxin concentrations and OR, 1.33, 95% CI, 0.17-10.58 versus OR, 4.29; 95% CI, 0.9-20.45 for the third tertile). If the endotoxin exposure is increased, subjects with the mutant allele have shown less respiratory symptoms such as wheeze, runny nose, or BHR compared with subjects carrying only the wild-type allele. The findings could be further substantiated by multivariate analysis. Carriers of only the wild-type allele are more frequently affected by asthma (OR, 6.24; 95% CI, 1.33-29.17 for the second tertile, OR, 4.54; 95% CI, 0.94-21.96 for the third tertile) and showed more respiratory symptoms as wheeze (OR, 2.16; 95% CI, 0.93-5.05 for the second tertile, OR, 2.44; 95% CI, 1.05-5.69 for the third tertile), whereas subjects with the *TLR4* variants exposed to elevated endotoxin levels revealed a statistically significant reduced BHR (OR, 0.02; 95% CI, 0.001-0.47 for the second tertile, OR, 0.08; 95% CI, 0.002-3.30 for the third tertile). Alternative approaches using 15% fall of FEV_{1,0} as cutoff or dose-response slopes as a continuous outcome measure showed similar results but weaker effect estimates (data not shown). Nonparametric correlation analysis revealed an inverse association between asthma and current endotoxin exposure in subjects with the mutant allele. This association was less clear for the outcomes wheeze and BHR (Fig 1). Analysis regarding atopic versus nonatopic wheeze could not be performed because of the small number of observations. In contrast, we could not associate the presence of *TLR4* polymorphisms with any atopic characteristics such as hay fever, atopic dermatitis, atopic sensitization, and elevated total IgE (Table IV).

TABLE II. Description of endotoxin exposure (EU/m²) in living room floor dust (N = 334)*

		TLR4 D299/T399*			TLR4 G299/I399		
		N	Geometric mean	SD	N	Geometric mean	SD
Center	Erfurt	147	15,764	170,180	33	11,264	72,331
	Hamburg	131	17,580	158,058	22	15,446	36,227
Sex	Male	139	16,179	87,052	27	13,588	75,671
	Female	139	17,021	215,250	28	12,047	41,024
Age (y)	30-39	97	14,047	193,978	20	10,273	36,808
	40-54	181	18,146	146,599	35	14,479	70,273
Physician-diagnosed diseases							
Asthma	Yes	23	28,963	305,200	5	14,827	19,465
	No	255	15,782	145,369	50	12,592	62,807
Hay fever	Yes	61	11,328	59,403	15	11,390	24,751
	No	217	18,475†	182,596	40	13,344	68,588
Atopic dermatitis	Yes	124	14,423	152,936	24	13,565	35,662
	No	154	18,579	173,220	31	12,204	74,226
Clinical symptoms							
Wheeze	Yes	52	27,387	230,157	7	20,853	58,743
	No	226	14,788†	144,369	48	11,900	60,713
Runny/stuffed nose	Yes	99	15,166	167,466	22	8,599	22,552
	No	179	17,442	163,086	33	16,645	73,699
Itchy skin	Yes	34	15,278	85,036	10	11,894	29,898
	No	244	16,787	172,578	45	12,986	65,145
BHR‡	Yes	45	16,915	123,123	8	4,167	29,062
	No	201	16,217	181,393	40	12,542	33,346
BHR or predicted FEV ₁ < 80%	Yes	50	18,788	230,026	9	6,048	43,826
	No	196	15,771	153,733	39	11,839	30,180
Allergic sensitization							
RAST ≥3.5 kU/L§	Yes	38	12,763	73,785	5	6,480	14,255
	No	210	17,669	157,937	44	14,255	65,920
Total IgE >100 kU/L	Yes	69	16,689	68,527	15	10,223	35,653
	No	190	16,799	164,604	37	14,418	69,327

EU, Endotoxin units.

*One subject without endotoxin value.

† $P < .05$.‡Bronchial hyperreactivity defined as FEV₁ fall of at least 20% after a cumulative dose of 2 mg methacholine.§At least 1 of 5 specific IgE against birch or grass pollen, mite or cat allergens, and *Cladosporium* ≥3.5 kU/L.

DISCUSSION

The Toll-like receptor 4 has been established as an essential receptor for the recognition of endotoxins derived from gram-negative bacteria. Subsequent to binding of endotoxins, the innate immune response is activated. Recent studies have suggested that *TLR4* also plays a role in dendritic cell maturation and initiation of the adaptive immune response.²⁷ Signaling by means of the transcription factor *NFκB* leads to the release of several cytokines, such as *TNFα* and *IL-1β*, neutrophil recruiting chemokines, and matrix metalloproteinases, and eventually to inflammation.^{28,29} Although environmental endotoxins are important triggers of asthma, the mechanisms underlying the different susceptibility are poorly understood. Because of the *TLR4* function and expression profile, variants in the *TLR4* gene might alter the risk of asthma development.

