



Association of Toll-like receptor 2 gene polymorphisms with lung function in workers in swine operations

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ABSTRACT

Background: Workers in swine operations are exposed to indoor dusts and gases and are at increased risk of respiratory problems. Toll-like receptor (TLR) 2 recognizes ligands from gram-positive bacteria, whereas TLR4 responds to endotoxin from gram-negative bacteria.

Objective: To investigate the effects of TLR2 and TLR4 polymorphisms on lung function in workers from swine operations and nonfarming rural dwellers.

Methods: A total of 374 full-time workers from large swine operations and 411 nonfarming rural dwellers from Saskatchewan were included. Information on demography, lifestyle, and occupation, lung function measurements, and blood samples for genotyping were obtained from the participants. Multiple regression analysis and Bonferroni correction were used in the statistical analysis.

Results: Workers with TLR2-16933T/A polymorphism (AA) had significantly greater mean values of lung function than workers with wild-type genotypes (AT+TT) after controlling for potential confounders (forced expiratory volume in 1 second, 3.7 vs 3.5 L; $P=.009$; forced expiratory flow between 25% and 75%, 3.7 vs 3.3 L; $P=.003$; predicted forced expiratory volume in 1 second; 100.3% vs 95.6%; $P=.005$; forced expiratory flow between 25% and 75%, 92.4% vs 83.4%; $P=.009$). These results were also observed for TLR2Arg677Trp polymorphism among the workers. No such significant differences were observed among nonfarming rural dwellers. For Asp299Gly and Thr399Ile polymorphisms in the *TLR4* gene, no significant differences were observed in the mean lung function values between the polymorphic and wild-type groups in both workers and rural dwellers.

Conclusion: Our study is the first, to our knowledge, to report protective effects of TLR2 polymorphisms on lung function among workers in swine operations and raises the possibility that TLR2 polymorphisms are protective of airway disease in individuals exposed to gram-positive organisms in the inhaled airborne dust.

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Introduction

Studies have demonstrated that working in swine operations is associated with a number of respiratory health problems, including accelerated lung function decline,¹ bronchitis-like symptoms,²

increased risk of developing chronic obstructive pulmonary disease (COPD),³ and asthma.^{4,5} Gram-positive and Gram-negative bacteria, dusts, and gases (hydrogen sulfide and ammonia) are found in the indoor air in swine operations and likely contribute to respiratory dysfunction among people who are exposed during work.^{6,7} Concentration of gram-positive bacteria was reported to be much higher than that of gram-negative bacteria in swine operations.⁸

The etiology of these respiratory health problems is not fully understood. However, individual susceptibility plays an important role in the respiratory health status of the workers. Toll-like receptors (TLRs) are pattern recognition receptors, are highly polymorphic, and play an important role in both innate and adaptive immunity.^{9–12} TLR2 mainly responds to cell wall structure components from gram-positive bacteria, such as peptidoglycan,

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and TLR4 mainly recognizes microbial membrane components from gram-negative bacteria, such as lipopolysaccharide (LPS) or endotoxin.^{13,14}

TLR4 polymorphisms have been associated with reduced risk of allergic rhinitis, atopy, and airway responsiveness in several studies.^{15–17} The reduced immune responses of TLR2 polymorphisms have also been investigated in experimental studies using human cells or animal models.^{18–20} However, to date there is limited study of the relationship between polymorphisms in the *TLR2* gene and lung function in humans.

In this study, we extended these observations from experimental studies using the human cells and animals and human population studies using naive healthy adults to full-time workers by examining the effects of TLR2 and TLR4 polymorphisms on lung function in full-time workers in swine operations and nonfarming rural dwellers, who are exposed to high and low levels of respiratory hazards, respectively.

Methods

Recruitment and data collection

The study was conducted in rural Saskatchewan, Canada. The workers in swine operations were recruited from the Pork Producers' Association in Saskatchewan. Large swine production companies were contacted and requested to circulate a description of the study to their workers. To participate in the study, workers in swine operations had to be 17 years or older and had to work in a confinement building for at least 4 days per week with a total work duration of 20 hours or more per week. The participation rate from the large swine operations was approximately 70%.

Nonfarming rural dwellers were recruited from nonfarming rural dwellers residing in close proximity (within a 100-km radius) to a swine production site by mail using taxation lists provided by rural towns. Nonfarming rural dwellers who were involved in mining, grain handling, metal, and autobody work were excluded because of their potential exposure to respiratory hazards.

All interviews and pulmonary function tests were conducted in communities located near the swine production sites and the residences of the nonfarming rural dwellers. The study was approved by the Biomedical Research Ethics Board of the University of Saskatchewan. Informed written consent was obtained from all study participants.

A previously validated questionnaire^{1,2,16,21} was used to collect information comprising anthropomorphic data, respiratory symptoms, smoking history, past illnesses, and occupational history. Each participant also had measurements of height, weight, and systolic and diastolic blood pressure. Pulmonary measurements included forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC), FEV₁/FVC ratio, and forced expiratory flow between 25% and 75% of FVC (FEF_{25%–75%}). These measurements were obtained using a volume displacement spirometer (model 1022; Sensor-Medics, Yorba Linda, California) by trained technicians who followed the American Thoracic Society recommendations.²² For each study participant, the lung function measurements were taken with the participant in the sitting position with a nose-clip in place. The technology has been used in our previous studies.^{2,23,24} Percentage predicted values for FEV₁, FVC, FEV₁/FVC ratio, and FEF_{25%–75%} were obtained using the equations developed by Crapo et al.²⁵

