ORIGINAL ARTICLE

Endotoxin exposure, *CD14* and wheeze among farmers: a gene–environment interaction

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ABSTRACT

Objectives Endotoxin-exposed workers are at an increased risk of non-atopic asthma and lung-function decline. Genetic variants may influence susceptibility to these effects. The objective of the present study was to assess whether the association between occupational endotoxin exposure and wheeze is modified by innate immunity gene variants.

Methods Twenty-four single nucleotide polymorphisms (SNPs) in *CD14*, Toll-like receptor 4 (*TLR4*), *TLR2*, *MD2* and *MyD88* were genotyped in 408 agricultural workers with spirometry and questionnaire data on asthma symptoms available. Personal airborne endotoxin exposure levels were estimated in 249 exposure measurements.

Results The association between endotoxin exposure and wheeze was modified by three *CD14* SNPs: -260 C/T (rs2569190), -1247 T/C (rs2569191) and -1721 A/G (rs2915863), and one *MD2* SNP (rs10808798 T/C). In individuals carrying the *CD14* and *MD2* major allele variants, the prevalence of wheeze increased with increasing endotoxin concentration, whereas this was the opposite in minor allele homozygotes. Interaction between endotoxin exposure and genotype was statistically significant under the best-fitting recessive model (p=0.05 to 0.006). Correction for multiple comparisons resulted in marginally significant p values for interaction ($p<0.06$) for *CD14* -260 C/T and -1247 T/C, and for *MD2* rs10808798 T/C. The *CD14* SNPs appeared to modify associations between endotoxin exposure and forced expiratory volume in 1 s in a similar direction (p interaction=0.07 to 0.15).

Conclusions The association between occupational endotoxin exposure and wheeze in agricultural workers was significantly modified by genetic variants in *CD14* and *MD2*. Our study suggests that carriers of the functional *CD14*/-260 C allele are more responsive to endotoxin exposure than T allele homozygotes.

What this paper adds

- It has been shown that *CD14* variants modulate the association between house-dust endotoxin levels and allergic outcomes.
- Innate immunity gene variants may also influence susceptibility to non-atopic asthma and lung-function decline in endotoxin-exposed workers.
- This study found that the association between occupational endotoxin exposure and wheeze in agricultural workers was significantly modified by genetic variants in *CD14* and *MD2*.

INTRODUCTION

Swine farmers, poultry farmers and agricultural processing workers are commonly exposed to very high organic dust and endotoxin levels.[1] Inhalation of organic dust or purified endotoxin (lipopolysaccharide (LPS)) causes an inflammatory response in the airways which is characterised by increased numbers of infiltrating neutrophils and levels of pro-inflammatory cytokines.[2 3] Epidemiological studies have shown an increased risk of non-atopic asthma, chronic obstructive pulmonary disease and lung-function decline among endotoxin-exposed workers.[4–6] Despite this increased risk, most farmers and agricultural workers appear to have a normal forced expiratory volume in 1 s (FEV_1) and do not report airway symptoms, suggesting important individual variation in sensitivity.[7] A differential response to inhaled LPS was evidenced in experimental studies in healthy subjects not exposed to endotoxin at work; a >5% fall in FEV_1 after LPS-containing cotton dust inhalation occurred in more than one-third, whereas almost two-thirds preserved their baseline FEV_1.[8] We have previously shown that ex vivo LPS-induced cytokine release was associated with susceptibility to adverse respiratory effects in a survey among endotoxin-exposed individuals.[7] Heritable differences in susceptibility are likely to account for these findings. For instance, single nucleotide polymorphisms (SNPs) in Toll-like receptor 4 (*TLR4*) and *CD14* have been associated with a differential response to LPS[9–11] and with respiratory health effects in endotoxin-exposed workers.[12–14] However, these studies did not investigate whether the shape and slope of exposure–response relationships differed according to genotype, as was done in studies in domestic settings. The latter studies provided evidence of gene–environment interaction between house dust endotoxin levels and *CD14*/-260 (a functional promoter SNP, also reported as *CD14*/-159) for allergic outcomes.[15–18] Inhalable endotoxin levels measured during a farmer's working day are approximately 100 to 10 000 times higher than levels in the air in domestic settings.[1 19] Therefore, studying an occupational population may provide

new insights in interactions between endotoxin exposure, *CD14*/-260, and variants in other innate immunity genes such as *TLR4* and *TLR2* at clearly higher exposure levels. *TLR2* encodes yet another pattern recognition receptor for bacterial and fungal pro-inflammatory agents that may also be present in organic dust.

