

Endotoxin Exposure, CD14, and Allergic Disease

An Interaction between Genes and the Environment

Angela Simpson, Sally L. John, Francine Jury, Rob Niven, Ashley Woodcock, William E. R. Ollier, and Adnan Custovic

Academic Division of Medicine and Surgery South, North West Lung Centre, Wythenshawe Hospital; and Centre for Integrated Genomic Medical Research, University of Manchester, Manchester, United Kingdom

Rationale: High endotoxin exposure may reduce the risk of allergic sensitization.

Objective: To determine the relationship between a promoter polymorphism in the *CD14* gene (*CD14*–159 C to T) and endotoxin exposure in relation to the development of allergic sensitization, eczema, and wheeze within the setting of a birth cohort.

Methods: We genotyped 442 children (*CD14*–159 C to T; rs2569190). We assessed children for allergic sensitization (IgE > 0.2 kU/L to at least one of seven allergens), eczema (physical examination), and parentally reported wheeze. Endotoxin was measured in house dust.

Main Results: Genotype frequencies were consistent with other populations (TT, 25%; CT, 47%; CC, 28%). Sensitization (present in 33% of children) was not associated with genotype. For children with TT and CT genotypes, there was no association between endotoxin and sensitization (odds ratio [OR], 0.95; 95% confidence interval [CI], 0.71–1.23; $p = 0.7$; and OR, 0.90; 95% CI, 0.77–1.04; $p = 0.16$, respectively) or endotoxin and eczema (OR, 0.99; 95% CI, 0.81–1.20; $p = 0.89$; and OR, 1.38; 95% CI, 0.83–2.30; $p = 0.22$, respectively). In children with the genotype CC, increasing endotoxin load was associated with a marked and significant reduction in the risk of sensitization (OR, 0.70; 95% CI, 0.55–0.89; $p = 0.004$) and eczema (OR, 0.73; 95% CI, 0.56–0.95; $p = 0.02$). However, we observed an increased risk of nonatopic wheeze with increasing endotoxin exposure in children with the CC genotype (OR, 1.42; 95% CI, 1.01–1.99; $p = 0.04$) but not other genotypes. No effect was seen for atopic wheeze.

Conclusions: Increasing endotoxin exposure is associated with reduced risk of allergic sensitization and eczema but with increased risk of nonatopic wheeze in children with the CC genotype at –159 of the *CD14* gene. The impact of environmental endotoxin may be enhanced in individuals with this genotype.

Keywords: allergy; asthma; genetics

Allergic sensitization is neither necessary nor sufficient for asthma development, but the association between the two is strong (1). Twin and family studies have confirmed a strong genetic component of both conditions (2). The increase in prevalence of asthma and sensitization suggests an important environmental component. Until recently, genetic and environmental factors have generally been studied independently. To increase the probability of identifying causative factors for complex diseases, it is neces-

sary to study the genes and the environment to determine their interactions (3, 4).

A locus on chromosome 5q31.1 is linked to atopy and asthma (5). One gene mapping to this region is *CD14*, part of the receptor complex for lipopolysaccharide, the major component of endotoxin (6, 7). Activation of this pathway results in strong interleukin (IL)-12 expression in early life, which favors Th1 differentiation of naive T cells, reducing the probability of an IgE response to allergens (8, 9). Although a promoter single nucleotide polymorphism (SNP) in this gene (*CD14*–159 C to T, rs2569190) was not associated with the frequency of sensitization, among sensitized children, C-allele homozygotes had higher total IgE levels and more positive skin tests (10). Similar results were reported in studies of Dutch adults with asthma (11), in Australian (12) and Chinese children (13), and in northern Indian populations (14). No association was found between *CD14*–159 polymorphism and atopy or total IgE in two German populations (15, 16) or among Japanese subjects (17). In contrast, in a founder population in the United States (the Hutterites), the T allele was associated with sensitization (18). This allele has recently been identified as a risk for eczema at 2 yr of age in high-risk children (19). However, a study of a high-risk, 1-yr-old children (including 14% ethnic minorities) found that T-allele homozygotes had a reduced risk of eczema but only if they were dog owners (20). The results of previous studies that investigated the association between *CD14* polymorphisms and clinical outcomes are presented in Table E1 of the online supplement.

The T allele was also associated with higher soluble *CD14* levels. *In vivo* (10) and *in vitro* studies showed that the T allele was transcribed more efficiently in monocytes (21), suggesting that this polymorphism is functional.

Conflicting results of the association between *CD14* polymorphisms and sensitization may be due to differing environmental exposures in the populations studied. Endotoxin exposure may be a plausible candidate and has been associated with allergic disease. Lower early-life endotoxin load was reported in sensitized compared with nonsensitized, high-risk children (22). Higher exposure reduced the risk of eczema at 6 mo (23) and 12 mo of age (24) and of sensitization and atopic asthma among schoolchildren (25). However, this association was not reproduced in all populations (26, 27).

Endotoxin exposure and the *CD14* polymorphism have been associated with allergy in some populations but not in others. Because the effect of endotoxin is mediated through *CD14*, we aimed to investigate the relationship between a promoter polymorphism in the *CD14* gene (*CD14*–159 C to T) and endotoxin exposure with allergic sensitization, eczema, and wheeze in a population-based birth cohort study. Some of the results of this study were reported in the form of an abstract (28, 29).

METHODS

Additional details on study population, environmental exposures, and genotyping are available in the online supplement.

(Received in original form September 6, 2005; accepted in final form April 13, 2006)

Supported by the Moulton Charitable Trust, and Asthma UK.

Correspondence and requests for reprints should be addressed to Adnan Custovic, M.D., Ph.D., North West Lung Centre, Wythenshawe Hospital, Manchester M23 9LT, UK. E-mail: adnan.custovic@manchester.ac.uk

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 174, pp 386–392, 2006

Originally Published in Press as DOI: 10.1164/rccm.200509-1380OC on April 13, 2006
Internet address: www.atsjournals.org

Study Population

The Manchester Asthma and Allergy Study is a population-based birth cohort study (30–32) set in Greater Manchester, United Kingdom (population, 6 million). Participants were recruited prenatally (30) and reviewed at 5 yr ($\pm$ 4 wk) of age. We collected domestic dust samples for measurement of environmental exposures at this time. The study was approved by the local research ethics committee. We obtained informed consent from all parents.

Outcomes

Allergic sensitization. We measured specific serum IgE (mite, cat, dog, grass, milk, egg, and peanut; ImmunoCAP; Pharmacia, Uppsala, Sweden) and defined sensitization as specific IgE greater than 0.2 kU/L to any allergen. We used a skin-prick test for *Dermatophagoides pteronyssinus*, cat, dog, grasses, molds, milk, and egg (Bayer, Elkhart, IN) and defined sensitization as a wheal diameter 3 mm greater than negative control.

Clinical assessment. A validated questionnaire (33) was interviewer administered to collect information on parentally reported symptoms, physician-diagnosed illnesses, and treatments received. Children were defined as having recent wheeze if they had wheezed within the previous 12 mo. Wheeze was defined as atopic if it occurred within the context of allergic sensitization and nonatopic if it was reported in children who were not sensitized.

Physical examination was conducted in a standardized manner. Eczema was defined using the U.K. diagnostic criteria (34).

Environmental Exposures

We collected two living-room floor dust samples by vacuuming 1-m² areas for 2 min (32). Endotoxin content was measured by a kinetic limulus assay (35). Results were expressed as endotoxin load (EU/m²) and level (EU/mg). Dust mite allergen Der p 1 was measured using ELISA (32). Results were expressed as allergen concentration (μ g/g).

Genotyping

We genotyped an SNP in the promoter region of CD14 (–159 T > C rs2569190) using a fluorescence-based primer extension method (SNaPshot; ABI, Warrington, UK). Polymerase chain reactions were performed in 10- μ l reaction volumes containing 10 ng genomic DNA (isolated from whole blood) per reaction. Three positive controls of each genotype and negative water controls were included on each plate.

Soluble CD14

Soluble CD14 (sCD14) was measured in plasma collected at 5 yr of age (R&D Systems, Abingdon, UK). Results were expressed as μ g/ml.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 11.0). Endotoxin and mite allergen data followed a log-normal distribution; results are presented as geometric means (GM) and 95% confidence intervals (CI). Deviation from Hardy-Weinberg equilibrium was tested for using a χ^2 test. Logistic regression was used to ascertain the effect of endotoxin load on sensitization, eczema, wheeze, and sCD14 in the whole population and within different genotype groups. The results are presented as odds ratios (OR) and 95% CI.

RESULTS

The study profile is shown in Figure 1. We reviewed 1,025 children; 122 children randomized to an environmental control regime (36) were excluded from this analysis. A full dataset was available in 442 children of mixed European ancestry. Where genotyping was not performed, this was generally because the child refused venipuncture. Children excluded from the analysis did not differ from those included in terms of family history, parental smoking, maternal age, socioeconomic status, gestational age, history of wheeze, allergic sensitization, and endotoxin exposure (data available on request).