We reported that the presence of 2 common cosegregating polymorphisms in the extracellular domain of the *TLR4* gene, which interrupt the signaling pathway, modify the effect of endotoxins on asthma in adults. If

exposed to even moderate endotoxin concentrations in house dust, carriers of at least 1 mutant allele showed a decreased risk of asthma and asthma-associated outcomes such as wheeze and BHR compared with those with the wild-type allele. This effect might be even stronger for homozygous individuals, because the mutant *TLR4* is compensated by the wild-type *TLR4* in heterozygous individuals. Because in our study population the D299G and the T399I polymorphisms are in complete linkage disequilibrium, the responsible variant could not be ascertained. However, an Italian study on atherosclerosis has recently reported an impaired immune response that has been associated with the D299G allele alone.²¹ Our findings suggest that *TLR4* gene variants might affect the pathogenesis of asthma. This conclusion can be supported by the fact that the polymorphism D299G has been related to hyporesponsiveness to inhaled endotoxins in healthy humans.²⁰

One implication of our results is that the inflammation causing the characteristic airway hyperresponsiveness seems to be mediated by the *TLR4* pathway. It has been proposed that inflammatory mechanisms other than

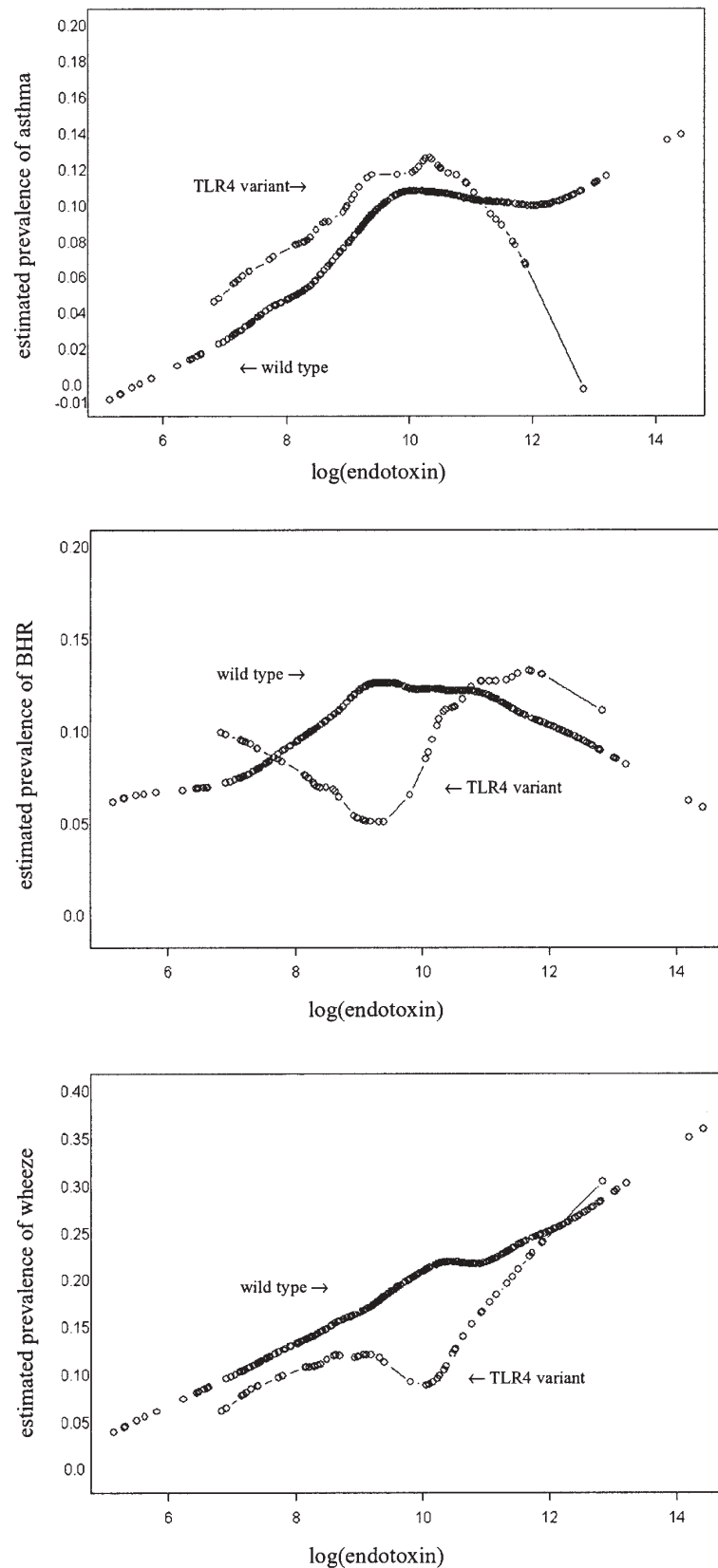


FIG 1. Smoothed plots of the prevalence of asthma, BHR, and wheeze in relation to the log-transformed endotoxin levels.

