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Gender-specific associations between polymorphisms in the *Toll-like receptor* (*TLR*) genes and lung function among workers in swine operations

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Abstract

Workers in swine operations are exposed to dust, bacteria, and virus, and are at increased risk of respiratory problems. Toll-like receptors (TLR) play an important role in human immune responses to respiratory hazards. Worker gender and age may significantly modify the involvement of TLR in the etiology of these respiratory outcomes. The aim of this study was to investigate whether modification effects of worker gender and age altered associations between polymorphisms in the *TLR* genes and lung function. This study included 374 full-time workers from large swine operations from Saskatchewan. Information on demography, lifestyle, pulmonary function, and blood samples were obtained. Multiple linear regression and decision tree model were used in the analysis. Among females aged <45.8 years, workers with polymorphisms of rs4696480 in the *TLR2* gene exhibited markedly better lung function than workers with wild-type. These associations were not observed among female workers aged ≥45.8 years and males. Among males, workers with polymorphisms of rs187084 in the *TLR9* gene displayed significantly lower lung function than those with wild-type. This male-specific association was not dependent on worker age. This is the first study to report gender-specific correlations between lung function and polymorphisms of *TLR* genes, and modification effects of worker age on these associations, suggesting the importance of considering gender and age in genetic association studies of airway diseases due to exposure of high concentration of respiratory hazards.

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Declarations of interest

None.

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Keywords

airway

Introduction

Gender differences in incidence, susceptibility, and severity of many lung diseases have been long recognized. Females are at increased risk of development of asthma, airway hyperresponsiveness, and severe exacerbation (Becklake and Kauffmann 1999; Leynaert et al. 1997; Manfreda et al. 2004; Townsend, Miller, and Prakash 2012). Males are more susceptible to bacteria and virus infections, infectious complications after surgery, severe sepsis and septic shock (Merkel et al. 2001; Offner, Moore, and Biffl 1999; Traub et al. 2012; Wichmann et al. 2000; Yamamoto et al. 1991). Although the biological mechanisms underlying gender differences in pulmonary responsiveness are not fully understood, recent evidence suggests the involvement of sex-related hormones (Lange et al. 2001; Redline and Gold 1994; Troisi et al. 1995). Epidemiological studies demonstrated significant differences in many lung diseases prior to and after puberty, menopause, and andropause, when sex hormones undergo marked changes. Before the onset of puberty, when sex hormones are generally present at low concentration levels, boys are at higher risk of developing asthma than girls (Redline and Gold 1994). However, after puberty, which is related to an increase in sex hormones levels, females are more likely to be diagnosed with asthma (Redline and Gold 1994). In women, menopause initiates complex biological changes which include alterations in sex hormone levels. The onset of menopause is associated with a decrease in sex hormone levels and thus reported to provide protection against asthma, while the use of postmenopausal hormones may reverse the protective effects of menopause (Lange et al. 2001; Troisi et al. 1995). Significant lung function changes during the menstrual cycle phases and enhanced asthma exacerbations during pregnancy were reported in many studies (Driver et al. 2005; Murphy, Clifton, and Gibson 2006; Stanford et al. 2006). Taken together results from these investigations suggested sex hormones play a major role in many chronic lung diseases.

Toll-like receptors (TLRs), a family of pattern recognition receptors (PRR), play an important role in both innate and adaptive immune responses (Iwasaki and Medzhitov 2004; Lee and Lawrence 2018; Tipping 2006). Several investigators demonstrated gender differences in immunological responses after stimulation of TLR ligand (Berghofer et al. 2006; Clifford et al. 2012; Griesbeck et al. 2015; Iyer and Cheng 2012; Torcia et al. 2012; Traub et al. 2012). Further ovariectomy and estradiol replacement therapy significantly modified these gender-specific immune responses (Marriott, Bost, and Huet-Hudson 2006; Soucy et al. 2005). However, fewer studies examined gender differences in these associations in a human population. The aim of this study was to (1) examine the gender-specific associations between lung function and polymorphisms of *TLR* genes and (2) determine the possible modification effects of age on these associations in a human population.