Demographic data of the subjects are presented in Table 1; 33% were sensitized by IgE (17% to mite) and 25% by skin

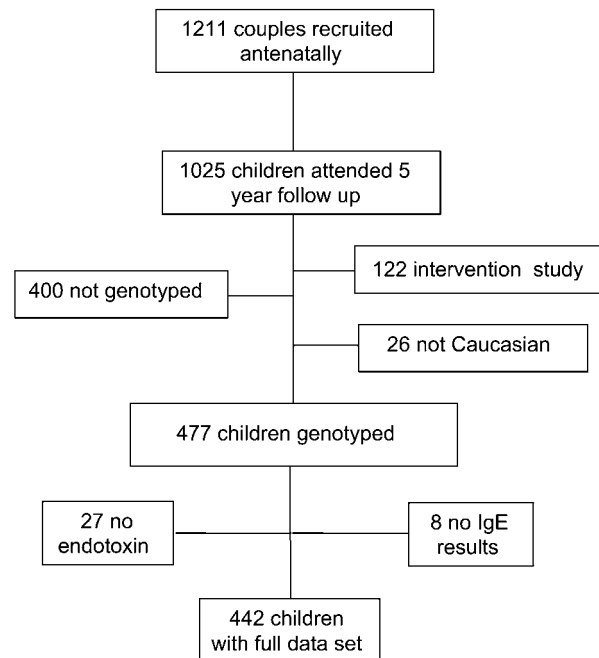


Figure 1. Schematic representation of trial profile.

tests (14% to mite), 38% reported wheeze ever, 21% reported current wheeze, and 14% had eczema on clinical examination. Endotoxin exposure covered a large range ($\sim$ 200,000-fold; load GM, 2,856 EU/m²; 95% CI, 2,420–3,370, and level GM, 16.1 EU/mg; 95% CI, 14.3–18.1) and was not associated with family history of allergic disease, socioeconomic status, position in the sibship, or parental smoking ($p > 0.2$). sCD14 was detectable in all children (mean, 1.77 μ g/ml; 95% CI, 1.73–1.80 μ g/ml). Genotype frequencies were in Hardy-Weinberg equilibrium and consistent with other populations (TT, 25%; CT, 47%; CC, 28%) (10).

Allergic Sensitization

There was no association between CD14/–159 genotype and sensitization defined by IgE ($p = 0.4$). For the whole population, increasing endotoxin load was significantly associated with a decreased risk of sensitization (OR, 0.85; 95% CI, 0.76–0.95; $p = 0.005$). However, when this analysis was repeated by genotype, there was no association between endotoxin load and sensitization for the TT and CT genotypes (OR, 0.95; 95% CI, 0.71–1.3; $p = 0.7$; and OR, 0.90; 95% CI, 0.77–1.04; $p = 0.16$, respectively). In contrast, in children with the CC genotype, increasing endotoxin load was associated with a marked and significant decrease in the risk of sensitization (OR, 0.70; 95% CI, 0.55–0.89; $p = 0.004$; Figure 2). For the CC genotype group only, for each log unit increase in endotoxin exposure in the home, there was a 30% decrease (95% CI, 11–45) in the odds of being sensitized to one or more allergen. We found some limited evidence of a statistical interaction between genotype and endotoxin load in their relationship with allergic sensitization when an interaction term was included in the logistic regression model (overall interaction, $p = 0.09$).

None of these relationships was significantly altered when sensitization was defined as one or more specific IgE greater than 0.35 kU/L. Similarly, defining allergic sensitization by skin tests or expressing endotoxin exposure as level or quartiles of load (Table 2) or inclusion of the environmental tobacco smoke

TABLE 1. DEMOGRAPHIC DATA OF THE STUDY POPULATION

Variable	Whole Group*	Genotype (%)			p Value
		TT (n = 109)	CT (n = 207)	CC (n = 126)	
Male	56	62	52	56	0.2 [†]
Maternal asthma	18.3	18.3	18.4	18.3	1 [†]
Paternal asthma	11.5	15.6	9.7	11.1	0.3 [†]
Maternal smoking	14.3	11.9	15	15.1	0.7 [†]
Paternal smoking	25.1	21	27	25	0.5 [†]
Maternal atopy	52.8	46.8	54.9	54.8	0.3 [†]
Paternal atopy	56.8	63.9	53.4	56.3	0.2 [†]
Cat owner	25.6	31.2	23.8	23.8	0.3 [†]
Dog owner	19.5	18.3	19.4	20.6	0.9 [†]
Older siblings	56.3	56	57	55.6	0.9 [†]
Attended nursery	68	68.9	66.1	70.1	0.7 [†]
Breast fed > 16 wk	39.2	42.9	39.3	35.7	0.1 [†]
Wheeze ever	38.2	34.9	39.1	39.7	0.7 [†]
Current wheeze	20.6	15.6	24.2	19.0	0.2 [†]
Eczema	14.1	8.3	16.4	15.2	0.14 [‡]
Sensitization (IgE)	32.8	28.4	35.7	31.7	0.4 [†]
Sensitization (SPT)	25.3	22.2	28.6	22.7	0.3 [†]
Mite sensitization (IgE)	17	16.5	17.4	16.7	1 [†]
Mite sensitization (SPT)	14	13	14	14	1 [†]
sCD14, mg/ml	1.77	1.83	1.78	1.70	0.02 [‡]
Total IgE, kU/L	26.99	24.79	24.67	33.67	0.18 [‡]
Endotoxin load, EU/m ²	2856	2885	2974	2647	0.84 [‡]

Definition of abbreviation: SPT = skin prick test.

* Values are percentages unless otherwise noted.

[†] p Value for χ^2 by genotype.

[‡] p value by analysis of variance.

exposure at 5 yr of age or maternal smoking during pregnancy did not materially alter the results.

To further explore this association with sensitization to individual allergens, we investigated the relationship between the *CD14* polymorphism, exposure to mite allergen, and endotoxin- and mite-specific IgE. There was no association between *CD14* genotype and mite sensitization ($p = 1$; see Table 1). In a multivariate logistic regression analysis, for the whole population

there was a trend toward an increase in the risk of sensitization to mite with increasing mite allergen exposure (OR, 1.14; 95% CI, 1.00–1.30; $p = 0.06$) and a significant decrease in the risk with increasing endotoxin exposure (OR, 0.84; 95% CI, 0.73–0.98; $p = 0.02$). In children with the CC genotype, an increase in the risk of mite sensitization was significantly associated with increasing mite allergen exposure and decreasing endotoxin exposure (OR, 1.34; 95% CI, 1.03–1.75; $p = 0.03$; and OR, 0.67; 95% CI, 0.50–0.90; $p = 0.007$, respectively; Figure 3). No such effect was seen in children with the CT and TT genotypes ($p > 0.4$). Defining mite sensitization by skin tests or using early-life or cumulative exposure did not alter the results (data not shown).

Eczema

For the whole population, there was no association between eczema and *CD14* genotype ($p = 0.14$) or endotoxin exposure ($p = 0.28$). However, in children with the CC genotype, increasing endotoxin load was associated with a decreased risk of eczema (OR, 0.73; 95% CI, 0.56–0.95; $p = 0.02$), which was not seen for the CT or TT genotypes (OR, 0.99; 95% CI, 0.81–1.20; $p = 0.89$; and OR, 1.38; 95% CI, 0.83–2.30; $p = 0.22$, respectively; Figure 4a). For the CC genotype group only, for each log unit increase in endotoxin exposure in the home there was a 27% decrease (95% CI, 5–44) in the odds of having eczema. We found a statistically significant interaction between genotype and endotoxin load, with evidence of a difference between the genotypes with respect to the relationship between eczema and endotoxin load (overall interaction, $p = 0.05$).

Wheeze

For the whole population, we found no association between wheeze and *CD14* genotype (wheeze ever, $p = 0.7$; recent wheeze, $p = 0.2$) or between wheeze and endotoxin exposure (wheeze ever, $p = 0.1$; recent wheeze, $p = 0.3$). However, in the CC genotype subgroup, increasing endotoxin load was associated with a

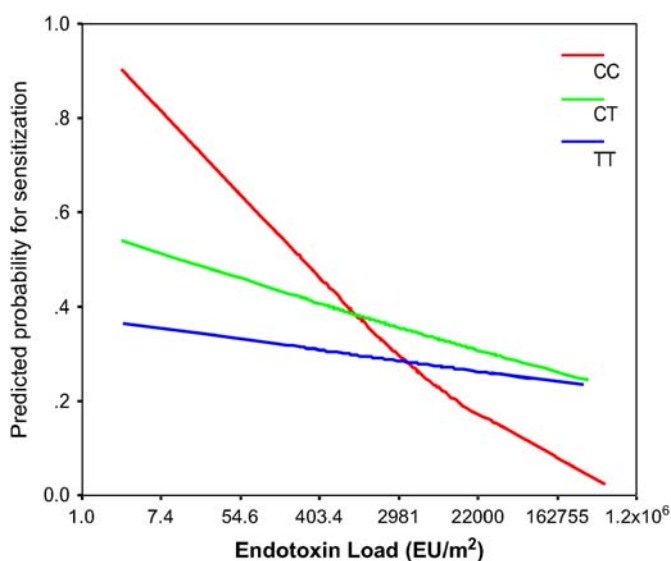


Figure 2. Fitted predicted probability curves for allergic sensitization at 5 yr of age in relation to environmental endotoxin load in children with CC, CT, and TT genotypes in the promoter region of the *CD14* gene (*CD14*–159 C to T) derived from the logistic regression analysis.

TABLE 2. EVIDENCE FOR ASSOCIATION AMONG THREE DIFFERENT DEFINITIONS OF ALLERGIC SENSITIZATION AND THREE DIFFERENT MEASURES OF ENDOTOXIN EXPOSURE IN CHILDREN WITH THE CC GENOTYPE (n = 126)

	IgE > 0.2 kU/L (n = 31 with allergic sensitization)		IgE > 0.35 kU/L (n = 27 with allergic sensitization)		Skin Prick Test (n = 24 with allergic sensitization)	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Endotoxin load, EU/m ²	0.70 (0.55–0.89)	0.004	0.77 (0.61–0.96)	0.023	0.81 (0.64–1.02)	0.067
Endotoxin level, EU/mg	0.61 (0.43–0.87)	0.006	0.70 (0.51–0.97)	0.032	0.76 (0.55–1.05)	0.093
Endotoxin load, quartiles	0.60 (0.42–0.87)	0.007	0.62 (0.43–0.91)	0.015	0.63 (0.42–0.94)	0.023

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

significant increase in the risk of recent wheeze (OR, 1.31; 95% CI, 1.00–1.73; $p = 0.05$), which was not seen in the CT or TT genotypes (OR, 1.00; 95% CI, 0.84–1.18; $p = 1.0$; and OR, 1.04; 95% CI, 0.72–1.50; $p = 0.8$, respectively). Children with recent wheeze were divided into atopic ($n = 42$) and nonatopic ($n = 49$) groups. Using nonwheezers as the reference group, there was no association between atopic wheeze and endotoxin exposure for the whole population ($p = 0.8$) or for individual genotypes ($p > 0.4$). For nonatopic wheeze, there was no association between endotoxin exposure and wheeze for the whole population ($p = 0.2$), but in children with CC genotype, increasing endotoxin was associated with an increase in wheeze (OR, 1.42; 95% CI, 1.01–1.99; $p = 0.04$; Figure 4b). This was not seen for the CT (OR, 1.05; 95% CI, 0.81–1.34; $p = 0.7$) or TT genotypes (OR, 0.97; 95% CI, 0.62–1.52; $p = 0.9$). We found no association between endotoxin exposure and wheeze ever for individual genotypes.