Our earlier findings showed positive associations between endotoxin exposure and wheeze in a highly exposed adult population.[20 21] This study aims to assess whether wheeze in this population is associated with SNPs in genes encoding the LPS–receptor complex (*CD14*, *TLR4*, *MD-2*, *MyD88*) and in *TLR2*, and whether the association between endotoxin exposure and wheeze is modified by these SNPs. In addition, we studied whether these SNPs are associated with LPS-induced cytokine production in an ex vivo whole-blood assay.

METHODS

Study population

A cross-sectional study was carried out in 434 Dutch farmers and workers from four agricultural processing industries (onion trade, flower bulb trade, animal feed industry and vegetable seed industry). Recruitment of the study population has been described earlier,[7 20] and a flow diagram is given in the supplementary material. Using questionnaires, data on respiratory symptoms, personal characteristics, smoking habits and farm childhood were collected.[20] Questions about respiratory symptoms were adopted from the Dutch version of the European Community Respiratory Health Survey questionnaire, and current wheezing was defined as wheezing at any time in the last 12 months. Venous blood samples were taken, and spirometry was assessed during a visit to the worksite at the start of the workday[7] and performed according to European Respiratory Society (ERS) standards.[22] Age- and standing-height-adjusted spirometric reference values of the European Community for Steel and Coal were used.[22] Atopy was defined as the presence of specific serum immunoglobulin E antibodies to one or more of the common allergens (house dust mite, grass pollen (mix of timothy and perennial ryegrass), cat and dog), which were determined by enzyme immunoassays as described previously.[21 23] In total, data from 408 out of 434 participating subjects were used (319 agricultural industry workers, 89 farmers); others were excluded because of non-Caucasian race (n=8), age <18 or >65 years (n=4), or because the DNA extraction or genotyping failed (n=14). Bronchial hyperresponsiveness (BHR) was available for a subgroup of 110 workers, and BHR was defined as a fall in FEV_1 of at least 20% at a methacholine dose of 2.5 mg or less as described previously.[21] The study protocol was approved by the institutional ethics committee, and all participants gave written informed consent.

Endotoxin exposure

Endotoxin exposure assessment and modelling have been reported in detail earlier.[20] Briefly, personal airborne endotoxin exposure (249 full-shift measurements) was measured in a sample of 198 participants using Gilian GilAir portable pumps (Gilian, West Caldwell, New Jersey) at a flow rate of 3.5 l/min using GSP sampling heads and 37 mm glass-fibre filters (Whatman GF/A, Maidstone, UK). Filters were extracted in pyrogen-free water with 0.05% Tween 20, and supernatants were analysed by the quantitative kinetic chromogenic Limulus amoebocyte lysate assay. Endotoxin exposure was modelled on the basis of work site and job title. Mixed-effects models were applied for each industry separately, including worker as random effect and job title as fixed effect, to calculate geometric mean exposure levels for different job titles. The resulting job–exposure matrix was combined with the job title of all participating subjects to assign endotoxin exposure. This type of grouping strategy is not very sensitive to the effects of exposure misclassification, and measures of association usually show very little bias.[24] Median endotoxin exposure was 289 endotoxin units (EU)/m^3, and exposure levels ranged from 10 to 10 000 EU/m^3.

Genotyping

We genotyped SNPs in *CD14* (five SNPs), *TLR2* (four SNPs), *TLR4* (nine SNPs), *MyD88* (two SNPs) and *MD2* (five SNPs) (supplementary material, table 1). SNPs were selected based on a literature search for SNPs known to have functional impact or to be associated with asthma or atopy. Furthermore, haplotype tagging SNPs were selected from the HapMap database[25] or from the Innate Immunity website[26] based on the largest number of SNPs with a minor allele frequency >0.1 available. Genotyping was performed as described before[27] by K-biosciences (Hoddesdon, UK) with genotype call rates >96%. All SNPs were in Hardy–Weinberg equilibrium ($p>0.05$), except for *TLR4* rs10759931 ($p<0.001$), which was excluded from analyses.