Methods and materials

Ethics approval

The study was approved by the Biomedical Research Ethics Board of the University of Saskatchewan. Informed written consent was obtained from all subjects.

Recruitment and data collection

Detailed information on the recruitment and data collection of this cross-sectional study are described elsewhere (Gao et al. 2013, 2014; Senthilselvan et al. 2009, 2007, 1997, 2008). Briefly, 374 full-time workers were recruited from large swine production companies in Saskatchewan. Only those workers who were 17 years or older and worked for a minimum of 4 days per week with a total work-duration of 20 hr or more per week were included in this study. The participation rate was approximately 70%.

A previously validated questionnaire was used to collect anthropomorphic data, respiratory symptoms, smoking history, past illnesses, and occupational history (Gao et al. 2013, 2014, 2009, 2007; Senthilselvan et al. 1997, 2008). Each participant also had measurements taken of height (cm), weight (kg), pulmonary function, and systolic and diastolic blood pressure (mmHg).

Pulmonary function measurements including forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of FVC ($FEF_{25\%–75\%}$) were obtained by trained technicians using a volume displacement spirometer (model 1022; Sensor-Medics, Yorba Linda, California) who followed the American Thoracic Society recommendations (1987, 136:1285–1298). All interviews and pulmonary function tests were conducted in local communities near the swine production sites.

Skin prick tests (SPT) for 5 allergens (Alternaria sp, swine, mixed grass allergens, house dust mite, and cat dander) were performed on the same occasion as the pulmonary function tests as described in previously (Gao et al. 2013, 2014; Senthilselvan et al. 2008). Solutions for the 5 allergens were obtained from Western Allergy Services Ltd, Burnaby, BC, Canada. Histamine and saline were also included as the positive and negative control (Gao et al. 2013, 2014; Senthilselvan et al. 2008). The tests were performed on normal skin of each worker's forearms with a distance of 2 cm between two SPT to avoid cross-contamination. A positive test was defined as one or more SPT had a raised wheal of 3 mm or larger than the saline control (Gao et al. 2013, 2014; Senthilselvan et al. 2008). The technicians conducting the SPT were not aware of the genotypes of each worker.

Smoking status was defined from the questionnaire as following: a current smoker (a person currently smoking cigarettes); an ex-smoker (a person who has smoked more than 400 cigarettes (or equivalent amount of tobacco) in his/her lifetime but was not currently smoking); and a non-smoker (a person who had not smoked more than 400 cigarettes (or equivalent amount of tobacco in his/her lifetime).

DNA isolation and genotyping

Detailed information on blood sample collection and genotyping methods was previously described (Pahwa et al. 2009). Briefly, Qiagen PAXgene tubes with blood cards spotted for each sample (S&S, catalog #10538414) were used to collect blood samples. DNA was isolated by a Gentra Autopure robot (Qiagen Corporation, Hilden, Germany), and 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. All other TLR single nucleotide polymorphisms (SNP) 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, CA, USA). The BeadStudio software (Illumina® GenomeStudio) was employed to cluster and clean raw genotyping data.

Selection of TLR SNP

The selection of single nucleotide polymorphism (SNP) in the *TLR* genes was based upon the following criteria as described previously (Gao et al. 2013, 2014; Senthilselvan et al. 2009): (i) SNP that were associated with one or more disease phenotypes in multiple cohorts or shown to alter gene function in biological assays; and (ii) tagging SNP (tSNP) of TLR genes identified using high-density SNP maps generated by the HapMap project. The tSNP of each TLR gene were selected using the SNPSelector software which prioritizes SNP on their tagging for linkage disequilibrium, SNP allele frequencies and source, function, regulatory potential, and repeat status (Xu et al. 2005). A total of 11 SNP from all *TLR* genes except *TLR8* were selected in our study.