Potential Confounders

We found no association between CD14 genotypes and factors previously shown to be associated with allergic sensitization and wheeze (*see* Table 1).

sCD14

sCD14 was lower among children with the CC genotype ($p = 0.02$; *see* Table 1). There was no linear association between endotoxin exposure and sCD14 level for the whole group ($p = 0.6$) or within genotype groups ($p > 0.2$). There was no association between sCD14 level and allergic sensitization or current wheeze ($p > 0.14$). Children with eczema had lower sCD14 (mean, 1.67 $\mu\text{g/ml}$; 95% CI, 1.59–1.74, vs. mean, 1.78; 95% CI, 1.74–1.82; $p = 0.02$).

DISCUSSION

This study provides clear evidence of the differing effect of domestic endotoxin exposure on allergic sensitization, eczema, and wheeze in children with different CD14 genotypes. Children homozygous for the C allele at position –159 in the promoter region of the *CD14* gene showed a dose-dependent response to endotoxin: high exposures were associated with a decreased risk of allergic sensitization and eczema, and low exposures were associated with an increased risk relative to the rest of the population. However, in the same children, high endotoxin was associated with an increase in nonatopic wheeze. Among the remaining children, there was no effect of endotoxin on sensitization, wheeze, or eczema.

Limitations

One potential limitation is that endotoxin was measured once at age 5 yr, raising a question whether this single level is an appropriate index of early-life exposure (arguably the most critical time). In 70 homes, we also measured early-life endotoxin

load and demonstrated a good correlation between the two measures ($r = 0.54$), similar to that reported by others over a 6-yr period ($r = 0.51$) (37). We also measured mite allergen at birth and at 3 and 5 yr of age and demonstrated the same relationship between exposure and mite sensitization when we used allergen levels at the individual time points or cumulative level. Thus, when assigning “index of exposure” to an environmental factor with huge between-individual variability within a population ($\sim 200,000$ -fold for endotoxin in our study), comparatively small within-individual variability due to timing of the measurement is outweighed by large between-individual differences. We therefore argue that endotoxin exposure measured at age 5 yr is a good estimate of early-life exposure. The levels of endotoxin in our study were similar to those in the nonfarming households but lower compared with the farming households in the ALEX (ALlergy and EndotoXin) study (25). The reported relationships seemed to be specific for endotoxin and were not observed when we repeated the analysis using total weight of dust.

CD14 contributes not only to TLR4-mediated recognition of endotoxin (more generally, Toll-like receptor-4 [TLR4] ligands) but is also involved in the detection of gram-positive and other products through TLR2 (38). Thus, endotoxin may act as a surrogate marker for a loosely related ligand rather than the CD14 ligand itself, which may be an alternative explanation for the lack of consistency between studies.

We have investigated the effect of a single SNP in the promoter region of the CD14 gene (CD14/–159 C to T; rs2569190). It is possible that the effects seen are a result of linkage disequilibrium between this and other SNPs in this gene or in the surrounding region. A further line of investigation might be to study the effects of maternal CD14 genotype, endotoxin exposure during pregnancy, and allergic outcomes in offspring.

Although retention in this population-based observational birth cohort is excellent, we analyzed data from approximately half of the subjects (mostly because some children refused venipuncture). There was no difference between children excluded or included in the analysis in any relevant parameter. Furthermore, the prevalence of allergic sensitization among the parents of the children is similar to that of young adults in the United Kingdom (39), suggesting the subjects are representative of the general population.

“Endotoxin Switch”

In 2003, Vercelli proposed the “endotoxin switch,” suggesting a bimodal relationship between environmental exposures to bacterial products and immune responses (being Th2-like at low and Th1-like at high exposures) (4). Our data support this hypothesis. We identified an endotoxin load above which children with CC genotype were at a reduced risk of sensitization relative to the remainder of the population. Immune responses to endotoxin in different CD14/–159 genotypes may be represented as a series of parallel curves positioned at different points along the bacterial load axis, implying that the dose of endotoxin at

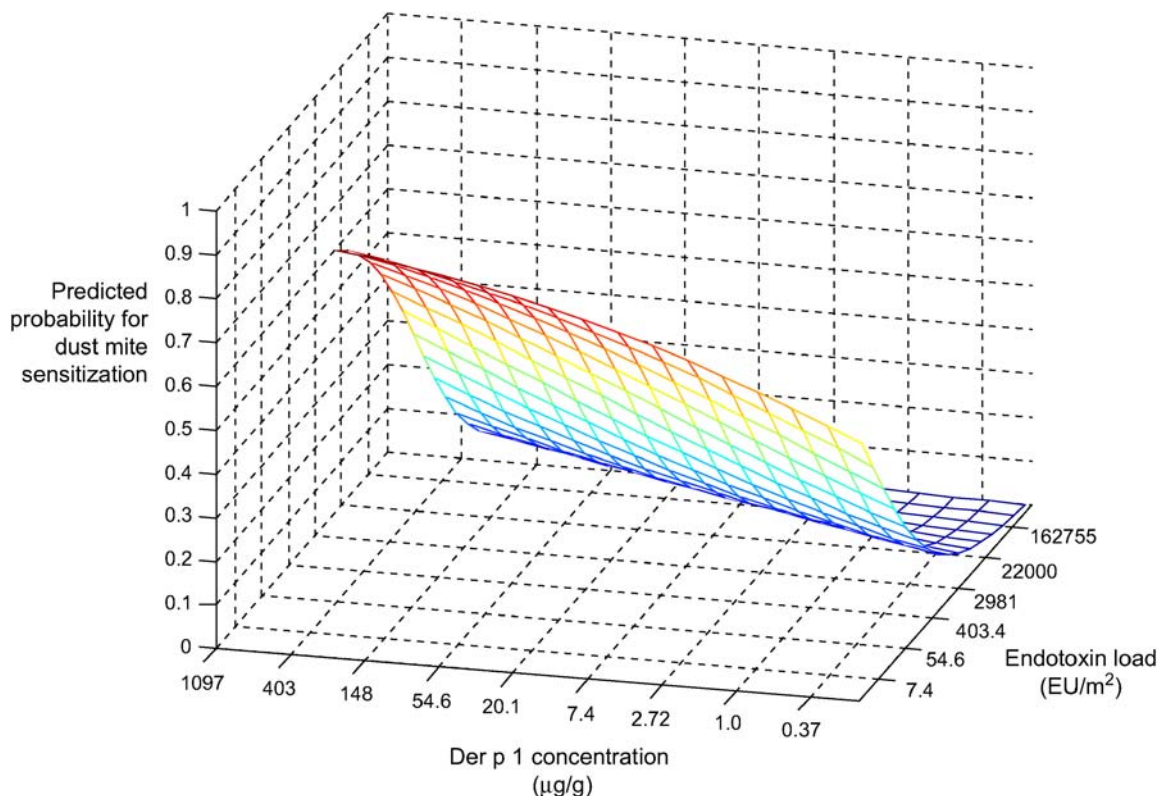


Figure 3. Fitted predicted probability for sensitization to mite at 5 yr of age in relation to environmental endotoxin load and Der p 1 exposure in children with CC genotype in the promoter region of the CD14 gene (CD14/–159 C to T) derived from the logistic regression analysis.

which they switch from a Th2 to a Th1 response is shifted in different genotypes (4). Rather than identifying the parallel response curves at differing endotoxin loads for the three CD14/–159 genotypes, our data suggest that the difference between genotypes in the relationship between endotoxin load and immune response is the gradient (not the position) of the dose–

response curve. This may suggest that individuals with TT and CT genotypes are much less susceptible to the effect of endotoxin compared with C-allele homozygotes.

In most studies that reported an association between CD14/–159 genotype and severity of sensitization, the increase in severity was associated with the C allele (10, 11). In contrast, a