Whole-blood assay

A whole-blood assay was performed as described previously.[7] Briefly, heparinised whole blood samples of each participant were stimulated with LPS (1 ng/ml) during 18 h at 37°C, 5% CO_2 and 96% relative humidity. Tumour necrosis factor (TNF)α, interleukin (IL)-1β and IL-10 were measured in supernatants using a Bio-plex assay as described before.[28] LPS-induced cytokine production was calculated by subtracting cytokine concentrations in the LPS-free control samples from corresponding LPS-stimulated samples. As in the earlier study, the population was dichotomised into low and high responders according to median cytokine concentrations for each cytokine separately (~0.7, 0.8 and 0.8 ng/ml for TNFα, IL1β and IL10, respectively).[7]

Data analysis

Associations between SNPs and wheeze were assessed by logistic regression analysis. The effect of each genotype was tested under a general, additive, dominant or recessive genetic model, and ORs and 95% CIs were calculated. ORs were adjusted for natural log-transformed endotoxin exposure levels, sex, age, smoking habits and farm childhood, unless stated otherwise. Effect modification of the association between endotoxin exposure and wheezing was tested by inclusion of an interaction term (genotype×endotoxin) in the model. Interaction was tested using the best-fitting genetic model according to the likelihood ratio test. The shape of the relationships between endotoxin and wheeze was explored by employing generalised additive modelling (smoothing). As in our earlier paper that did not take into account the subjects' genetic make-up,[21] the associations seemed log-linear. False-discovery-rate-adjusted p values were calculated to take multiple comparisons (n=24 SNPs) into account.[29] Associations between genotype and cytokine response (high/low responders) were assessed by χ^2 test.

RESULTS

Descriptive characteristics of 51 subjects who reported wheeze and 357 subjects without wheeze are shown in table 1. Higher

Table 1 Characteristics of 408 agricultural workers, stratified by wheeze

	Wheeze	
	No	Yes
Total no of subjects	357	51
Males (%)	87.7	86.3
Age (years), mean±SD	42.0±10.5	40.9±10.7
Smoking habits (%)		
Never	43.0	23.5
Former	31.5	35.3
Current	25.6	41.2*
Farm childhood (%)	46.8	41.2
Endotoxin exposure (endotoxin units/m^3), median (interquartile range)	289 (93 to 720)	335 (219 to 1086)*
Forced expiratory volume in 1 s (% predicted), mean±SD	107.0±13.9	99.7±17.1*
Bronchial hyper-responsiveness (%)	20.8	64.3*
Atopy (%)	17.4	29.4*
Total immunoglobulin E (IU/ml), median (interquartile range)	29 (8 to 91)	71 (11 to 350)*
Interleukin-1β high responders (%)	50.9	49.0
Tumour necrosis factor-α high responders (%)	49.4	57.1
Interleukin-10 high responders (%)	48.8	65.3*

*p<0.05, χ^2 test or t test.
Bronchial hyper-responsiveness was available in a subgroup of 110 workers, and was defined as a fall in forced expiratory volume in 1 s of at least 20% at a methacholine dose of 2.5 mg or less.[21]

endotoxin exposure, current smoking and objectively measured respiratory and allergic outcomes such as presence of bronchial hyper-responsiveness and atopy, lower FEV_1 and higher total immunoglobulin E were significantly associated with a higher prevalence of wheeze. Despite this, more than 70% of the subjects reporting wheeze were non-atopic. None of the *CD14*, *TLR2*, *TLR4*, *MD2* or *MyD88* SNPs were significantly associated with wheeze (adjusted or unadjusted models p>0.05, supplementary material, table 2).