Statistical analysis

Descriptive statistics, mean (STD) and N (%) were provided for continuous and categorical variables, respectively. Significant differences in characteristics and genotypes between male and female workers were examined by chi-square test for categorical variables and Student's t-test for continuous variables. Fisher's exact test was used if the expected count for any cell was less than 5. Only those SNP which followed HWE (Hardy–Weinberg Equilibrium) and are common variances, Minor Allele Frequency (MAF) ≥5%, were included in this study. HWE was examined among females for the SNP of the *TLR7* and *TLR8* genes on the X chromosome. For the SNP in high Linkage disequilibrium (LD), D'>0.95, the SNP with the highest polymorphism information content (PIC) value was selected. Both dominant and recessive inheritance models were considered. However, only the inheritance model with the smallest AIC value (the best-fitted model) was selected.

To investigate the gender-specific association between lung function and polymorphisms of *TLR* genes, gender-stratified multivariable analysis was carried out after controlling for potential confounders including age, height, weight, and smoking status.

To examine the modification effects of age on the gender-specific associations between lung function and a SNP, a decision tree analysis was utilized by including age, gender, and a SNP (dominant and recessive inheritance) as independent variables in the model (SAS

2015). This method is based upon recursively splitting a dataset by independent variables into non-overlapping segments in such a way that all individuals within each segment are as similar as possible, which is measured by Gini index, entropy, and residual sum of squares (SAS 2015). In this study, age was included as a continuous variable. In order to reduce the number of computations for searching candidate cut-points of the age variable, the INTERVALBINS = 5 was defined. A minimum of 5 observations was also defined in child segments of a split. Based upon the results from a decision model, age group-stratified multivariable analysis was carried to further examine these gender-specific associations between a SNP and lung function in each age group after controlling for potential confounders including height, weight and smoking habit. The Bonferroni adjustment was applied in this study. The criterion for significance was set at $p < 0.05$.

Sensitivity analysis

To confirm the modification effects of age on the gender-specific associations between lung function and a SNP, in addition to age, gender, and a SNP variable, smoke, height, and weight were also included in the decision tree models. As the cut-points of age variable given by a decision tree analysis were based upon maximizing the homogeneity in all segments, a series of sensitivity analyses were conducted by using different cut-points of the age variable at 40, 45, and 50 years, respectively.

Results

As indicated in Table 1, male workers were significantly heavier and taller on average than females accompanied by significantly higher mean values of lung function parameters except for FEV_1/FVC ratio, which was significantly lower in males. The genotype distributions of most SNP were similar between female and male workers, except for *TLR5*-rs5744168 and *TLR10*-rs4129009 polymorphisms. The two polymorphisms in the *TLR4* gene (rs4986790 and rs4986791) were in high linkage disequilibrium (D' = 0.97) and TLR4-rs4986790 polymorphisms were selected for further analysis because of its higher polymorphism information content (PIC) value. The *TLR2*-rs 5743708 and the *TLR5*-rs5744168 polymorphisms were excluded due to their minor allele frequency (MAF) less than 5%. This yielded a total of 7 SNP.

The gender-specific associations from multivariate analysis after controlling for potential confounders (age, height, weight, and smoking habit) are shown in Table 2. Among males, workers with the *TLR3*-rs 3775291 polymorphisms exhibited significantly higher mean values of FEV_1 and FVC than those with wild-type, and individuals with the *TLR6*-rs 5743810 polymorphisms displayed significantly higher mean values of FEV_1/FVC and FEF25%−75% than those with wild-type. Workers with the *TLR9*-rs187084 polymorphisms on average exhibited significantly higher FEV_1/FVC, but significantly lower FVC than those with wild-type. After adjusting for multiple comparisons using Bonferroni's correction at $\alpha = 0.007$ (0.05/7) significance level, only the association between the *TLR9*-rs187084 polymorphisms and FVC remained significant among males. Among females with the *TLR2*-rs4696480 polymorphisms they on average displayed significantly higher lung function in FEV_1, FVC and FEF25%−75% than those with wild-type. After adjusting for

multiple comparisons, at $\alpha = 0.007$ (0.05/7) significance level, associations with FEV_1 and FVC remained significant among females.