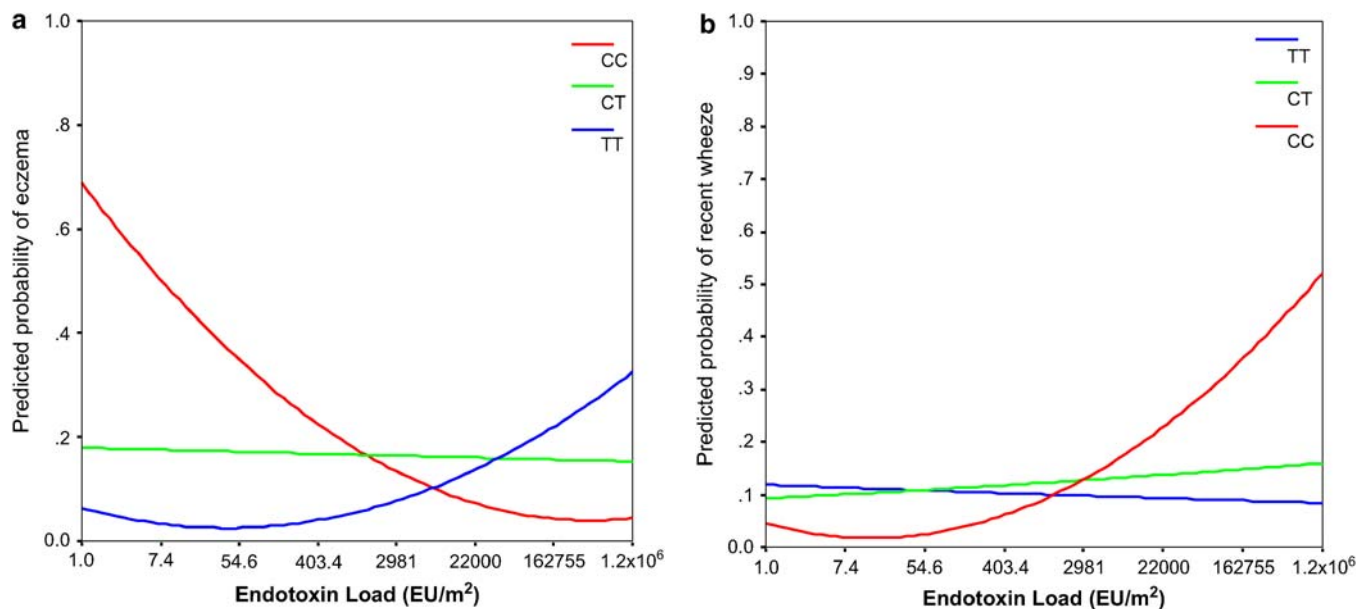


Figure 4. (a) Fitted predicted probability curves for eczema at 5 yr of age in relation to environmental endotoxin load in children with CC, CT, and TT genotypes in the promoter region of the CD14 gene (CD14/–159 C to T) derived from the logistic regression analysis. (b) Fitted predicted probability curves for current nonatopic wheeze at 5 yr of age in relation to environmental endotoxin load in children with CC, CT, and TT genotypes in the promoter region of the CD14 gene (CD14/–159 C to T) derived from the logistic regression analysis.

study in the Hutterites of a farming community likely to be exposed to high endotoxin levels (40) suggested that the T allele was associated with sensitization (18). Extrapolating from our results, in populations with high endotoxin exposure, CC homozygotes would be at reduced risk of sensitization relative to the T-allele carriers, making this the risk genotype. In studies where the C allele conferred increased risk, it is likely that the population exposures to endotoxin were generally lower. Our data can explain how, in cross-sectional studies, the same genotype may confer an increase, a decrease, or no effect on sensitization, dependent on the population endotoxin exposure. A recent family-based study of adults with asthma from Barbados suggested that T-allele homozygotes were less likely to have asthma as a group. However, when stratified by endotoxin exposure (highest quartile vs. rest), T-allele homozygotes with high exposure were at increased risk compared with the CC genotype group (albeit in a small number of subjects) (41). This finding is in agreement with our data. Further confirmation of this important effect was reported in children of the ALEX study, where carriers of the C allele exposed to the highest tertile of endotoxin had less allergic sensitization (42). Although the studies are of different design, there are important similarities replicating the findings.

Although we confirmed previous reports of lower sCD14 in the CC genotype subgroup (10), we found no effect of endotoxin on sCD14, suggesting that the mechanism by which endotoxin exposure affects sensitization is not by altering sCD14 levels. As well as sCD14, in humans CD14 is expressed on the membrane of monocytes and macrophages (mCD14). Studies of the effect of mutations on the function of CD14 protein as an LPS receptor suggest that the critical regions may be different for the membrane and the soluble form of CD14 (43). The relationship between the density of mCD14 on monocytes and CD14 genotype is also variable (44–46). Therefore, our findings of an effect of endotoxin on allergic sensitization in the CC genotype group may be mediated by complex effects on the function of membrane-bound CD14.

Environmental Exposures and Mite-specific Sensitization

A dose–response relationship between mite allergen exposure and specific sensitization has been shown for some populations (47) but not others (48). In our study, increasing allergen exposure was associated with increased risk of sensitization, but the size of this effect was dampened at higher endotoxin exposure. When the analysis was repeated by genotype, this complex relationship was seen only among children with CC genotype at CD14/–159. Thus, the development of allergen-specific sensitization is influenced not only by allergen exposure, but also by other environmental exposures (e.g., endotoxin) and genetic predisposition. We found no such effect for cat and dog sensitization and exposure to the respective allergens (data not shown). This may be a consequence of lower rates of sensitization to pet allergens (cat, 9%; dog, 5.9%).

Symptomatic Disease

Similar to the relationship between endotoxin and sensitization, eczema was decreased in children with the CC genotype exposed to high endotoxin levels. We confirmed eczema by clinical examination rather than by relying only on parental reports of itchy rash.

In marked contrast to the findings for eczema, nonatopic wheeze was increased in children with the CC genotype with increasing endotoxin exposure. No such association was seen for atopic wheeze. Others have also reported an increase in nonatopic wheeze with increasing endotoxin exposure (25). The hygiene hypothesis implicates reduced exposure to microbials as a cause for the increase in prevalence of allergy (49). The

results of the current study may help explain why this hypothesis is more consistent in its association with allergic sensitization than with wheeze and asthma (50).

CONCLUSIONS

One aim of epidemiologic studies is to identify potentially modifiable risk factors and to design primary prevention strategies for asthma and allergic diseases. Success in the identification of genetic polymorphisms that confer an increase in risk for sensitization and asthma has been limited. This reflects the difficulties in phenotype definition and the fact that the relevant environmental exposures have rarely been taken into account. We have shown that exposure to a specific environmental factor (endotoxin) may be relevant only in individuals with a particular genetic polymorphism (CC genotype for CD14/–159). Therefore, within the context of primary prevention strategies, only individuals with particular genotypes may benefit from an intervention directed at an increase in endotoxin (or microbial) exposure. Primary prevention requires the development of tailor-made measures targeting subjects with specific susceptibilities who will benefit from a particular intervention rather than blanket advice aimed at the whole population. Thus, in assessing the impact of genetic factors on complex disease, it is imperative not to investigate genetic factors in isolation but to consider potential gene–environment interactions.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank the children and parents of the Manchester Asthma and Allergy Study for their participation; Bridget Simpson and Patricia Kissen for the clinical follow-up of the cohort; Dr. Gael Tavernier for the endotoxin measurements; Mark Craven, M.Sc., for Der p 1 measurement; and Julie Morris, M.Sc., and Lars Soderstrom for help in the statistical analysis.

References

1. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, Campbell J, Woodcock A. NAC Manchester Asthma and Allergy Study (^{NAC}MAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001;31:391–399.
2. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990; 42:1351–1358.
3. Baldini M, Vercelli D, Martinez FD. CD14: an example of gene by environment interaction. *Allergy* 2002;57:188–192.
4. Vercelli D. Learning from discrepancies: CD14 polymorphisms, atopy and the endotoxin switch. *Clin Exp Allergy* 2003;33:153–155.
5. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Friedhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH. Linkage analysis of I14 and other chromosome 5q31.1 markers and total serum IgE concentrations. *Science* 1994;264:1152–1156.
6. Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. *Science* 1988;239:497–500.
7. Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M, Glauser MP, Tobias PS, Ulevitch RJ. CD14 is a pattern recognition receptor. *Immunity* 1994;1:509–516.
8. Heinzel FP, Kerko RM, Ling P, Hakimi J, Schoenhaut DS. Interleukin 12 is produced in vivo during endotoxaemia and stimulates synthesis of gamma interferon. *Infect Immun* 1994;62:4244–4249.
9. Trinchieri G, Wysocka M, D'Andrea A, Rengaraju M, Aste-Amezaga M, Kubin M, Valiant NM, Chehimi J. Natural killer cell stimulatory factor (NKSF) or interleukin-12 is a key regulator of immune response and inflammation. *Prog Growth Factor Res* 1992;4:355–368.
10. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum IgE. *Am J Respir Cell Mol Biol* 1999;20:976–983.
11. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, Postma DS, Bleeker ER. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163:965–969.