A current smoker was defined from the questionnaire as a person currently smoking cigarettes. An ex-smoker was defined from the questionnaire as a person who had smoked more than 400 cigarettes (or an equivalent amount of tobacco) in his/her lifetime but was not currently smoking. A lifetime nonsmoker was defined from the questionnaire as a person who had not smoked more than 400 cigarettes (or an equivalent amount of tobacco) in his/her lifetime. Allergy skin prick tests (SPTs) were performed for

Table 1

Characteristics of workers in swine operations and nonfarming rural dwellers^a

Characteristic	Workers	Rural dwellers	P value
Age, y			
Male	36.2 ± 11.9	40.1 ± 13.5	.002
Female	34.9 ± 10.7	38.7 ± 12.4	.004
Height, cm			
Male	176.8 ± 6.1	178.3 ± 6.7	.02
Female	164.4 ± 5.8	164.7 ± 6.5	.67
Weight, kg			
Male	88.1 ± 16.6	90.4 ± 17.8	.17
Female	75.5 ± 15.0	75.6 ± 16.9	.96
Male, No. (%)	240 (64.2)	184 (44.8)	<.001
Smoking, No. (%)			<.001
Current smoker	117 (31.3)	74 (18.0)	
Ex-smoker	101 (27.0)	115 (28.0)	
Nonsmoker	156 (41.7)	222 (54.0)	
Atopic, No. (%)	123 (35.3)	169 (42.1)	.06
Observed FEV ₁ , L			
Male	4.1 ± 0.7	4.1 ± 0.8	.63
Female	3.1 ± 0.6	3.1 ± 0.6	.76
FVC, L			
Male	5.3 ± 0.8	5.3 ± 1.0	.63
Female	3.9 ± 0.6	3.9 ± 0.6	.68
FEV ₁ /FVC, %			
Male	77.8 ± 6.6	77.9 ± 6.5	.88
Female	80.0 ± 6.2	80.1 ± 6.1	.87
FEF _{25%–75%} , L			
Male	3.8 ± 1.2	3.8 ± 1.3	.90
Female	3.2 ± 1.0	3.1 ± 1.0	.73
Percent predicted			
FEV ₁	96.9 ± 12.6	99.3 ± 13.3	.01
FVC	102.4 ± 12.0	103.9 ± 13.7	.10
FEV ₁ /FVC	82.9 ± 2.4	82.6 ± 2.6	.10
FEF _{25%–75%}	86.9 ± 25.6	90.1 ± 25.0	.08
TLR4 Asp299Gly (rs4986790) ^b			.20 ^c
GG	0 (0.0)	2 (0.7)	
AG	35 (12.8)	29 (9.8)	
AA	238 (87.2)	266 (89.6)	
TLR4 Thr399Ile (rs4986791) ^b			.91
CC	0 (0)	0 (0)	
CT	33 (12.1)	35 (11.8)	
TT	240 (87.9)	262 (88.2)	
TLR4 LD (D') ^c	0.97	0.97	
TLR2-16933T/A (rs4696480) ^b			.58
AA	68 (24.5)	66 (20.9)	
AT	144 (51.8)	172 (54.4)	
TT	66 (23.7)	78 (24.7)	
TLR2-Arg753Gln (rs5743708) ^b			.99
AA	0 (0)	0 (0)	
AG	18 (6.6)	20 (6.4)	
GG	257 (93.4)	294 (93.6)	
TLR2 LD (D') ^d	0.55	0.47	

Abbreviations: FEF_{25%–75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; LD, linkage disequilibrium.

^aData are presented as mean ± SD unless otherwise indicated.

^bHardy-Weinberg equilibrium was followed in both workers in swine operations and nonfarming rural dwellers.

^cFisher exact test.

^dLD between the 2 single-nucleotide polymorphisms in the *TLR4* and *TLR2* genes, respectively.

5 allergens (*Alternaria* sp, swine, mixed grass allergens, house dust mite, and cat dander; from Western Allergy Services Ltd, Burnaby, British Columbia, Canada), as described in another study reporting the reciprocal association between atopy and respiratory symptoms using the same cohort.²⁶ Histamine and saline were also included in the SPT as the positive and negative control. The tests were conducted on the same occasion as the pulmonary function testing using methods in previous studies.^{17,26} The SPT was performed on the normal skin on each participant's forearms, and the distance between 2 SPTs was 2 cm to avoid cross-contamination.

Results were considered positive if one or more SPT result had a raised wheal of 3 mm or larger compared with the saline

control.²⁷ The technicians conducting the SPT were masked to the genotypes of each participant.

DNA isolation and genotyping

Blood sample collection and genotyping methods have been previously described.²⁸ Briefly, blood samples were collected in Qiagen PAXgene tubes with blood cards spotted for each sample (Schleicher & Schuell, catalog No. 10538414). A Gentra Autopure robot (Qiagen Corporation, Hilden, Germany) was used to isolate DNA. DNA for genotyping was quantified by PicoGreen assays. Human TLR4 Asp299Gly and Thr399Ile polymorphisms were genotyped using TaqMan assays and standard protocols on the ABI 7900 Sequence Detection System. Plasmids carrying mutant and wild-type sequences were included as controls. TLR2 single-nucleotide polymorphisms (SNPs) were genotyped as a part of a 96-plex GoldenGate genotyping assay on Veracode beads. Assays were performed according to manufacturer's protocols and scanned on a BeadXpress reader (Illumina Inc, San Diego, California). Raw genotyping data were clustered and cleaned in the BeadStudio software (Illumina GenomeStudio).