The modification effects of work's age on female-specific associations between *TLR2*-rs4696480 polymorphisms and lung function are presented in Table 3. The age cut-off from the decision tree models was at 45.8 years in females and 36.2 years in males (see the outputs from decision tree models in Figures 1 and 2). The Figures 1 and 2 illustrate the *TLR2*-rs4696480 polymorphisms selected by the decision tree among females aged <45.8 years in the model of FEV_1 and FVC, and were not selected among females aged ≥45.8 years and males. Among females who were younger than 45.8 years, individuals with the *TLR2*-rs4696480 polymorphisms exhibited significantly higher average lung function in FEV_1 and FVC than those with wild-type after adjusting for height, weight and smoking habit. These associations remained significant after adjusting for multiple comparisons at $\alpha = 0.007$ (0.05/7) significance level. No marked associations were observed among female workers who were older than 45.8 years, and among males.

Sensitivity analysis confirmed the female-specific associations between *TLR2*-rs4696480 polymorphisms and lung function (FEV_1 and FVC) were significant among the females only in the younger age group, but not in the older age group using different cut-offs of age at 40, 45 or 50 years (Table 4). Further, decision tree analysis was conducted by including age, gender, *TLR2*-rs4696480, weight, height, and smoke status. The results from the decision tree analysis also confirmed modification effects of age on these female-specific associations only in the younger age group since *TLR2*-rs4696480 polymorphism was not selected by the decision tree among female workers aged ≥45 years (see the outputs from decision tree models in Figures 3 and 4).

Discussion

Data demonstrated that female workers with *TLR2* -rs 4696480 polymorphisms displayed significantly higher mean values of FEV_1 and FVC than females with wild-type, but this relationship was not observed in males. Further, these female-specific associations were dependent upon age and only noted in the younger but not older age group. Male-specific association was also found where male workers with *TLR9*-rs187084 polymorphisms were significantly correlated with lower mean FVC values than males with wild-type. However, this male-specific association was not dependent upon age.

Workers in animal confinement facilities are at higher risk of lung dysfunction due to exposure to a large variety of hazards including bacteria (gram-positive and gram-negative), viruses, fungi, dust particles, and gases (Dosman et al. 2004; Eduard, Pearce, and Douwes 2009; Harting et al. 2012; May, Romberger, and Poole 2012, 2007; Senthilselvan et al. 1997; Viegas et al. 2013). Several investigators showed that the involvement of TLR2 in the airway diseases among workers in swine operation and the important role of TLR9 in lung inflammation following exposure to chicken barn air (Gao et al. 2013; Harting et al. 2012; Just, Duchaine, and Singh 2009; May, Romberger, and Poole 2012; Schneberger et al. 2016). TLR2 mainly responds to cell wall structure components such as peptidoglycan from gram-positive bacteria, and the ligand of TLR9 is un-methylated DNA which is found in bacterial

and viral DNA (Hemmi et al. 2000; Iwasaki and Medzhitov 2004). The activation of TLR induces a series of intracellular signaling cascades to produce both pro- and anti-inflammatory cytokines and chemokines, which all work together to respond to invading pathogens (Hemmi et al. 2000; Iwasaki and Medzhitov 2004; Tipping 2006).