12. O'Donnell AR, Toelle BG, Marks GB, Hayden CM, Laing IA, Peat JK, Goldblatt J, Le Souef PN. Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years. *Am J Respir Crit Care Med* 2004;169:615-622.
13. Leung TF, Tang NL, Sung YM, Li AM, Wong GW, Chan IH, Lam CW. The C-159T polymorphism in the CD14 promoter is associated with serum total IgE concentration in atopic Chinese children. *Pediatr Allergy Immunol* 2003;14:255-260.
14. Sharma M, Batra J, Mabalirajan U, Goswami S, Ganguly D, Lahkar B, Bhatia NK, Kumar A, Ghosh B. Suggestive evidence of association of C-159T functional polymorphism of the CD14 gene with atopic asthma in northern and northwestern Indian populations. *Immunogenetics* 2004;56:544-547.
15. Sengler C, Haider A, Sommerfeld C, Lau S, Baldini M, Martinez F, Wahn U, Nickel R; German Multicenter Allergy Study Group. Evaluation of the CD14 C-159 T polymorphism in the German Multicenter Allergy Study Cohort. *Clin Exp Allergy* 2003;33:166-169.
16. Kabesch M, Hasemann K, Schickinger V, Tzotcheva I, Bohnert A, Carr D, Baldini M, Hackstein H, Leupold W, Weiland SK, et al. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. *Allergy* 2004;59:520-525.
17. Gao PS, Mao XQ, Baldini M, Roberts MH, Adra CN, Shirakawa T, Holt PG, Martinez FD, Hopkin JM. Serum total IgE levels and CD14 on chromosome 5q31. *Clin Genet* 1999;56:164-165.
18. Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet* 2000;67:1154-1162.
19. Litonjua AA, Belanger K, Celedon JC, Milton DK, Bracken MB, Kraft P, Triche EW, Sredl DL, Weiss ST, Leaderer BP, et al. Polymorphisms in the 5' region of the CD14 gene are associated with eczema in young children. *J Allergy Clin Immunol* 2005;115:1056-1062.
20. Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, Neaville WA, Carlson-Dakes K, Adler K, Hamilton R, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004;113:307-314.
21. LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, Vercelli D. A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001;167:5838-5844.
22. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, Liu AH. Relation between house dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-1683.
23. Gehring U, Bolte G, Borte M, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. The LISA study group. Lifestyle-related factors on the immune system and the development of allergies in childhood: exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol* 2001;108:847-854.
24. Phipatanakul W, Celedon JC, Raby BA, Litonjua AA, Milton DK, Sredl D, Weiss ST, Gold DR. Endotoxin exposure and eczema in the first year of life. *Pediatrics* 2004;114:13-18.
25. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, et al.; the Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school age children. *N Engl J Med* 2002;347:869-877.
26. Botcher MF, Bjorksten B, Gustafson S, Voor T, Jenmalm MC. Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003;33:295-300.
27. Bolte G, Bischof W, Borte M, Lehmann I, Wichmann HE, Heinrich J. Early endotoxin exposure and atopy development in infants: results of a birth cohort study. *Clin Exp Allergy* 2003;33:770-776.
28. Simpson A, Custovic A, Woodcock A, Niven R, Jury F, Ollier WER, John S. CD14 polymorphisms and endotoxin exposure interact in the development of allergic sensitization. *Am J Hum Genet* 2004;75(Suppl):24.
29. Simpson A, John S, Jury F, Niven R, Woodcock A, Ollier W, Custovic A. CD14 polymorphisms, endotoxin and Der p1 exposures and sensitization to dust mite in the Manchester Asthma and Allergy Study. *J Allergy Clin Immunol* 2005;115:S61.
30. Custovic A, Simpson BM, Murray CS, Lowe L, Woodcock A. The National Asthma Campaign Manchester Asthma and Allergy Study. *Pediatr Allergy Immunol* 2002;13:32-37.
31. Lowe L, Murray C, Custovic A, Simpson BM, Kissen P, Woodcock A. Specific airway resistance in three year old children. *Lancet* 2002;359:1904-1908.
32. Simpson A, Simpson B, Custovic A, Woodcock A. Stringent environmental control in pregnancy and early life: the long term effects on mite, cat and dog allergen. *Clin Exp Allergy* 2003;33:1183-1189.
33. Pearce N, Weiland S, Keil U, Langridge P, Anderson HR, Strachan D, Bauman A, Young L, Gluyas P, Ruffin D. Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. *Eur Respir J* 1993;6:1455-1461.
34. Williams HC, Burney PGJ, Pembroke AC, Hay RJ. Validation of the UK diagnostic criteria for atopic dermatitis in a population setting. *Br J Dermatol* 1996;135:12-17.
35. Tavernier GO, Fletcher GD, Francis HC, Oldham LA, Fletcher AM, Blacklock G, Stewart L, Gee I, Watson A, Frank TL, et al. Endotoxin exposure in asthmatic children and matched healthy controls: results of IPEADAM study. *Indoor Air* 2005;15:25-32.
36. Custovic A, Simpson BM, Simpson A, Kissen P, Woodcock A. Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during the first year of life: a randomised trial. *Lancet* 2001;358:188-193.
37. Topp R, Wimmer K, Fahlbusch B, Bischof W, Richter K, Wichmann HE, Heinrich J; INGA Study Group. Repeated measurements of allergens and endotoxin in settled house dust over a time period of 6 years. *Clin Exp Allergy* 2003;33:1659-1666.
38. Manukyan M, Triantafyllou K, Triantafyllou M, Mackie A, Nilsen N, Espevik T, Wiesmuller KH, Ulmer AJ, Heine H. Binding of lipopeptide to CD14 induces physical proximity of CD14, TLR2 and TLR1. *Eur J Immunol* 2005;35:911-921.
39. Broadfield E, McKeever TM, Scrivener S, Venn A, Lewis SA, Britton J. Increase in the prevalence of allergen skin sensitization in successive birth cohorts. *J Allergy Clin Immunol* 2002;109:969-974.
40. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230-1234.
41. Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, Beaty TH, Barnes KC. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005;115:1203-1209.
42. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, Nowak D, Martinez FD; the Allergy and Endotoxin (ALEX) Study Team. Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *J Allergy Clin Immunol* 2005;116:601-607.
43. Viriyakosol S, Mathison JC, Tobias PS, Kirkland T. Structure-function analysis of CD14 as a soluble receptor for LPS. *J Biol Chem* 2000;275:3144-3149.
44. Heeson M, Blomeke B, Shulter B, Heussen N, Rossaint R, Kunz D. Lack of association between -260 C to T promoter polymorphism of the CD14 gene and the CD14 density of unstimulated human monocytes and sCD14 plasma levels. *Intensive Care Med* 2001;27:1770-1775.
45. Hubacek JA, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R, Schmitz G. C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999;99:3218-3220.
46. Ito D, Murata M, Tanahashi N, Sato H, Sonoda A, Saito I, Watanabe K, Fukuuchi Y. Polymorphism in the promoter of lipopolysaccharide receptor CD14 and ischemic cerebrovascular disease. *Stroke* 2000;31:2661-2664.
47. Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, Wahn U. Early exposure to house dust mite and cat allergens and development of childhood asthma: a cohort study. *Lancet* 2000;356:1392-1397.
48. Burr ML, Limb ES, Maguire MJ, Amarah L, Eldridge BA, Layzell JC, Merrett TG. Infant feeding, wheezing, and allergy: a prospective study. *Arch Dis Child* 1993;68:724-728.
49. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259-1260.
50. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis." *Thorax* 2000;55:S2-S10.

**ENDOTOXIN EXPOSURE, CD14, AND ALLERGIC DISEASE: AN
INTERACTION BETWEEN GENES AND THE ENVIRONMENT**

Angela Simpson, MD, Sally L. John, PhD, Francine Jury, MSc, Rob Niven, MD, Ashley
Woodcock, MD, William E.R. Ollier, PhD, Adnan Custovic, MD, PhD

ONLINE SUPPLEMENT

INTRODUCTION

The published studies investigating the relationship between CD14/-159 CtoT SNP and asthma and allergy outcomes are summarised in Table E1. Those suggesting the C allele is the risk allele are listed first, followed by those suggesting no association, then those suggesting the T allele is the risk allele. Finally are the studies that have looked for interactions.

METHODS

The Manchester Asthma and Allergy Study is a prospective study which follows the development of asthma and other atopic disorders in a cohort of infants. The setting is the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, comprising of 50 square miles of South Manchester and Cheshire, UK, a stable mixed urban-rural population. Study was approved by the Local Research Ethics Committee. Informed consent was obtained from all parents.

Screening & Recruitment

All pregnant women were screened for eligibility at 'Booking' antenatal visits (8th-10th week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin prick testing was obtained. Both parents completed a questionnaire about their and their partner's history of asthma and allergic diseases and smoking habits.

If the pregnant woman's partner was not present at the antenatal clinic visit, an invitation was sent for him to attend an open-access evening clinic at Wythenshawe Hospital for skin prick testing and questionnaire. Once both parents had completed questionnaires and skin prick testing, a full explanation of the proposed future follow-up for the child

was given. Of the 1499 couples who met the inclusion criteria (≤ 10 weeks of pregnancy, maternal age ≥ 18 years, questionnaire and skin test data available for both parents), 288 declined to take part in the study. Of 1211 couples who initially agreed to take part, 1085 had a successful full-term pregnancy (>36 weeks gestation) and gave consent to a further follow-up. Of those, 128 were prenatally randomized into an environmental control group (see below), and 957 were followed as an observational arm of the cohort.

Allocation to study groups

Children were assigned to risk groups according to parental sensitisation status. Nested within the cohort is a primary prevention study in which a subgroup of children at high risk (both parents atopic) without pets in home was randomised to either stringent environmental control (allergen impermeable covers for the parental and infant bed, hot washing of bedding weekly, HEPA vacuum cleaner, hard floor for the nursery) or a normal regime. Children randomized to the environmental control group were excluded from this analysis.

Follow-up

The children have been followed prospectively, and attended review clinics at age 3 and 5 years (± 4 weeks). We completed assessment at age 5 years in April 2003. The overall follow-up rate during the first five years of life in the observational part of the cohort was exceptionally high (874/957 [91.3%] age 3 years; 840/957 [87.8%] age 5 years).

Outcomes

Clinical assessment: A validated questionnaire^{E1} was interviewer-administered to collect information on parentally-reported symptoms, physician-diagnosed illnesses and treatments received. Children were defined as having recent wheeze if they had wheezed

within the previous 12 months. Physical examination was conducted in a standardized manner and eczema defined using the UK diagnostic criteria^{E2}.

Allergic sensitization: We measured specific serum IgE (mite, cat, dog, grass, milk, egg and peanut; ImmunoCAPTM, Pharmacia, Uppsala, Sweden) and defined sensitization as specific IgE >0.2 kU/L to any allergen. We also skin prick tested the children (*D pteronyssinus*, cat, dog, grasses, moulds, milk, egg [Bayer, Elkhart, IN, USA]) and defined sensitization as a weal diameter 3mm greater than negative control.

Environmental exposures

We collected 2 living-room floor dust samples by vacuuming a 1m² area using a Medivac dust sampler (airflow 45 l/s; Medivac Plc, Wilmslow, Cheshire, UK) for 2 minutes in a standardised fashion. The sampling head was loaded with a stainless steel mesh filter, to remove particles >300µm diameter, allowing a sample of fine dust to be collected onto a 5 µm pore size vinyl filter (Plastok Associates Ltd, Wirral, UK). Immediately after collection, the dust sample was transferred into pre-weighed petri dishes and coded. The filled petri dish was weighed to calculate the mass of fine dust collected and stored at 4°C until extraction. After each sample collection the sampling head was cleaned using 70% isopropyl alcohol.

Endotoxin content was measured by a kinetic limulus assay^{E3-4}. Briefly, 50 mg of fine dust were extracted by shaking in pyrogen-free tubes with 5-ml endotoxin-free water (both Charles River Endosafe) for 1hr at room temperature. Following centrifugation (3000 rpm, 30 min RT), aliquots (100 µl, 1:100-1:100,000) were transferred to a microtiter plate (96 wells, Becton Dickinson Labware) and 100 µl LAL (Limulus Amebocyte Lysate) were added. Optical densities were read every 30s at 405 nm for 1 hr

(BIO-TEK Instruments). Results were calculated from a standard curve run on each plate using Biolise software (Charles River Endosafe). Results were expressed as endotoxin load (EU/m²) and level (EU/mg). Mite allergen was measured using enzyme-linked immunoassay. Results were expressed as allergen concentration (µg/g).