The selection of TLR2 SNPs was based on the following criteria. First, we selected ones that have been associated with one or more disease phenotypes in multiple cohorts or have been shown to alter gene function in biological assays. Second, we used high-density SNP maps generated by the HapMap project and selected sets of tagging SNPs for *TLR2* gene using the SNPSelector software.²⁹ Using these criteria, we genotyped 3 SNPs of the *TLR2* gene (TLR2-16933T/A, Arg753Gln, and Arg677Trp). However, TLR2 Arg677Trp polymorphism failed to satisfy the Hardy-Weinberg equilibrium (HWE) in the workers and was excluded from this study.

Statistical analyses

Differences in genotype frequencies or descriptive characteristics between workers in swine operations and nonfarming rural dwellers were determined by the χ^2 test or Fisher exact test when the expected count for any cell was less than 5. For each genotype, HWE was tested, and only those SNPs that follow HWE were included. Three inheritance models (additive, dominant, and recessive) were fitted to determine the best-fitted model with the smallest Akaike information criterion values.

Multiple linear regression analysis was used to test the differences in the mean values of lung function parameters between genotypes of each SNP after controlling for age, sex, height, weight, and smoking habit. In the multiple regression analysis, interaction

between exposure groups (workers and nonfarming rural dwellers) and genotypes of each SNP was included, and post hoc contrasts were used to test the differences in the mean lung function parameters between polymorphic and wild-type groups in workers and nonfarming rural dwellers, respectively. The Bonferroni correction was used to adjust the *P* values from the multiple regressions for testing multiple TLR2 SNPs. The effect of years of exposure in swine operation on the results was tested by including the interaction between years of exposure and the dichotomous variable indicating polymorphic or wild-type group in the multiple regression of 4 lung function measurements. We considered years of exposure as both a continuous and dichotomous variables (<10 vs ≥ 10 years of swine work). SAS statistical software, version 9.2 (SAS Institute Inc, Cary, North Carolina), was used for the statistical analysis.

Results

Table 1 lists the characteristics of workers in swine operations and rural dwellers. Workers in the swine operations were significantly younger in both males and females and somewhat shorter in males than nonfarming rural dwellers. Mean weight was similar between the 2 groups in both males and females. There were more males among the workers (64.2%) than rural dwellers (44.8%). Smoking was more prevalent among the workers than among the rural dwellers. The mean values of lung function parameters were similar between the 2 groups in both males and females, except mean values of percent predicted FEV₁, which was significantly lower in the workers than rural dwellers. Atopy was less common in workers in swine operations than nonfarming rural dwellers (35.3% vs 42.1%), but the difference was not statistically significant (Table 1).

As indicated in Table 1, the genotype distributions of Asp299Gly and Thr399Ile polymorphisms in the *TLR4* gene and -16933T/A and Arg753Gln polymorphisms in the *TLR2* gene were similar between the workers and nonfarming rural dwellers. The 2 SNPs in the *TLR4* gene were in high linkage disequilibrium ($D' = 0.97$).

The recessive model (AA vs AT+TT) of TLR2-16933T/A had the best fit with the smallest Akaike information criterion values in the regression analysis of all the 4 lung function parameters compared with the additive (AA=2, AT=1, and TT=0) and dominant models (AA+AT vs TT) (data not shown). Under the recessive model, as indicated in Table 2 and Figure 1, workers with the AA genotype (polymorphic group) of -16933T/A polymorphism in the *TLR2* gene had significantly greater mean values of lung function parameters

Table 2
Differences in mean values of lung function parameters between TLR2-16933T/A polymorphism (AA) and wild type (AT+TT) for workers in swine operations and nonfarming rural dwellers in the recessive inheritance model

Lung function measurements	Workers in swine operations (n=278)			Nonfarming rural dwellers (n=316)		
	Polymorphism, mean (95% CI)	Wild type, mean (95% CI)	Comparison difference (95% CI)	Polymorphism, mean (95% CI)	Wild type, mean (95% CI)	Comparison difference (95% CI)
Observed values^a						
FEV ₁ , L	3.7 (3.6 to 3.8)	3.5 (3.5 to 3.6)	0.2 (0.0 to 0.3) ^b	3.6 (3.5 to 3.7)	3.6 (3.6 to 3.7)	0.0 (−0.1 to 0.1)
FVC, L	4.7 (4.6 to 4.8)	4.6 (4.5 to 4.6)	0.1 (−0.0 to 0.3)	4.6 (4.5 to 4.8)	4.6 (4.6 to 4.7)	0.0 (−0.1 to 0.2)
FEV ₁ /FVC, %	79.0 (77.5 to 80.3)	77.7 (76.8 to 78.5)	1.3 (−0.3 to 2.9)	78.5 (77.1 to 78.0)	78.8 (78.1 to 79.6)	−0.3 (−1.9 to 1.3)
FEF _{25%–75%} , L/s ^c	3.7 (3.4 to 3.9)	3.3 (3.1 to 3.4)	0.4 (0.1 to 0.6) ^b	3.5 (3.3 to 3.7)	3.5 (3.4 to 3.5)	0.0 (−0.2 to 0.3)
Predicted values, %^d						
FEV ₁ ^c	100.3 (97.4 to 103.2)	95.6 (93.9 to 97.2)	4.8 (1.4 to 8.1) ^b	98.3 (95.4 to 101.3)	98.2 (96.7 to 99.8)	0.1 (−3.2 to 3.4)
FVC	105.4 (102.6 to 108.3)	101.9 (100.2 to 103.5)	3.6 (0.3 to 6.8) ^b	103.9 (101.0 to 106.8)	103.4 (101.8 to 104.9)	0.5 (−2.7 to 3.7)
FEV ₁ /FVC	83.1 (82.6 to 83.7)	82.7 (82.3 to 83.0)	0.5 (−0.2 to 1.2)	82.5 (82.0 to 83.1)	82.3 (82.0 to 82.6)	0.3 (−0.4 to 0.9)
FEF _{25%–75%} ^c	92.4 (86.5 to 98.3)	83.4 (80.0 to 86.8)	9.0 (2.2 to 15.8) ^b	87.7 (81.6 to 93.7)	88.0 (84.8 to 91.2)	−0.3 (−7.1 to 6.4)

Abbreviations: CI, confidence interval; FEF_{25%–75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

^a*P* value from the multiple regression analysis after adjusting for age, sex, height, weight, and smoking habit.