TABLE III. Clinical characteristics by *TLR4* status and endotoxin exposure

	<i>TLR4</i> D299/T399*				<i>TLR4</i> G299/I399			
	Endotoxin [EU/m ²]				Endotoxin [EU/m ²]			
	<8,152†	[8,152;32,533]	>32,533‡		<8,152†	[8,152;32,533]	>32,533‡	
	N	N = 87	N = 97	N = 94	N	N = 24	N = 14	N = 17
Physician-diagnosed diseases								
Asthma (ever; N = 334)	23	2.3%	13.4%	8.5%	5	8.3%	7.1%	11.8%
Hay fever (ever; N = 334)	61	26.4%	23.7%	16.0%	15	33.3%	21.4%	23.5%
Atopic dermatitis (ever; N = 334)	124	46.0%	47.4%	40.4%	24	33.3%	57.1%	47.1%
Clinical symptoms (N = 334)								
Wheeze (last 12 mo)	52	10.3%	22.7%	22.3%	7	12.5%	7.1%	17.7%
Runny/stuffed nose (ever)	99	35.6%	39.2%	31.9%	22	54.2%	28.6%	29.4%
Itchy skin (last 12 mo)	34	11.5%	13.4%	11.7%	10	16.7%	14.3%	23.5%
BHR§ (N = 295)	45	21.3%	13.8%	19.8%	8	26.1%	7.7%	8.3%
Allergic sensitization								
RAST ≥3.5 kU/L (N = 298)¶	38	19.7%	11.9%	14.8%	5	18.2%	0.0%	6.3%
Total IgE >100 kU/L (N = 312)	69	22.8%	31.1%	25.6%	15	34.8%	23.1%	25.0%

Subjects homozygous (N = 2) and heterozygous (N = 53).

EU, Endotoxin units.

*One subject without endotoxin value.

†First tertile.

‡Third tertile.

§Bronchial hyperreactivity defined as FEV₁ fall of at least 20% after a cumulative dose of 2 mg methacholine.¶At least 1 of 5 specific IgE against birch or grass pollen, mite or cat allergens, and *Cladosporium* ≥3.5 kU/L.

eosinophilic inflammation result in enhanced bronchial reactivity. To a great extent, airway hyperreactivity might also be based on neutrophilic inflammation in which *TLR4* signaling is involved.³⁰ Disruption of this pathway by the D299G polymorphism might account for the significantly reduced BHR shown in subjects with this mutant allele. In addition to LPS, *TLR4* is also involved in the recognition of other ligands such as proteins from the respiratory syncytial virus,³¹ toxic lipoteichoic acid from gram-positive bacteria, bacterial heat shock proteins, mycobacterial lipids, and fibrinogen. Hence, an effective immune response to such diverse ligands might require a functional *TLR4* pathway. From several studies there is also evidence that LPS and other microbial products derived from different microorganisms might stimulate different *TLR* subtypes such as *TLR2* or *TLR9*. Thus, subtype-specific and shared *TLR* signal transduction pathway might be activated.³²

Although it has been proposed that endotoxin exposure early in life might protect against allergic sensitization by enhancing the T_H1-driven immunity,^{4,33-35} we did not find an association between endotoxin exposure and atopic symptoms. Although atopic wheezing in children was inversely related to higher endotoxin levels,⁸ this study could not observe this association in the adult population. As reported from studies in mice, *TLR4* seems to be required for the development of T_H2 immune response, and thus, variants in *TLR4* might be protective against the development of atopy.²⁷ Moreover, genetic factors were suggested to be different for atopy and bronchial hyperresponsiveness.³⁶

Although our findings show a clear association, sources of bias should be considered. First, exposure was

only measured in parallel for 3 months after the medical examination and not over a longer period. Little data exist on endotoxin exposure variation over time so far; however, repeated measurements of endotoxin in settled house dust clearly showed a higher between-home variance than within-home variance.³⁷ Thus, we might assume that measured concentrations reflect the middle or even long-term exposure. Second, because allergic diseases and asthma often occur in infancy, the association based on current endotoxin exposure might be biased by reverse causation. The current data, however, do not show significant differences in exposure between affected and nonaffected subjects. Even though this reverse effect cannot be completely excluded, it is not very likely. Third, the dropout rate because of the first stage of the survey is remarkable. Nevertheless, the study population is comparable to the initial sample of 1631 individuals regarding demographic characteristics and clinical outcomes (data not shown).