For measurement of Der p 1 content, a 100 mg aliquot of the dust was extracted by rotation with 2 ml borate-buffered saline with 0.1% Tween 20 pH 8.0, at room temperature for 2 hours before being centrifuged for 20 minutes at 2500 rpm at 4°C. The supernatant was stored at -20°C until analysed for allergen content.

Der p 1 level was measured using a two-site monoclonal antibody based ELISA (Indoor Biotechnologies, Chester, UK). The standard used to establish the control curve for Der p 1 assay (UVA 93/02) was considered to contain 2500 ng Der p 1/ml (relative to WHO/IUIS *D. pteronyssinus* standard NIBSC 82/518 which has been estimated to contain 12.5 µg Der p 1 per ampoule). Doubling dilutions of this standard extract were used to construct a control curve with each individual assay; the dilutions used were from 250-0.5 ng/ml. Results were then calculated as µg Der p 1 per gram of fine dust collected (µg/g) – allergen concentration.

Genotyping methods

A SNP found in the promoter region of CD14 (-159 T>C RS2569190) was selected for genotyping in the MAAS asthma cohort. A fluorescence-based primer extension method (SNaPshot™ (ABI, Warrington, UK) was used to genotype the SNP on the ABI Prism® 3100 Genetic Analyser. A set of PCR primers and probes were selected for the assay with 3' terminus of the probe terminating directly adjacent to the SNP and extended by a single fluorescently labelled ddNTP. PCR's were performed in 5µl reaction volumes in

384 well plates and contained 10ng genomic DNA (isolated from whole blood) per reaction. The forward probe was used for the post PCR extension step. Genotypes were validated on a subset of the total cohort using the reverse probe.

SNAPSHOT

CD14F	TTAGGCTCCCGAGTCAACAG
CD14R	GATCACCTCCCCACCTCTC
CD14Fpro	CCTGCAGAATCCTTCCTGTTACGG
CD14Rpro	CAATGAAGGATGTTTCAGGGAGGGGG

The SNP is a T>C in its forward orientation.

RESULTS

Allergic sensitization

The analysis was repeated using quintiles of endotoxin and allergic sensitisation using chi square test for each genotype group separately. For the TT and CT genotype group there was no association between quintile of endotoxin exposure and sensitisation ($p=0.68$, $p=0.50$ respectively). For the CC genotype group those exposed to higher quintiles of endotoxin were less likely to be sensitised ($p=0.005$) as illustrated in Figure E1.

Eczema

The analysis was repeated using quintiles of endotoxin and eczema present in clinic using chi square test for each genotype group separately. For the TT and CT genotype group there was no association between quintile of endotoxin exposure and sensitisation ($p=0.44$, $p=0.71$ respectively). For the CC genotype group those exposed to higher quintiles of endotoxin were less likely to be sensitised ($p=0.019$) as illustrated in Figure E2.

In addition we present the data for non-atopic wheeze (Figure E3)

REFERENCES

- E1. J Pearce N, Weiland S, Keil U et al. Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. *Eur Respir J*. 1993;6:1455-61.
- E2. Williams HC, Burney PGJ, Pembroke AC, Hay RJ. Validation of the UK diagnostic criteria for atopic dermatitis in a population setting *Br J Dermatol* 1996;135:12-17
- E3. F. Cooper, M. E. Weary and F. T. Jordan. The impact of non-endotoxin LAL-reactive materials on *Limulus* Amebocyte Lysate analysis. *PDA Journal of Pharmaceutical Science and Technology*, 51: 2-6, 1997.
- E4. J. Levin and F. Bang. Clotable protein in *Limulus*: its localization and kinetics of its coagulation by endotoxin. *Thromb Diath Haemorrh*, 19: 186, 1968.
- E5. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum IgE. *Am J Respir Cell Mol Biol* 1999;20:976-83
- E6. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, Postma DS, Bleecker ER. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163:965-969
- E7. Kabesch M, Hasemann K, Schickinger V, Tzotcheva I, Bohnert A, Carr D, Baldini M, Hackstein H, Leupold W, Weiland SK, Martinez FD, Mutius E, Bein G. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. *Allergy* 2004;59:520-5.

- E8. O'Donnell AR, Toelle BG, Marks GB, Hayden CM, Laing IA, Peat JK, Goldblatt J, Le Souef PN. Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years *Am J Respir Crit Care Med*. 2004;169:615-22
- E9. Heinzmann A, Dietrich H, Jerkic SP, Kurz T, Deichmann KA. Promoter polymorphisms of the CD14 gene are not associated with bronchial asthma in Caucasian children. *Eur J Immunogenet*. 2003;30:345-8.
- E10. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C-->T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol*. 2003;112:438-44
- E11. Sengler C, Haider A, Sommerfeld C, Lau S, Baldini M, Martinez F, Wahn U, Nickel R; German Multicenter Allergy Study Group. Evaluation of the CD14 C-159 T polymorphism in the German Multicenter Allergy Study Cohort. *Clin Exp Allergy* 2003;33:166-169
- E12. Wang JY, Wang LM, Lin CG, Chang AC, Wu LS. Association study using combination analysis of SNP and STRP markers: CD14 promoter polymorphism and IgE level in Taiwanese asthma children. *J Hum Genet*. 2005;50:36-41
- E13. Sharma M, Batra J, Mabalirajan U, Goswami S, Ganguly D, Lahkar B, Bhatia NK, Kumar A, Ghosh B. Suggestive evidence of association of C-159T functional polymorphism of the CD14 gene with atopic asthma in northern and northwestern Indian populations. *Immunogenetics*. 2004;56:544-7

- E14. Buckova D, Holla LI, Schuller M, Znojil V, Vacha J. Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy. *Allergy*. 2003 Oct;58(10):1023-6.
- E15. Leung TF, Tang NL, Sung YM, Li AM, Wong GW, Chan IH, Lam CW. The C-159T polymorphism in the CD14 promoter is associated with serum total IgE concentration in atopic Chinese children. *Pediatr Allergy Immunol*. 2003;14:255-60.
- E16. Gao PS, Mao XQ, Baldini M, Roberts MH, Adra CN, Shirakawa T, Holt PG, Martinez FD, Hopkin JM. Serum total IgE levels and CD14 on chromosome 5q31 *Clin Genet*. 1999;56:164-5.
- E17. Litonjua AA, Belanger K, Celedon JC, Milton DK, Bracken MB, Kraft P, Triche EW, Sredl DL, Weiss ST, Leaderer BP, Gold DR. Polymorphisms in the 5' region of the CD14 gene are associated with eczema in young children. *J Allergy Clin Immunol*. 2005 May;115(5):1056-62.
- E18. Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, Beaty TH, Barnes KC. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol*. 2005 Jun;115(6):1203-9
- E19. Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, Neaville WA, Carlson-Dakes K, Adler K, Hamilton R, Anderson E, Gilbertson-White S, Tisler C, Dasilva D, Anklam K, Mikus LD, Rosenthal LA, Ober C, Gangnon R, Lemanske RF Jr. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol*. 2004 ;113:307-14.

- E20. Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet.* 2000;67:1154-62
- E21. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, Nowak D, Martinez FD; Allergy And Endotoxin Alex Study Team. Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *J Allergy Clin Immunol.* 2005 ;116:601-7.
- E22. Choudhry S, Avila PC, Nazario S, Ung N, Kho J, Rodriguez-Santana JR, Casal J, Tsai HJ, Torres A, Ziv E, Toscano M, Sylvia JS, Alioto M, Salazar M, Gomez I, Fagan JK, Salas J, Lilly C, Matallana H, Castro RA, Selman M, Weiss ST, Ford JG, Drazen JM, Rodriguez-Cintron W, Chapela R, Silverman EK, Burchard EG. CD14 tobacco gene-environment interaction modifies asthma severity and immunoglobulin E levels in Latinos with asthma. *Am J Respir Crit Care Med.* 2005 ;172:173-82.

LEGEND FOR FIGURE

Figure E1

Bar chart showing percentage of children with allergic sensitisation within each quintile of endotoxin exposure, by genotype group

Figure E2

Bar chart showing percentage of children with eczema present in clinic within each quintile of endotoxin exposure, by genotype group

Figure E3

Bar chart showing percentage of children nonatopic wheeze within each quintile of endotoxin exposure, by genotype group

