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 (rs2568190), -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.

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 (FEV1) and do not report airway symptoms, suggesting important individual variation in sensitivity.7 8 A differential response to inhaled LPS was evidenced in experimental studies in healthy subjects not exposed to endotoxin at work; a >5% fall in FEV1 after LPS-containing cotton dust inhalation occurred in more than one-third, whereas almost two-thirds preserved their baseline FEV1.9 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 9 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 LPS10-11 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 Inhaled 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 12 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. 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, 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. 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 and performed according to European Respiratory Society (ERS) standards. Age- and standing-height-adjusted spirometric reference values of the European Community for Steel and Coal were used. 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. In total, data from 408 out of 454 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 FEV1 of at least 20% at a methacholine dose of 2.5 mg or less as described previously. 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. 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. Median endotoxin exposure was 289 endotoxin units (EU)/m3, and exposure levels ranged from 10 to 10 000 EU/m3.

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 or from the Innate Immunity website based on the largest number of SNPs with a minor allele frequency >0.1 available. Genotyping was performed as described before by K-biosciences (Hoddesdon, UK) with genotype call rates >96%. All SNPs were in Hardy–Weinberg equilibrium (p>0.05), except for TLR4 rs10759951 (p<0.001), which was excluded from analyses.

Whole-blood assay
A whole-blood assay was performed as described previously. Briefly, heparinised whole blood samples of each participant were stimulated with LPS (1 ng/ml) during 18 h at 37°C, 5% CO2 and 96% relative humidity. Tumour necrosis factor (TNFα), interleukin (IL)-1β and IL-10 were measured in supernatants using a Bio-plex assay as described before. 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).

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, the associations seemed log-linear. False-discovery-rate-adjusted p values were calculated to take multiple comparisons (n=24 SNPs) into account. Associations between genotype and cytokine response (high/low responders) were assessed by χ² test.

RESULTS
Descriptive characteristics of 51 subjects who reported wheeze and 557 subjects without wheeze are shown in table 1. Higher
endotoxin exposure, current smoking and objectively measured respiratory and allergic outcomes such as presence of bronchial hyper-responsiveness and atopy, lower FEV₁ 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 subjects were currently smoking. For the association between endotoxin exposure and wheeze, the number of subjects in the analysis is less than 408, owing to genotype call rates for the SNP: CD14 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 CD4 SNPs were in high linkage disequilibrium (r² 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, 0.55 (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.

Modification of the association between endotoxin exposure and FEV₁ by genotype
We investigated whether the significant gene–environment interactions for wheeze were also present when FEV₁ was studied as an outcome. CD14 -1721 A/G, -1247 T/C, and -260 C/T appeared to modify associations between endotoxin exposure and FEV₁ in a similar direction as in the case of wheeze,

### Table 1
Characteristics of 408 agricultural workers, stratified by wheeze

<table>
<thead>
<tr>
<th>Wheeze</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no of subjects</td>
<td>357</td>
<td>51</td>
</tr>
<tr>
<td>Males (%)</td>
<td>87.7</td>
<td>86.3</td>
</tr>
<tr>
<td>Age (years), mean±SD</td>
<td>42.0±10.5</td>
<td>40.9±10.7</td>
</tr>
<tr>
<td>Smoking habits (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>43.0</td>
<td>23.5</td>
</tr>
<tr>
<td>Former</td>
<td>31.5</td>
<td>35.3</td>
</tr>
<tr>
<td>Current</td>
<td>25.6</td>
<td>41.2*</td>
</tr>
<tr>
<td>Farm/childhood (%)</td>
<td>46.8</td>
<td>41.2</td>
</tr>
<tr>
<td>Endotoxin exposure (endotoxin units/m³), median (interquartile range)</td>
<td>289 (93 to 720)</td>
<td>355 (219 to 1086)*</td>
</tr>
<tr>
<td>Forced expiratory volume in 1 s (% predicted), mean±SD</td>
<td>107.0±13.9</td>
<td>99.7±17.1*</td>
</tr>
<tr>
<td>Bronchial hyper-responsiveness (%)</td>
<td>20.8</td>
<td>64.3*</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>17.4</td>
<td>29.4*</td>
</tr>
<tr>
<td>Total immunoglobulin E (IU/mL), median (interquartile range)</td>
<td>29 (8 to 91)</td>
<td>71 (11 to 350)*</td>
</tr>
<tr>
<td>Interleukin-1β high responders (%)</td>
<td>50.9</td>
<td>49.0</td>
</tr>
<tr>
<td>Tumour necrosis factor-α high responders (%)</td>
<td>49.4</td>
<td>57.1</td>
</tr>
<tr>
<td>Interleukin-10 high responders (%)</td>
<td>48.8</td>
<td>65.3*</td>
</tr>
</tbody>
</table>

* p < 0.05, γ test or t test.

Forced expiratory volume in 1 s 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

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

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

Data are presented as the OR for an interquartile range increase in endotoxin exposure levels (endotoxin units/m³) with 95% CI (95% CI) as described earlier.21 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%.

### 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.

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**Workplace**
showing lower FEV1 % predicted values with increasing endotoxin exposure (p≤0.01) in subjects carrying the major allele for the three CD14 SNPs, and a non-significant association between endotoxin exposure and higher FEV1 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 FEV1 (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 (56 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, 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 paralleled 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/-260 C/T.

Wheeze and BHR in the present population of agricultural workers were characterised by a predominantly non-atopic phenotype. Furthermore, wheeze was associated with a relatively low FEV1 (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. CD14 variants modified the association between endotoxin exposure and wheeze in a similar direction as for FEV1, 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 atopic and atopic asthma. Four studies that related domestic endotoxin exposures to atopic outcomes found a relatively consistent protective effect of endotoxin exposure on atopy and atopic asthma, but only among carriers of the CD14/-260
C allele.\textsuperscript{15–18} Carrying the C allele also increased the risk for non-atopic wheeze at high exposures, as was demonstrated by Simpson et al.\textsuperscript{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.\textsuperscript{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 Th2 responses,\textsuperscript{15–18} an increase in non-atopic respiratory effects,\textsuperscript{16} and to increased serum sCD14 levels.\textsuperscript{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.\textsuperscript{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\textsubscript{1} and LPS-induced IL-1\textbeta 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\textsubscript{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.\textsuperscript{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.\textsuperscript{31}

Previously, we reported highly consistent associations between endotoxin and wheeze between the various agricultural sectors included in the study.\textsuperscript{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.\textsuperscript{2, 4} In our study, inhalable dust and endotoxin measurements were highly correlated (r=0.82, p<0.001),\textsuperscript{20} and a relatively high correlation between endotoxin and other microbial agents, such as fungal β (1→3) glucan, can be expected as well.\textsuperscript{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.\textsuperscript{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,\textsuperscript{15–18} even though the inhalable endotoxin levels measured in our study are 100 to 1000 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\textsuperscript{3} or EU per m\textsuperscript{2}, 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\textsuperscript{15, 27} or farm childhood\textsuperscript{12, 32} or studies that did not include an environmental component at all showed more heterogeneous results.\textsuperscript{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. GIK has received a research grant from GSK (€610 000, 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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