In conclusion, these data indicate one of the first major examples in which genetic variation and environmental factors interact to cause disease in a population-based sample. The D299G polymorphism in *TLR4*, which affects endotoxin recognition and reduces subsequent inflammatory processes, was associated with the risk of asthma in adults. The subgroup specified by the presence of this variant seems to be hyporesponsive even at elevated endotoxin exposure. Because this subset of the population is less than 10% of the total population, the attributable risk of asthma for endotoxin is less affected by the small subset of "protected" individuals. However, the functional relationship between G299/I399 polymorphisms and the modified response to endotoxin still needs clarification.

TABLE IV. Association (univariate and multivariate) between endotoxin exposure and respiratory or atopic outcomes stratified by *TLR4* variants (odds ratios and 95% CI)

	<i>TLR4</i> D299/T399			<i>TLR4</i> G299/I399		
	Endotoxin [EU/m ²]			Endotoxin [EU/m ²]		
	<8,152*	[8,152;32,533]	>32,533†	<8,152*	[8,152;32,533]	>32,533†
Univariate analysis						
Physician-diagnosed diseases						
Asthma	1	5.66‡ (1.23,26.04)	4.29 (0.90,20.45)	1	0.67 (0.06,8.06)	1.33 (0.17,10.58)
Hay fever	1	0.85 (0.44,1.66)	0.51 (0.25,1.07)	1	0.55 (0.12,2.53)	0.62 (0.15,2.51)
Atopic dermatitis	1	1.00 (0.56,1.78)	0.80 (0.45,1.44)	1	2.67 (0.69,10.36)	1.78 (0.50,6.37)
Clinical symptoms						
Wheeze	1	2.24 (0.97,5.21)	2.51‡ (1.08,5.82)	1	0.42 (0.04,4.48)	1.36 (0.24,7.75)
Runny nose	1	1.20 (0.66,2.20)	0.90 (0.49,1.68)	1	0.34 (0.08,1.39)	0.35 (0.10,1.32)
Itchy skin	1	1.07 (0.44,2.62)	1.10 (0.45,2.69)	1	0.83 (0.13,5.26)	1.54 (0.33,7.26)
BHR§	1	0.65 (0.29,1.49)	0.93 (0.43,2.00)	1	0.18 (0.02,1.68)	0.23 (0.02,2.17)
Allergic sensitization						
RAST ≥3.5 kU/L¶	1	0.45 (0.19,1.11)	0.72 (0.32,1.61)	1	0.47 (0.04,5.11)	0.38 (0.04,4.03)
Total IgE >100 kU/L	1	1.43 (0.72,2.82)	1.07 (0.53,2.17)	1	0.56 (0.12,2.65)	0.63 (0.15,2.59)
Multivariate analysis (adjusted for age, sex, center, smoking, and season of dust sampling)						
Physician-diagnosed diseases						
Asthma	1	6.24‡ (1.33,29.17)	4.54 (0.94,21.96)	1	**	**
Hay fever	1	0.87 (0.44,1.72)	0.52 (0.25,1.08)	1	0.32 (0.06,1.82)	0.73 (0.16,3.32)
Atopic dermatitis	1	0.99 (0.54,1.82)	0.80 (0.43,1.47)	1	2.15 (0.49,9.53)	2.06 (0.51,8.32)
Clinical symptoms						
Wheeze	1	2.16 (0.93,5.05)	2.44‡ (1.05,5.69)	1	0.20 (0.01,3.04)	1.99 (0.28,14.41)
Runny nose	1	1.19 (0.64,2.21)	0.89 (0.47,1.67)	1	0.19 (0.04,1.01)	0.34 (0.08,1.42)
Itchy skin	1	1.12 (0.45,2.80)	1.17 (0.47,2.91)	1	**	**
BHR§	1	0.61 (0.26,1.42)	0.86 (0.39,1.89)	1	0.02‡ (0.001,0.47)	0.08 (0.002,3.30)
Allergic sensitization						
RAST ≥3.5 kU/L¶	1	0.46 (0.18,1.15)	0.72 (0.32,1.66)	1	0.37 (0.02,5.92)	0.44 (0.03,5.78)
Total IgE >100 kU/L	1	1.46 (0.73,2.93)	1.04 (0.51,2.16)	1	0.32 (0.05,1.89)	0.85 (0.18,4.13)

EU, Endotoxin units.

*First tertile.

†Third tertile.

‡Statistically significant.

§Bronchial hyperreactivity defined as FEV₁ fall of at least 20% after a cumulative dose of 2 mg methacholine.

¶At least 1 of 5 specific IgE against birch or grass pollen, mite or cat allergens, and *Cladosporium* ≥3.5 kU/L.

**No convergence because of empty cells.

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