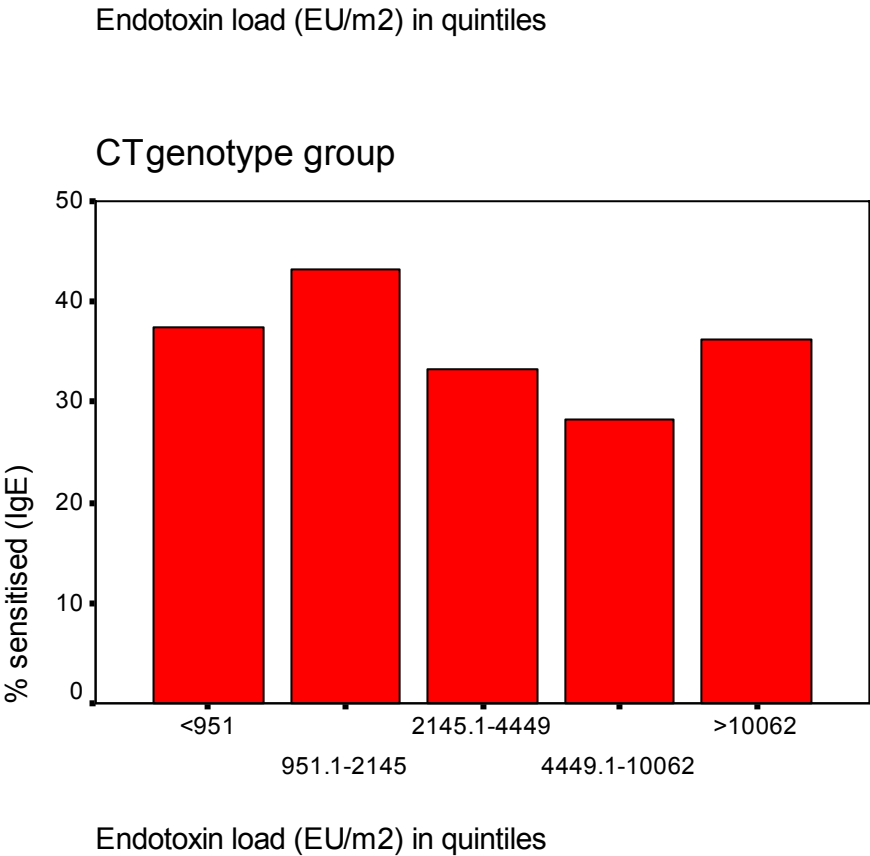
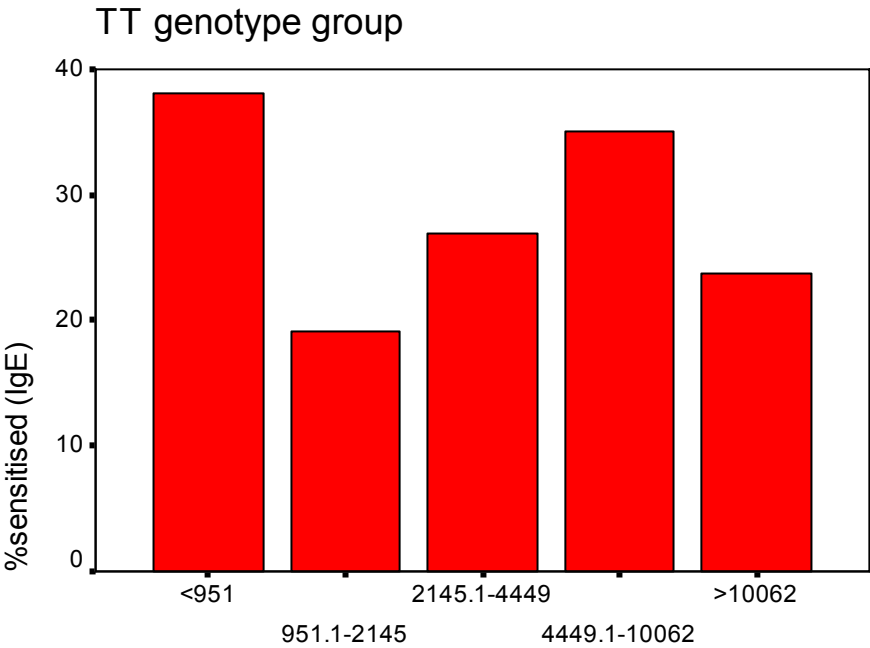
Table E1 Summary of Published Studies on the relationship between CD14/-159 C to T (rs2569190) and asthma and allergy outcome measures

Study	Population	Outcome measure	Association	Comment
Baldini 1999 ^{E5}	Tucson Children's Respiratory Study 481 children from 4 ethnic groups	atopy	No association	TT children with allergies are 'less allergic' and have higher sCD14
		Total IgE in children with positive skin tests	Lower in TT group	
		Number of positive skin tests in atopic children	Fewer in TT group	
Koppelman 2001 ^{E6}	Dutch adults with asthma and their spouses as controls (n=317)	Number of positive skin tests, Total IgE in subjects with positive skin tests	Higher in CC group	
		Hay fever	More common in CC group	
		MD asthma	No association	
Kabesch 2004 ^{E7}	German children from ISAAC study n~2000	Total IgE, number of positive skin tests, asthma, rhinitis, eczema	No association	Higher sCD14 in TT group
O'Donnell 2004 ^{E8}	Cohort of Australian children, n=305, followed from age 8 years to age 25 years	atopy	Associated with CC genotype in childhood but some effect lost in adulthood; CC have increased risk of early onset of atopy	CC associated with atopy and AHR in childhood, with much of the effect lost by adulthood
		AHR	Associated with CC genotype at age 8 years, with trend at other time points	
Heinzmann 2003 ^{E9}	Asthma case control study (n=452 children)	asthma	No association with CD14 genotype	Controls were not tested for asthma. Found total linkage disequilibrium between -159 and -1359 SNPs.

Woo 2003 ^{E10}	Asthma (n=175 adults) and food allergy (n=77 children) and controls (n=61 adults)	Non atopic asthma, food allergy	Higher frequency of T allele relative to controls	Lower rate of T allele in controls than in other published studies (39%)
Sengler 2003 ^{E11}	German children from MAS90 study (n~600)	Asthma, rhinitis, eczema, polysensitisation, IgE	No association	
Wang 2005 ^{E12}	Taiwanese children with asthma (n=190)	high IgE in children with asthma	T allele associated but only if carried on haplotype with microsatellite marker D5S2011	
Sharma 2004 ^{E13}	Case control study of adults with atopic asthma from India (n=414)	Atopic asthma, total IgE	C allele was risk allele Higher in carriers of C allele	
	Trios, n=106 (probands with asthma or allergy)		Preferential transmission of C allele to affected offspring	
Buckova 2003 ^{E14}	Case control Czech adults with asthma or rhinitis (n=882)	Asthma, rhinitis, total IgE, lung function	No association	Found SNP -1359 assoc with number of positive skin prick tests
		Sensitisation to moulds	Associated with C allele	
Leung 2003 ^{E15}	Chinese children asthma case control (n=350)	Total IgE in atopic children	Higher in CC genotype	
		Asthma, allergic sensitisation	Not associated	
Gao 1999 ^{E16}	Japanese (n=200), British (n=300)	Asthma, total IgE	No association	Amongst British skin test negative children total IgE was higher in those with CC genotype

Litonjua 2005 ^{E17}	Birth cohorts, n=379	eczema	Carriers of T allele more likely to have eczema	SNP rs 256913 also associated with eczema. No effect of dog ownership
		Total IgE	Lower in CC group	
Zambelli-Weiner 2005 ^{E18}	Barbados, cases (n=221) with family members as controls (n=230)	asthma	Increased risk with C allele	Only 7 cases in this group, but could be interpreted as at high exposure to endotoxin T allele at increased risk relative to C allele group
		asthma, asthma severity	Increased in TT genotype group with high endotoxin exposure	
Gern 2004 ^{E19}	High risk cohort of children (COAST) N=285	eczema	CD14 genotype associated with eczema TT with dog had lower risk compared to TT without dogs	Do not state which genotype group is a risk for eczema. Only 17 cases of TT with dogs
		Allergic sensitisation, total IgE	No association	
Ober 2000 ^{E20}	Hutterites (n=693)	Allergic sensitisation	Transmission Disequilibrium Test showed T allele to be over transmitted	Hutterites practice a communal agrarian lifestyle
Eder 2005 ^{E21}	Children from Bavaria and Austria (n=624)	Total and specific IgE	No association	The effects of endotoxin and pets were independent
		Allergic sensitisation	C allele protective at high endotoxin, C allele protective in those exposed to stable animals T allele protective in those with dogs/cats	
Choudhry 2005 ^{E22}	684 children with asthma and their families of Mexican and Puerto Rican origin	Total IgE	Lowest in TT genotype group exposed to Environmental tobacco smoke	SNP +1437 associated with asthma severity
		Asthma, total IgE	No association	