^bSignificant after the Bonferroni correction for multiple comparisons ($P < .025$).

^cThe *P* value (type 3) of the interaction term from the multiple regression analysis is significant.

^d*P* value from the multiple regression analysis after adjusting for smoking habit.

than workers with AT or TT genotypes (wild type) after applying the Bonferroni correction and controlling for age, sex, height, weight, and smoking habit (FEV_1 , 3.7 vs 3.5 L; $P=.009$; $FEF_{25\%-75\%}$, 3.7% vs 3.3%; $P=.003$; predicted FEV_1 , 100.3% vs 95.6%; $P=.005$; predicted FVC, 105.4% vs 101.9%; $P=.03$; predicted $FEF_{25\%-75\%}$, 92.4% vs 83.4%; $P=.009$). Among nonfarming rural dwellers, no such associations were observed.

As indicated in Table 3 and Figure 2, workers with AG genotype (polymorphic group) of Arg753Gln polymorphism in the *TLR2* gene had significantly greater mean values of lung function parameters than workers with GG genotype (wild type) after applying the Bonferroni correction and controlling for age, sex, height, weight, and smoking habit (FEV_1/FVC , 80.9% vs 77.8%; $P=.03$; $FEF_{25\%-75\%}$, 4.0 vs 3.3 L/s; $P=.004$; predicted FEV_1 , 103.4% vs 96.3%; $P=.02$; predicted FEV_1/FVC , 84.1% vs 82.7%; $P=.02$; predicted $FEF_{25\%-75\%}$, 102.5% vs 84.6%; $P=.003$). Among nonfarming rural dwellers, no

significant associations were observed between Arg753Gln genotypes and lung function measurements.

As indicated in eTable 1, the effects of the interaction term between the *TLR2*-16933T/A polymorphism and an exposure group variable indicating workers with more than 10 years of swine work and nonfarming rural dwellers, were not significant in the multiple regression analysis of all lung function parameters except $FEF_{25\%-75\%}$ ($P=.03$) and percentage predicted $FEF_{25\%-75\%}$ ($P=.05$). Among workers with more than 10 years of swine work, subjects with the *TLR2*-16933T/A polymorphism had significantly better mean values of $FEF_{25\%-75\%}$ (4.0 vs 3.3 L/s, $P=.02$) and percentage predicted $FEF_{25\%-75\%}$ (98.5% vs 82.0%, $P=.04$) than those with the wild-type after controlling for age, sex, height, weight, and smoking habit. However, none of these associations remained significant after adjusting for Bonferroni correction. As indicated in eTable 2, none of the interaction terms between *TLR2* Arg753Gln polymorphism