Modification of the association between endotoxin exposure and wheeze by genotype

The association between endotoxin exposure and wheeze was modified by three *CD14* SNPs: -1721 A/G (rs2915863), -1247 T/C (rs2569191) and -260 C/T (rs2569190), and one *MD2* SNP: rs10808798 T/C, intron 1 (table 2). p Values for interaction were statistically significant under the best-fitting recessive model (p=0.05 to 0.006; table 2). Correction for multiple comparisons resulted in marginally significant p values for interaction (p<0.06) for *CD14* -260 C/T and -1247 T/C, and for *MD2* rs10808798 T/C, and abolished the significant interaction of *CD14* -1721 A/G. The *CD14* SNPs were in high linkage disequilibrium (r^2 between 0.74 and 0.99, supplementary material figure II). Logistic regression plots for the association between endotoxin exposure and wheeze are shown for *CD14*/-260 CC/CT subjects and TT subjects (figure 1). Figure 1 shows a clear dose–response curve for endotoxin exposure and wheeze: in individuals with the *CD14*/-260 CT and CC variants, the prevalence of wheeze increased with increasing endotoxin concentration, whereas this was the opposite in those with the TT variant. Similar curves were observed for *CD14* -1721 A/G and -1247 T/C, and for *MD2* rs10808798 T/C (not shown). Adjustment for atopy, or stratification for atopy, resulted in similar findings: among *CD14*/-260 CC/CT subjects, ORs (95% CI) for the association between endotoxin exposure and current wheeze were 1.68 (1.19 to 2.35) for non-atopics and 1.71 (0.98 to 2.97) for atopics, and among *CD14*/-260 TT subjects, 0.58 (0.16 to 2.13) for non-atopics and 0.70 (0.22 to 2.28) for atopics. Endotoxin exposure or the number of job years in the current job was not associated with *CD14* or *MD2* genotype (p>0.5). SNPs in *TLR2*, *TLR4* and *MyD88* did not show any gene–environment interaction with endotoxin exposure (p interaction >0.05, data not shown) and were not considered in further analyses.

Table 2 Association between endotoxin exposure and current wheeze in agricultural workers stratified by *CD14* and *MD2* genotype

Single nucleotide polymorphism	Genotype	No of subjects	Wheeze (%)	OR (95% CI)	p Interaction
CD14 /-1721	AA+AG	342	12.9	1.50 (1.12 to 2.00)	
rs2915863	GG	54	7.4	0.62 (0.22 to 1.75)	0.05
CD14 /-1247	TT+CT	326	13.5	1.59 (1.18 to 2.13)	
rs2569191	CC	73	9.6	0.38 (0.13 to 1.09)	0.007
CD14 /-260	CC+CT	330	13.3	1.58 (1.18 to 2.13)	
rs2569190	TT	72	9.7	0.49 (0.21 to 1.13)	0.006
MD2	TT+CT	314	12.4	1.70 (1.25 to 2.32)	
rs10808798	CC	91	12.1	0.58 (0.30 to 1.13)	0.006

Data are presented as the OR for an interquartile range increase in endotoxin exposure levels (endotoxin units/m^3) with 95% CI (95% CI) as described earlier.[20] ORs are adjusted for sex, age, smoking habits and farm childhood. p Interaction represents p values for the interaction between endotoxin exposure and genotype under the best-fitting recessive model. The number of subjects in the analysis is less than 408, owing to genotype call rates <100%.

Modification of the association between endotoxin exposure and FEV_1 by genotype

We investigated whether the significant gene–environment interactions for wheeze were also present when FEV_1 was studied as an outcome. *CD14* -1721 A/G, -1247 T/C, and -260 C/T appeared to modify associations between endotoxin exposure and FEV_1 in a similar direction as in the case of wheeze,