Figure E1



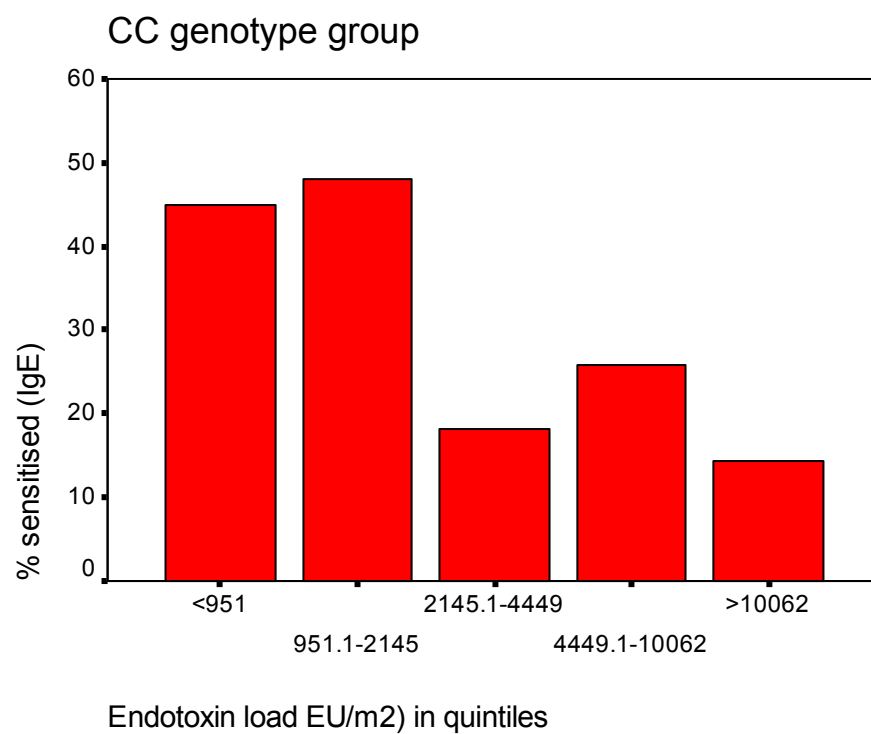
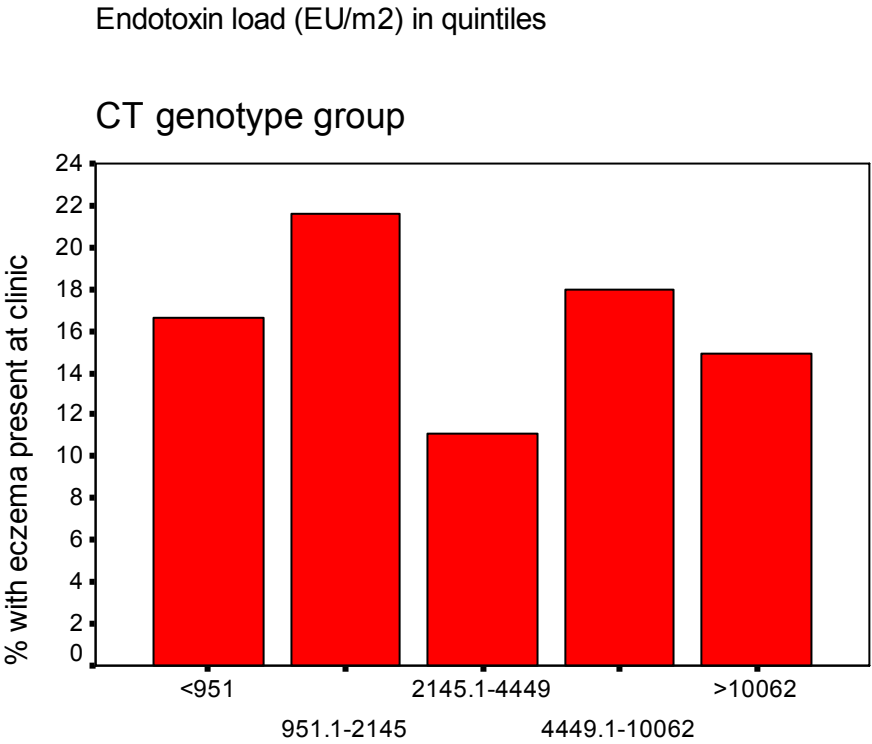
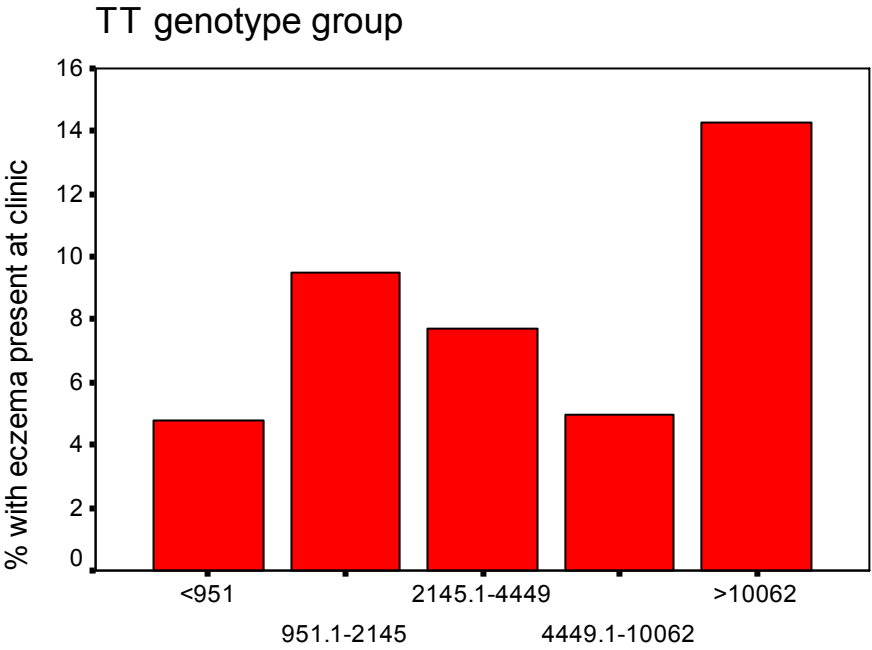


FIGURE E2



Endotoxin load (EU/m2) in quintiles

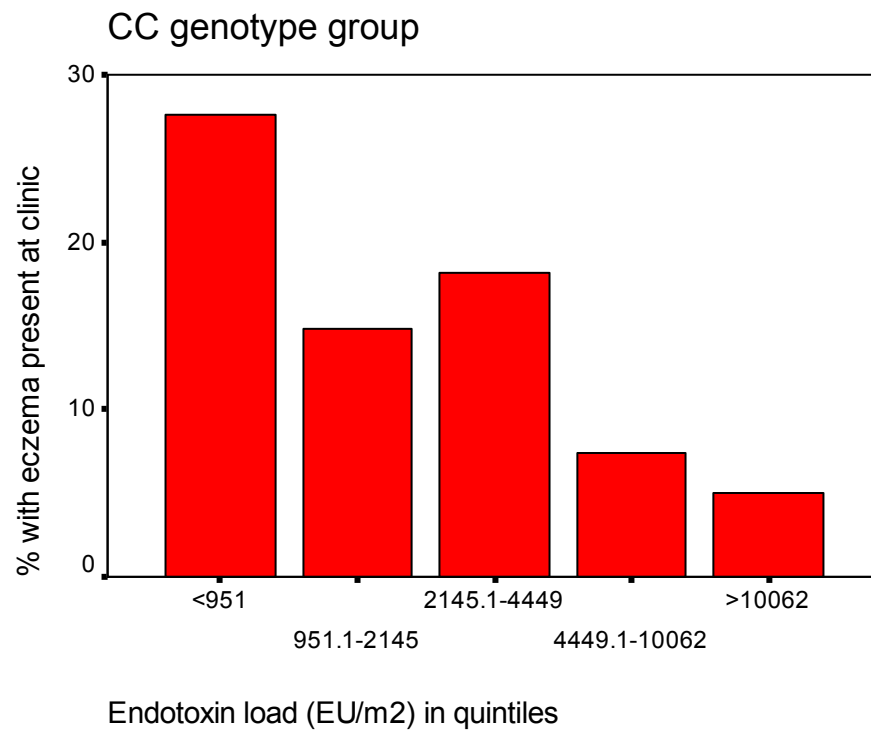
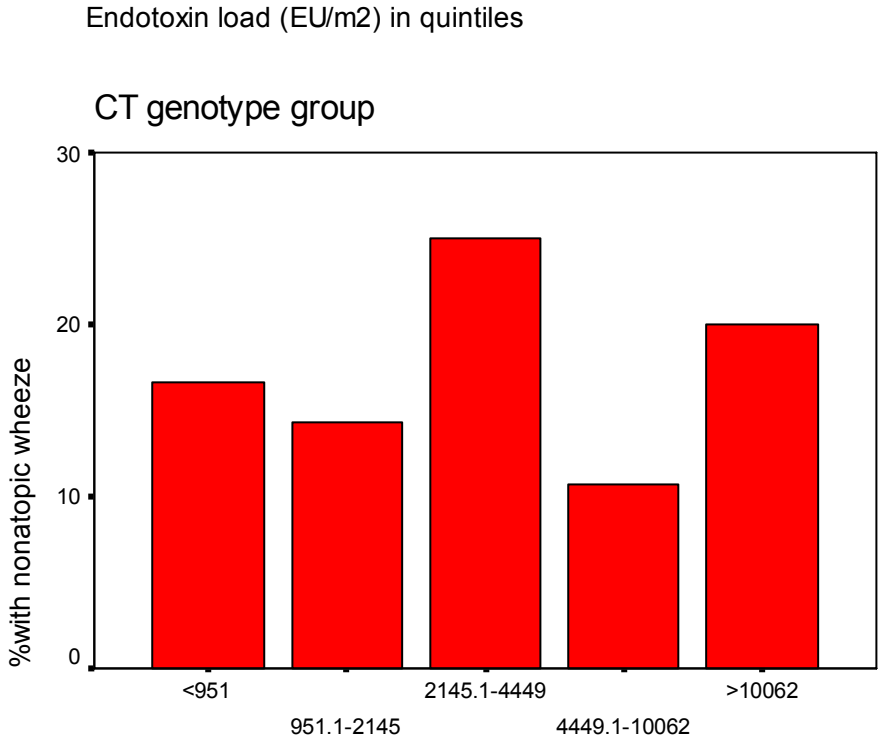
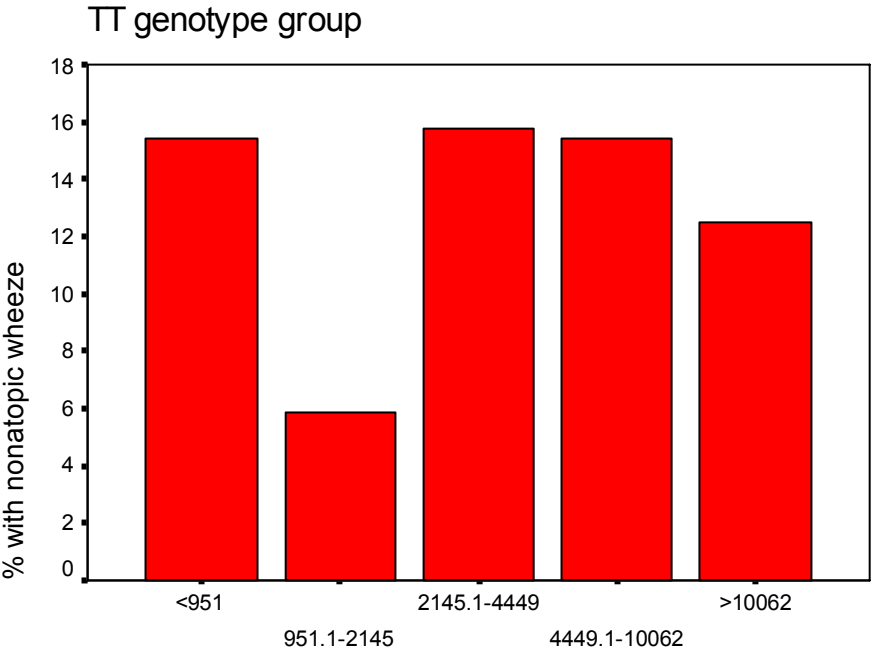


Figure E3



Endotoxin load (EU/m2) in quintiles