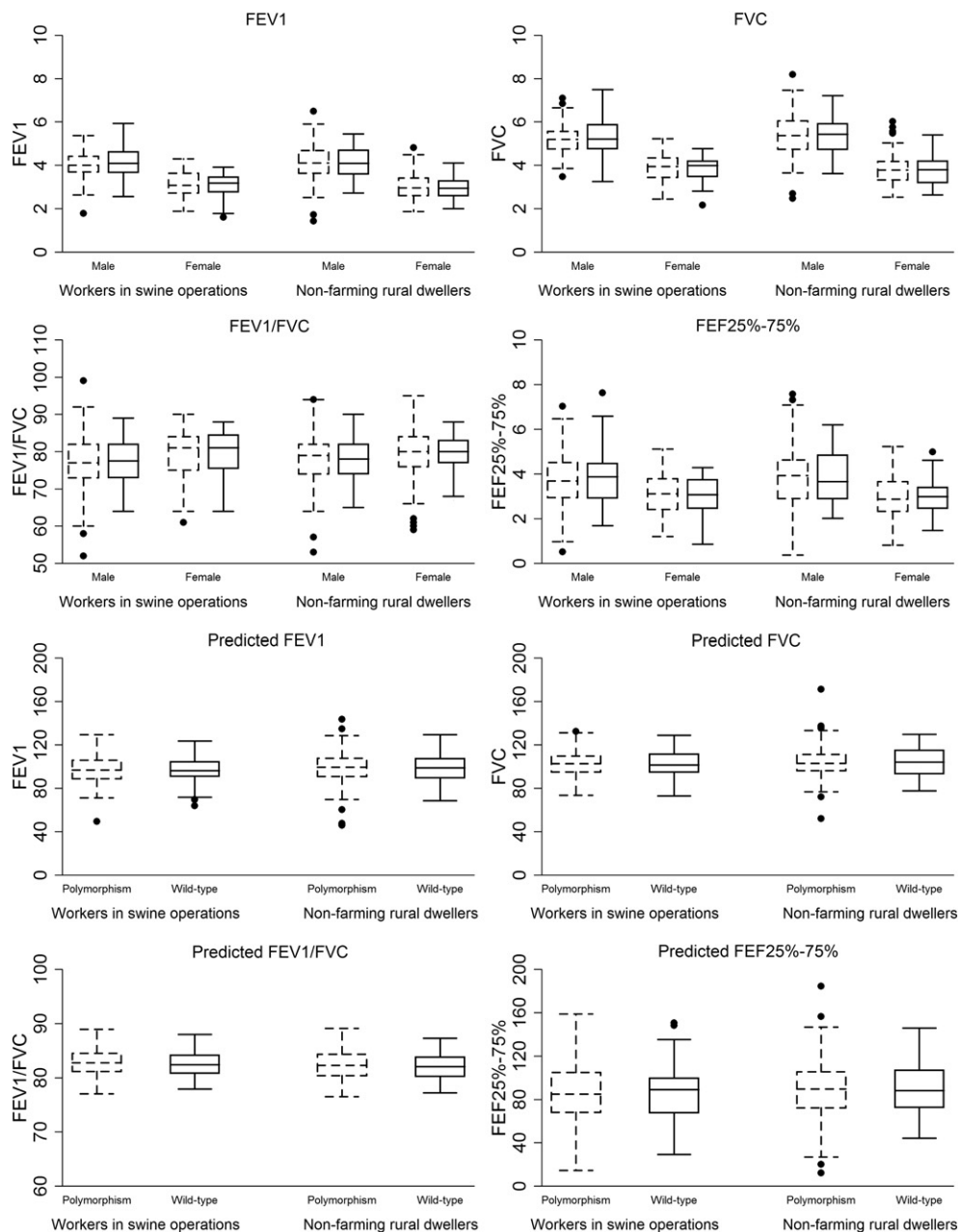


Figure 1. Box plot of lung function between *TLR2*-16933T/A polymorphic and wild-type groups in rural dwellers and workers. Short dashed line indicates polymorphism group; solid line, wild-type group.

Table 3
Differences in mean values of lung function parameters between TLR2 Arg753Gln polymorphism (AG) and wild type (GG) for workers in swine operations and nonfarming rural dwellers

Lung function parameter	Workers in swine operations (n=275)			Nonfarming rural dwellers (n=314)		
	Polymorphism, mean (95% CI)	Wild type, mean (95% CI)	Comparison difference (95% CI)	Polymorphism, mean (95% CI)	Wild type, mean (95% CI)	Comparison difference (95% CI)
Observed values^a						
FEV ₁ , L ^b	3.8 (3.6 to 4.0)	3.6 (3.5 to 3.6)	0.2 (0.0 to 0.4)	3.5 (3.3 to 3.7)	3.6 (3.6 to 3.7)	–0.1 (–0.3 to 0.1)
FVC, L	4.7 (4.5 to 5.0)	4.6 (4.5 to 4.6)	0.1 (–0.1 to 0.4)	4.5 (4.3 to 4.7)	4.6 (4.6 to 4.7)	–0.1 (–0.4 to 0.1)
FEV ₁ /FVC, %	80.8 (78.1 to 83.6)	77.8 (77.1 to 78.6)	3.1 (0.2 to 5.9) ^c	78.4 (75.8 to 81.0)	78.8 (78.1 to 79.5)	–0.3 (–3.0 to 2.3)
FEF _{25%–75%} , L/s ^b	4.0 (3.6 to 4.4)	3.3 (3.2 to 3.4)	0.7 (0.2 to 1.1) ^c	3.4 (2.9 to 3.8)	3.5 (3.4 to 3.6)	–0.1 (–0.6 to 0.3)
Predicted values, %^d						
FEV ₁ ^b	103.4 (97.8 to 109.0)	96.3 (94.8 to 97.8)	7.1 (1.3 to 12.9) ^c	96.2 (90.9 to 101.6)	98.4 (97.0 to 99.9)	–2.2 (–7.7 to 3.3)
FVC	106.2 (100.7 to 111.8)	102.4 (100.9 to 103.9)	3.8 (–1.9 to 9.5)	101.3 (96.1 to 106.6)	103.6 (102.2 to 105.1)	–2.3 (–7.7 to 3.1)
FEV ₁ /FVC ^b	84.1 (82.9 to 85.2)	82.7 (82.4 to 83.0)	1.4 (0.2 to 2.5) ^c	81.6 (80.5 to 82.6)	82.4 (82.1 to 82.7)	–0.8 (–1.9 to 0.3)
FEF _{25%–75%} ^b	102.5 (91.0 to 114.0)	84.6 (81.6 to 87.7)	17.9 (6.0 to 29.8) ^c	86.8 (75.8 to 97.7)	88.1 (85.1 to 91.1)	–1.3 (–12.6 to 9.9)

Abbreviations: CI, confidence interval; FEF_{25%–75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

^aP value from the multiple regression analysis after adjusting for age, sex, height, weight, and smoking habit.

^bThe P value (type 3) of the interaction term from the multiple regression analysis is significant.

^cSignificant after the Bonferroni correction for multiple comparisons ($P < .025$).

^dP value from the multiple regression analysis after adjusting for smoking habit.

and the exposure group variable were significant in the multiple regression models.

Among workers in swine operations, when the variable of years of swine work was treated as a continuous variable in the interaction term with each of the TLR2 SNPs, none of the interaction terms was significant in the multiple regression analyses (data not shown).

Discussion

In our study, Arg753Gln and –16933T/A polymorphisms in the *TLR2* gene, rather than Asp299Gly and Thr399Ile polymorphisms in the *TLR4* gene, were significantly associated with better lung function in full-time workers in swine operations. In contrast, these findings were not observed in nonfarming rural dwellers, a group likely exposed to low levels of respiratory hazards.