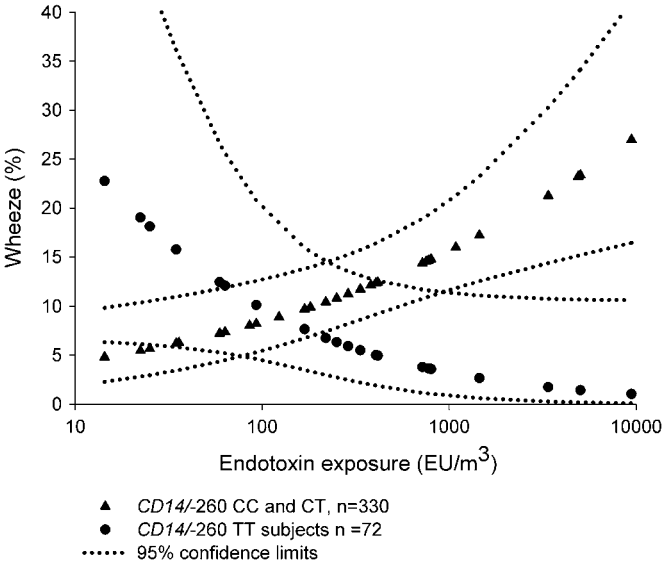

Figure 1 Logistic regression plots for the association between endotoxin exposure and current wheeze, stratified by *CD14*/-260 genotype. Each symbol represents a group of workers with the same estimated exposure level. Associations were adjusted for sex, age, smoking habits and farm childhood.

Table 3 Association between endotoxin exposure and forced expiratory volume in 1 s (% predicted) in agricultural workers stratified by *CD14* and *MD2* genotype

Single nucleotide polymorphism	Genotype	No of subjects	β (95% CI)	p Interaction
CD14 /-1721	AA+AG	340	−1.65 (−2.95 to −0.35)	
rs2915863	GG	54	2.31 (−0.78 to 5.41)	0.07
CD14 /-1247	TT+CT	324	−1.88 (−3.20 to −0.57)	
rs2569191	CC	73	1.78 (−1.16 to 4.73)	0.09
CD14 /-260	CC+CT	328	−1.77 (−3.06 to −0.49)	
rs2569190	TT	72	1.58 (−1.42 to 4.54)	0.15
MD2	TT+CT	313	−1.30 (−2.64 to 0.03)	
rs10808798	CC	90	−1.88 (−4.56 to 0.81)	0.99

Data are presented as the change in forced expiratory volume in 1 s (% predicted) at an interquartile range increase in endotoxin exposure levels (endotoxin units/m^3). Regression coefficients (β) and 95% CI are adjusted for smoking habits and atopy. p Interaction represents p values for the interaction between endotoxin exposure and genotype under the best-fitting recessive model. The number of subjects in the analysis is less than 408, owing to genotype call rates <100% and missing data on spirometry.

showing lower FEV_1 % predicted values with increasing endotoxin exposure ($p \leq 0.01$) in subjects carrying the major allele for the three *CD14* SNPs, and a non-significant association between endotoxin exposure and higher FEV_1 levels ($p > 0.05$) in minor allele homozygotes (table 3). The interaction between *CD14* genotype and endotoxin exposure was, however, not statistically significant (p=0.07 to 0.15; table 3). The *MD2* rs10808798 SNP did not modify the association between endotoxin exposure and FEV_1 (p=0.99; table 3).

Association of genotype and ex vivo cytokine production

The *CD14* SNPs that modified the association of endotoxin exposure and wheeze were also associated with ex vivo monocyte IL-1β production in response to LPS (p=0.02 to 0.06). The percentage of high IL-1β responders was relatively low among *CD14* -1721 GG, -1247 CC and -260 TT individuals (36 to 41%), whereas the percentage of high IL-1β responders among carriers of the major alleles was 53% (table 4). Adjustment for potential confounders including atopy in a multiple logistic regression model did not change these associations. For *MD2* rs10808798, LPS-induced cytokine production did not differ according to genotype (table 4).

DISCUSSION

Our study presents new evidence for the hypothesis that carriers of the *CD14*/-260 C allele are more responsive to endotoxin exposure than T allele homozygotes,[30] even at extremely high occupational exposure levels. The exposure–response curve among *CD14*/-260 CT and CC subjects showed a steeply increasing prevalence of (mainly non-atopic) wheeze at higher occupational endotoxin exposure levels. In TT subjects, a non-significant, inverse association between endotoxin exposure and wheeze was seen. A statistically significant interaction between *CD14*/-260 C carriage and endotoxin exposure on the presence of wheeze was found. These observations were parallelled by increased ex vivo LPS-induced pro-inflammatory cytokine IL-1β in *CD14*/-260 CC and CT subjects compared with TT subjects. Similar associations were found for *CD14* -1721 A/G and -1247 T/C, two SNPs in high linkage disequilibrium (LD) with *CD14*/-260C/T.