TLR2-16934 (rs4696480) is a promoter variant of *TLR2* gene. An experimental study of cord blood mononuclear cells showed the carriers of AA genotype of *this SNP* exhibited significantly increased gene expression of several Treg (regulatory T cells) marker genes including FOXP3 (forkhead box protein p3), GITR (glucocorticoid-induced tumor necrosis factor receptor) and LAG3 (lymphocyte activation gene 3) as well as Th2 (T helper cell type 2) cytokines and TNF-α (tumor necrosis factor alpha) secretion in the presence of maternal atopy and postulated modification effects of maternal atopy status on this association may be partially explained by epigenetic regulation (Liu et al. 2011). Eder et al. (2004) reported that farm children from rural areas in Austria and Germany with a T allele of *TLR2*-16934 (rs4696480) were less susceptible to the diagnosis of asthma and atop, but not among children who did not reside on a farm. Kerkhof et al (2010) noted that this SNP significantly modified the influence of $PM_{2.5}$ exposure on doctor-diagnosed asthma in children in the Netherlands.

Animal studies showed the *TLR2* gene is responsive to sex hormones (Li and Bai 2014; Poole et al. 2011; Scotland et al. 2011; Soucy et al. 2005; Yang et al. 2009). Soucy et al. (2005) demonstrated that a single injection of LPS to mice increased transcriptional activation of *TLR2* gene in the brain, which was completely inhibited by ovariectomy. Further estradiol replacement therapy was able to reverse the inhibitory effects of ovariectomy by stimulation of estrogen receptor (ER) α. Scotland et al. (2011) noted an elevated number of leukocytes in naïve peritoneal and pleural cavities of females compared to males, and resident peritoneal macrophages from females exhibited significantly higher expression of *TLRs* including *TLR2, TLR3*, and *TLR4* than males. It is of interest that ovariectomy significantly reduced mRNA expression levels of these *TLR*, as well as protein expression of *TLR2* and *TLR4* in female resident macrophages. Li and Bai (2014) using human THP-1 cells confirmed not only the involvement of 17β-estradiol (E2) in promoting transcription activity of *TLR2* mRNA, but also identified an ER response element in the 5′-flanking region, 251 bases upstream of the *TLR2* promoter region, which regulates E2-mediated upregulation of transcription activity and protein expression of the *TLR2* gene. Our study extended current knowledge from experimental studies using cells and animal models to a human population by providing direct evidence showing female-specific associations between polymorphisms of *TLR2* gene and lung function.

In humans, levels of sex hormones not only vary between males and female, but change according to age. Loss of sex hormones begins around the age of 35–40 in males and 40–50 in females, and these natural alterations in sex hormones provide a unique opportunity to study sex-hormone sensitive genes (Horstman et al. 2012). Population studies demonstrated the median age at natural menopause in Caucasian women is approximately 50 years, and perimenopause starts at 47.5 years, when female hormone levels start to markedly decrease (Horstman et al. 2012; McKinlay 1996). As a result of female hormone levels falling due to aging and menopause, female hormone sensitive genes may function differently, especially

after menopause. In our study, female-specific associations between *TLR2* -rs 4696480 polymorphisms and lung functions were only found among the younger age group, when female hormones are at higher levels.

Our study showed a male-specific association between FVC and the *TLR9*-rs187084 polymorphisms. Sex differences in susceptibility to infection were reported in various experimental and population studies. In comparison with female mice, males are more susceptible to infection and endotoxin shock induced by LPS (Merkel et al. 2001; Yamamoto et al. 1991). Human population investigations also confirmed males are more susceptible to bacterial infection (Offner, Moore, and Biffl 1999; Wichmann et al. 2000). A large prospective cohort of surgical intensive care patients from Germany demonstrated female patients displayed a lower chance to be referred to the ICU, and were less likely to develop severe sepsis/septic shock than male patients (Wichmann et al. 2000). Another prospective cohort study of 545 trauma patients from the USA also noted that males were 1.6-fold more likely to develop major infectious complications after surgery (Offner et al. Biffl 1999).