Although there is no hypothesis suggesting that inhalation is more relevant than contact through the skin or ingestion, results from several studies indicate that inhalation is relevant for acute and chronic health effects in individuals exposed to the indoor environment in the swine facilities.^{1–5,21,23,30} In a study investigating the acute inflammation reactions associated with bacteria concentration in the airborne dust in healthy naive individuals exposed in a swine facility for 3 hours, endotoxin (from gram-negative bacteria) and peptidoglycan (mainly from gram-positive bacteria) concentrations in the airborne dust were associated with bronchial hyperresponsiveness and lower vital capacity and increased blood granulocyte concentration and body temperature, respectively.²⁶ A study investigating the effect of using respiratory protective device in swine operations in Saskatchewan reported that healthy naive individuals wearing respiratory protective devices for 5 hours had decreased acute symptoms, including cough, chest tightness, and phlegm and a 10% decrease in lung function compared with those not wearing respiratory protective devices.²⁵ However, individual susceptibility to these exposures seems to be quite variable. TLRs, a family of immune receptors that are involved in recognition of pathogen-association molecular patterns, play a pivotal role in the modulation of both innate and adaptive immune responses.^{10–12} Human immune responses are important mechanisms of defense against pathogenic microorganisms by release of inflammatory cytokines. However, many cytokines, such as tumor necrosis factor α (TNF- α) and interferon γ , have been associated with many respiratory diseases, such as asthma.^{31–33}

TLR2 has been found to respond to a wide range of ligands of bacterial products, including gram-positive bacteria,³⁴ whereas

TLR4 seems to respond only to LPS, a major component of the outer cell membrane of gram-negative bacteria. In an animal model, Hoshino et al³⁵ reported that TLR4-deficient mice are hyporesponsive to LPS.³⁵ Arbour et al³⁶ provided the first direct evidence that polymorphisms in the *TLR4* gene in healthy adults were associated with hyporesponsiveness when exposed to a total of 41.5 μ g of LPS (daily LPS exposure level of an agricultural worker).³⁶ In a study of healthy adults, Asp299Gly and Thr399Ile polymorphisms in the *TLR4* gene were associated with a blunted response to inhaled LPS.¹⁵ Another study of healthy young adults reported that those with polymorphisms in the *TLR4* gene had less reduction in the percentage across-shift change in FEV₁ from baseline than did those in the wild-type group when they were exposed for 5 hours in a swine operation.¹⁶ Our study found that among full-time workers in swine operations, Asp299Gly and Thr399Ile polymorphisms in the *TLR4* gene were not significantly associated with lung function, raising the possibility that full-time workers might acclimatize to repeated endotoxin exposure in the workplace.

Workers in swine operations are subjected to long-term workplace exposure to a complex indoor environment, which includes high concentration of gram-positive bacteria.⁸ Experimental studies found reduced immune responses in TLR2-deficient macrophages when exposed to cell walls of gram-positive bacteria¹⁴ and in human embryonic kidney 293 cells when exposed to bleomycin in vitro.¹⁸ A study using an animal model found that the expression of CD86 on lung cells (a protein expressed on antigen-presenting cells for T-cell activation and survival) and tissue-infiltrating M1 macrophages (proinflammatory phenotype) was completely inhibited during the entire bleomycin-induced pulmonary inflammation stage in TLR2-deficient mice; plasmacytoid dendritic cells, M2 macrophages, and FoxP3⁺ T-regulatory cells were significantly blocked at the later stage of pulmonary inflammation in TLR2-deficient mice.¹⁹ In this study, significant protection from apoptosis of pulmonary cells was also observed in TLR2-deficient mice.¹⁹ A recent study found that TLR2 knockout mice had reduced TNF- α and interleukin 6 expression from lung macrophages and significant reductions in total cellularity, neutrophil influx, TNF- α , interleukin 6, and CXCL1 from bronchoalveolar lavage fluid and lung tissue when exposed to swine facility dust extract.²⁰

There are few published studies reporting the effects of polymorphisms in the *TLR2* gene on respiratory systems in the human population. A recent study of 916 children from Netherlands found that children with minor allele A on –16933T/A polymorphism in the *TLR2* gene were significantly less likely to have