Wheeze and BHR in the present population of agricultural workers were characterised by a predominantly non-atopic phenotype.[21] Furthermore, wheeze was associated with a relatively low FEV_1 (99.7 and 107.0% predicted in subjects with and without wheeze, respectively). Rather than asthma, wheeze in these subjects resembles an 'asthma-like syndrome,' which results primarily from non-antigenic exposures, and is characterised by non-allergic, transient airway responsiveness, chest symptoms and possibly longitudinal decline in lung function.[1] *CD14* variants modified the association between endotoxin exposure and wheeze in a similar direction as for FEV_1, suggesting a similar mechanism by which endotoxin exposure has an effect on wheeze and airway obstruction.

Many domestic studies have shown inverse dose–response relationships between exposure to endotoxin and the risk of atopy and atopic asthma.[30] Four studies that related domestic endotoxin exposures to atopic outcomes found a relatively consistent protective effect of endotoxin exposure on atopy and atopic athma, but only among carriers of the *CD14*/-260

Table 4 Percentage of high interleukin-1β, tumour necrosis factor-α, and interleukin-10 responders in an ex vivo lipopolysaccharide-stimulation assay, stratified by *CD14* and *MD2* genotype

Single nucleotide polymorphism	Genotype	n	Interleukin-1β high responders (%)	Tumour necrosis factor-α high responders (%)	Interleukin-10 high responders (%)
CD14 /-1721	AA+AG	330	53.0	50.6	50.9
rs2915863	GG	53	35.9	49.1	50.9
p Value			0.02	0.83	0.99
CD14 /-1247	TT+CT	315	53.0	50.8	52.1
rs2569191	CC	72	40.3	50.0	50.0
p Value			0.05	0.90	0.75
CD14 /-260	CC+CT	318	53.1	50.3	50.9
rs2569190	TT	71	40.9	49.3	49.3
p Value			0.06	0.88	0.80
MD2	TT+CT	307	52.1	50.8	49.5
rs10808798	CC	85	44.7	48.2	56.5
p Value			0.23	0.67	0.26

The expected prevalence of high cytokine responders is 50% for each genotype: for each cytokine, the population was dichotomised into low and high responders according to median cytokine concentrations for each cytokine separately.[7] The number of subjects in the analysis is less than 408 owing to genotype call rates <100% and missing data on cytokine response.

C allele.[15–18] Carrying the C allele also increased the risk for non-atopic wheeze at high exposures, as was demonstrated by Simpson *et al*.[16] In addition, levels of serum sCD14 increased after endotoxin inhalation in an experimental setting among healthy volunteers carrying the C allele, but not in those carrying TT variants, and this effect was not modified by atopic status.[11] These studies suggested that carriers of the *CD14*/-260 C allele are more responsive to endotoxin exposure than T allele homozygotes, because increased endotoxin responsiveness in *CD14*/-260 C allele carriers could lead to increased suppression of atopic Th_2 responses,[15–18] an increase in non-atopic respiratory effects,[16] and to increased serum sCD14 levels.[11] At high airborne endotoxin levels encountered in the occupational environment, endotoxin exposure leads to a higher risk of respiratory health effects owing to its pro-inflammatory nature, both among atopic and among non-atopic subjects.[21] Given the supposedly increased endotoxin responsiveness in *CD14*/-260 C allele carriers, the higher risk of respiratory health effects among CT or CC subjects in the present study agrees with previous findings that carriers of the *CD14*/-260 C allele appear to be more susceptible to the (protective and adverse) effects of endotoxin exposure than TT subjects.