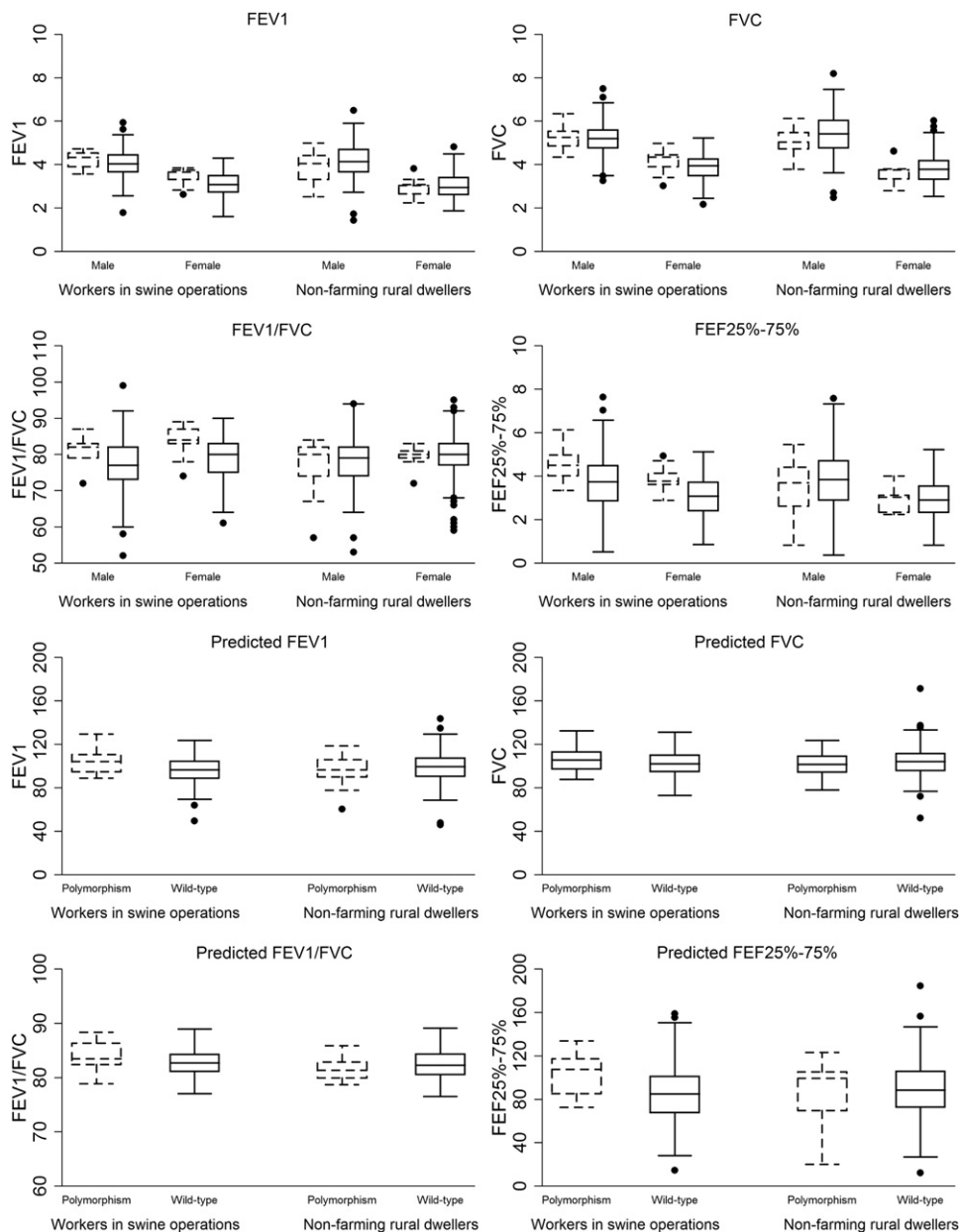


Figure 2. Boxplot of lung function between TLR2 Arg753Gln polymorphic and wild-type groups in rural dwellers and workers. Short dashed line indicates polymorphism group; solid line, wild-type group.

physician-diagnosed asthma compared with children with homozygotes of the T allele (odds ratios, 0.61 for TA vs TT and 0.53 for AA vs TT), and polymorphisms in the *TLR4* gene (Asp299Gly and Thr399Ile) were not significantly associated with physician-diagnosed asthma in this study.³⁷ In a study of 229 farmers' children and 380 nonfarmers' children in Austria and Germany, the *TLR2* -16933T/A polymorphisms were significantly associated with a diagnosis of asthma, current asthma symptoms, atopic sensitization, and current hay fever symptoms among farmers' children but not among children from the same rural communities not living on farms.³⁸ No significant association between polymorphisms of the *TLR4* gene and any asthma and asthma-related symptoms was observed in either farmers' children or nonfarmers' children.³⁸ This study concluded that the *TLR2* gene was a major determinant of asthma in children of European farmers.³⁸

Our study extended the current knowledge from experimental studies using human cells and animal models and human studies

using naive healthy adults to full-time workers who are exposed to high concentration of indoor respiratory hazards at their workplace. Our findings were the first to provide direct evidence that TLR2 Arg753Gln and -16933T/A polymorphisms, rather than *TLR4* Asp299Gly and Thr399Ile polymorphisms, are associated with protective effects on lung function among full-time workers in swine operations possibly due to their reduced proinflammation responses to their workplace exposure through the *TLR2* pathway. This also explains the importance of gram-positive bacteria in causing the adverse health effects among workers in swine operations.

In our study, workers with TLR2-16933T/A polymorphism had statistically significant greater mean values of lung function than workers with the wild-type sequence (160 mL in FEV₁ and 390 mL/s in FEF_{25%-75%}) and a bigger difference in FEF_{25%-75%} (670 mL/s) between the polymorphism and wild-type sequence of TLR2 Arg753Gln. A longitudinal study of 217 swine confinement workers

and 179 nonfarming rural dwellers from 1990–1991 to 1994–1995 found that swine confinement workers had an excess annual decrease of 26.1 mL in FEV₁ and 42.0 mL/s in FEF_{25%–75%} over non-farming rural dwellers.¹ Compared with the annual decrease, the observed differences in FEV₁ and FEF_{25%–75%} between the polymorphic and wild-type groups are clinically relevant for the respiratory health of workers in swine operations.

There were some limitations to our study. Lack of objective measurement of exposures, including endotoxin levels associated with gram-negative bacteria and peptidoglycan levels associated with gram-positive bacteria, in our study limits our ability to examine the dose-response relationship between exposure and lung function among workers with polymorphisms and the wild-type sequence in the *TLR2* and the *TLR4* genes. However, inclusion of nonfarming rural dwellers in our study allowed us to examine the effects of these polymorphisms in the *TLR2* and the *TLR4* genes at low-level exposure.

In conclusion, this study is the first, to our knowledge, to report the airways protective effects of *TLR2* polymorphisms among workers in swine operations. These findings raise the possibility that *TLR2* polymorphisms are protective of airway disease in those exposed to gram-positive organisms in the inhaled airborne dust and genetic testing might provide useful information for identifying workers at higher risk of respiratory problems when working in swine operations.

Supplementary Data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anaai.2012.11.003>.