The modest sample size of the present study limits interpretation of the results. However, despite the limited power, results on the functional *CD14*/-260 C/T SNP were statistically significant and consistent with many previous reports, and results for wheeze showed consistency with FEV_1 and LPS-induced IL-1β release. We are well aware that studying a large number of SNPs has the downside of multiple testing, which may result in false-positive results. Nevertheless, adjustment for multiple comparisons resulted in marginally significant p values for interaction ($p<0.06$) between endotoxin exposure and *CD14* -260 C/T and -1721 A/G, and *MD2* rs10808798 T/C on the presence of wheeze. Whether *CD14*/-260 C/T is responsible for these findings, or otherwise *CD14* -1721 A/G, -1247 T/C or yet another *CD14* SNP in LD is unknown. The interaction between *MD2* rs10808798 T/C and endotoxin exposure on the presence of wheeze is more difficult to appreciate. *MD2* rs10808798 T/C did not modify the association of endotoxin and FEV_1; nor was this SNP associated with cytokine production. Furthermore, until now, this SNP has not been associated with wheeze or asthma. Although we studied four central genes of the LPS signalling complex and *TLR2*, at least 25 other TLR-related pathway genes such as LPS binding protein and nucleotide-binding oligomerisation domain 1 (*NOD1*) are involved in LPS sensing as well.[31] In addition, our study was not large enough to further study gene–gene interactions between SNPs, and it is likely that relationships between TLR-related pathway genes and wheeze are highly complex.[31]

Previously, we reported highly consistent associations between endotoxin and wheeze between the various agricultural sectors included in the study.[20] In the present study, further stratification of the gene-by-environment analysis by sector was not possible, owing to the smaller numbers. Including industry in the model (four dummy variables) resulted in a similar OR estimate but wider confidence limits among *CD14*/-260 CC/CT subjects (1.76 (1.15 to 2.68)). Among *CD14*/-260 TT subjects, the model that included industry did not fit well. Endotoxin is only one of the active components of organic dust, and it has been shown that other agents in farm dust contribute to inflammation and respiratory effects as well.[2 4] In our study, inhalable dust and endotoxin measurements were highly correlated ($r=0.82$, $p<0.001$),[20] and a relatively high correlation between endotoxin and other microbial agents, such as fungal β (1→3) glucan, can be expected as well.[4 5] Therefore, we also analysed SNPs in *TLR2*, which is involved in the recognition of microbial motifs of a wide range of Gram-positive bacteria, fungi and mycobacteria. Moreover, SNPs in *TLR2* have been shown to modify associations between living in a rural area or on a farm during childhood and the development of asthma.[32 33] However, significant and consistent results were found only for *CD14*, which argues in favour of endotoxin as the causative agent in the observed associations between endotoxin exposure and wheeze.

To our knowledge, our study is the first to show an interaction between *CD14*/-260 and endotoxin exposure in an occupational setting. A limitation of using a heavily exposed workforce-based population is that a 'healthy worker effect' may have resulted in attenuated risk estimates for wheeze, because individuals with airway problems may avoid agricultural jobs with the highest exposures. However, it is unlikely that a healthy worker effect explains our results, because endotoxin exposure level and duration of exposure were not associated with genotype. Furthermore, as discussed earlier, it is encouraging that our findings appear to agree with domestic studies that used endotoxin levels in floor or mattress dust as a proxy of domestic endotoxin exposures,[15–18] even though the inhalable endotoxin levels measured in our study are 100 to 10 000 times higher than levels in the air in domestic settings. It should be noted that these four domestic studies and our study all used clearly different methods of exposure assessment. For example, dust was sampled as inhalable dust in the present study, and from mattresses, living room floors and bedroom floors in domestic studies. Furthermore, endotoxin levels were expressed as EU per mg dust, EU per m^2 or EU per m^3, which complicates a direct comparison between these studies. Nevertheless, studies using quantitatively assessed endotoxin exposures have clearly shown similar patterns and relatively homogeneous associations between *CD14*/-260 and asthma or atopy. Studies that used a proxy of exposure such as pet ownership[15 27] or farm childhood[12 32] or studies that did not include an environmental component at all showed more heterogeneous results.[30]

In conclusion, we found that the association between occupational endotoxin exposure and wheeze in agricultural workers was significantly modified by genetic variants in *CD14* and *MD2*. Our findings on interaction between *CD14*/-260 and endotoxin exposure agreed with studies in domestic settings using quantitatively assessed endotoxin exposure levels. This apparent replication of a gene–environment interaction once again argues for the use of high-quality exposure assessment in genetic-association studies.

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Competing interests DSP has been consultant to AstraZeneca, Boehringer Ingelheim, Chiesi, GSK, Nycomed, TEVA. GHK has received a research grant from GSK (<€10 000, money to institution), and has spoken at an annual Dutch conference for Paediatricians on asthma organised by an independent committee (GSK; €1250, money to institution).

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