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eTable 1

Differences in mean values of lung function parameters between TLR2 -16933T/A polymorphism (AA) and wild type (AT+TT) for workers in swine operations (≥ 10 years of swine work) and non-farming rural dwellers

Lung function parameters	Workers in swine operations (≥ 10 years of swine work)			Non-farming rural dwellers			P value (interaction term)
	Polymorphism Mean (95%CI)	Wild-type Mean (95%CI)	Comparison Diff (95%CI)	Polymorphism Mean (95%CI)	Wild-type Mean (95%CI)	Comparison Diff (95%CI)	
Observed ^a							
FEV ₁ , (L)	3.9 (3.7:4.1)	3.6 (3.5:3.8)	0.3 (0.0:0.5)	3.6 (3.5:3.7)	3.6 (3.6:3.7)	0.0 (−0.1:0.1)	.11
FVC, (L)	4.9 (4.6:5.2)	4.7 (4.5:4.9)	0.2 (−0.2:0.5)	4.6 (4.5:4.8)	4.6 (4.6:4.7)	0.0 (−0.1:0.2)	.46
FEV ₁ /FVC, (%)	80.0 (76.7:83.2)	77.4 (75.5:79.4)	2.6 (−1.2:6.3)	78.5 (77.1:78.0)	78.8 (78.1:79.6)	−0.3 (−1.9:1.3)	.16
FEF _{25%–75%} , (L/s)	4.0 (3.43:4.48)	3.3 (2.9:3.6)	0.7 (0.1:1.3)	3.5 (3.3:3.7)	3.5 (3.4:3.5)	0.0 (−0.2:0.3)	.03
Predicted (%) ^b							
FEV ₁	104.6 (98.0:111.2)	97.0 (93.1:101.0)	7.5 (−0.2:15.3)	98.3 (95.4:101.3)	98.2 (96.7:99.8)	0.1 (−3.2:3.4)	.09
FVC	109.0 (102.5:115.5)	103.9 (100.0:107.8)	5.0 (−2.5:12.6)	103.9 (101.0:106.8)	103.4 (101.8:104.9)	0.5 (−2.7:3.7)	.30
FEV ₁ /FVC	82.2 (80.9:83.5)	81.4 (80.6:82.2)	0.8 (−0.7:2.3)	82.5 (82.0:83.1)	82.3 (82.0:82.6)	0.3 (−0.4:0.9)	.57
FEF _{25%–75%}	98.5 (84.9:112.2)	82.0 (73.8:90.1)	16.6 (0.7:32.4)	87.7 (81.6:93.7)	88.0 (84.8:91.2)	−0.3 (−7.1:6.4)	.05

Bold values, $P < .05$.

^aP value from the multiple regression analysis after adjusting for age, sex, height, weight, and smoking habit.

^bP value from the multiple regression analysis after adjusting for smoking habit.

eTable 2

Differences in mean values of lung function parameters between TLR2 Arg753Gln polymorphism (AG) and wild type (GG) for workers in swine operations (≥ 10 years of swine work) and non-farming rural dwellers

Lung function parameters	Workers in swine operations (≥ 10 years of swine work)			Non-farming rural dwellers			P value (Interaction term)
	Polymorphism Mean (95%CI)	Wild-type Mean (95%CI)	Comparison Diff (95%CI)	Polymorphism Mean (95%CI)	Wild-type Mean (95%CI)	Comparison Diff (95%CI)	
Observed ^a							
FEV ₁ , (L)	4.0 (3.39:4.61)	3.7 (3.6:3.8)	0.3 (−0.3:0.9)	3.5 (3.3:3.7)	3.6 (3.6:3.7)	−0.1 (−0.3:0.1)	.19
FVC, (L)	5.3 (4.5:6.0)	4.7 (4.6:4.9)	0.5 (−0.2:1.3)	4.5 (4.3:4.7)	4.6 (4.6:4.7)	−0.1 (−0.4:0.1)	.09
FEV ₁ /FVC, (%)	77.1 (69.0:85.3)	78.2 (76.4:80.0)	−1.1 (−9.4:7.3)	78.4 (75.8:81.0)	78.8 (78.1:79.5)	−0.3 (−3.0:2.3)	.90
FEF _{25%–75%} , (L/s)	3.5 (2.2:4.9)	3.5 (3.2:3.7)	0.1 (−1.3:1.4)	3.4 (2.9:3.8)	3.5 (3.4:3.6)	−0.1 (−0.6:0.3)	.74
Predicted (%) ^b							
FEV ₁	111.8 (94.9:128.6)	98.6 (95.0:102.1)	13.2 (−4.0:30.4)	96.2 (90.9:101.6)	98.4 (97.0:99.9)	−2.2 (−7.7:3.3)	.10
FVC	119.8 (103.2:136.3)	104.7 (101.2:108.1)	15.1 (−1.8:32.0)	101.3 (96.1:106.6)	103.6 (102.2:105.1)	−2.3 (−7.7:3.1)	.06
FEV ₁ /FVC	83.3 (80.0:86.7)	81.5 (80.8:82.2)	1.8 (−1.6:5.2)	81.6 (80.5:82.6)	82.4 (82.1:82.7)	−0.8 (−1.9:0.3)	.16
FEF _{25%–75%}	94.2 (59.7:128.7)	86.4 (79.1:93.6)	7.8 (−27.3:43.0)	86.8 (75.8:97.7)	88.1 (85.1:91.1)	−1.3 (−12.6:9.9)	.64

^aP-value from the multiple regression analysis after adjusting for age, sex, height, weight, and smoking habit

^bP-value from the multiple regression analysis after adjusting for smoking habit.