The involvement of the *TLR9* gene in the regulation of inflammation was reported in animal model by Schneberger et al. (2016). Expression of *TLR9* and production of cytokines following TLR9 stimulation varied significantly between males and females (Torcia et al. 2012; Traub et al. 2012). The *TLR9* mRNA expression was 5-fold higher in male than female mice following infection of mouse cytomegalovirus (MCMV) (Traub et al. 2012). Torcia et al. (2012) examined IL10 production from the peripheral blood mononuclear cell (PBMC) and reported that following TLR9 stimulation or infection with Herpes simplex virus (HSV-1), IL10 production was significantly higher in males and females in post-menopausal age than females in reproductive age. A large case-control study from Taiwan also showed the male-specific association between polymorphisms in the *TLR9* gene and Graves' disease (Liao et al. 2010). The mechanisms underlying sex hormones modification of the *TLR9* gene are still unknown. However, several studies demonstrated that testosterone may upregulate production of IL10, which plays a key role in limiting host immune response to pathogens by activating anti-inflammatory immune pathways, reducing the production of inflammatory cytokines and enhancing activation of regulatory T cells, whereas estrogens downregulate the *TLR9* expression in ER + breast cancer cells (Iyer and Cheng 2012; Jukkola-Vuorinen et al. 2009; Liva and Voskuhl 2001; Torcia et al. 2012).

Sex hormones significantly modulate cell signaling pathways to control gene regulation and expression. Activation of the NF-κB family of transcription factors is a major result of TLR ligation with pathogen-associated molecular patterns and plays an important role in inflammatory responses. Estrogen receptors (ERs) might inhibit NF-κB activity in multiple ways in cytoplasm and in nucleus (Kalaitzidis and Gilmore 2005) by the following mechanisms (as illustrated in Supplementary Figure 1): (a) ERs directly interacting with NF-κB to block its binding to DNA, (b) ERs inhibiting IκB kinase complex (IKK) activity, (c) ERs inhibiting IκB degradation, (d) ERs inhibiting NF-κB activity by competing for NF-κB coactivators, and (e) ERs interfering with binding to NF-κB coactivators.

Decision tree analysis has gained attention among computational, biomedical, and medical researchers. The advantages and disadvantages of this method were comprehensively reviewed by Song and Zhang (2014) and Song and Lu (2015). Recently, due to rapid advances in microarray technology, investigators are facing the challenge of how to effectively analyze large numbers of genetic markers from limited numbers of samples in microarray data. Machine learning has been widely used for microarray analysis to identify genetic markers to improve diagnosis and prediction of prognosis or responses in patients receiving a particular treatment of many diseases such as systemic lupus erythematosus, primary antiphospholipid syndrome (Armananzas et al. 2009) and cancers (Deist et al. 2018; Wang 2014). Simplicity and easy interpretation makes this method popular. However, this method has the disadvantage of being unstable, i.e. the optimal decision tree based upon a small dataset is generally unstable, and suffers from overfitting, which limits its generalizability and robustness.

There were some limitations to our study. This investigation did not have an objective measure of peptidoglycan exposure levels associated with gram-positive bacteria. Lack of sex hormone measurement or self-report of menopause information also limited our ability to examine gender-specific associations and compare the female-specific associations prior to and post-menopause. The age cut-off at 45.8 years from decision tree is a rough measure of loss of female hormones generally beginning at the age of 40–50 years in females (Horstman et al. 2012). However, consistent results from our sensitivity analyses using three different cut-offs of age at 40, 45 and 50 years confirmed these female-specific associations in the younger age group. Small sample size of this study (n = 374) also limits further stratification of our statistical analysis and results from this study need to be confirmed in a large cohort.

Conclusions

This is the first human population study, to our knowledge, to report gender-specific associations between polymorphisms of *TLR* genes and lung function among workers in swine operations, and modification effects of age on these gender-specific associations. These findings emphasize the importance of considering gender and age in all genetic association studies of respiratory diseases since the underlying causes of these diseases might vary not only between males and females, but also between different age groups. Our study provides useful information for the prevention and treatment of lung diseases among workers exposed to high levels of respiratory hazards.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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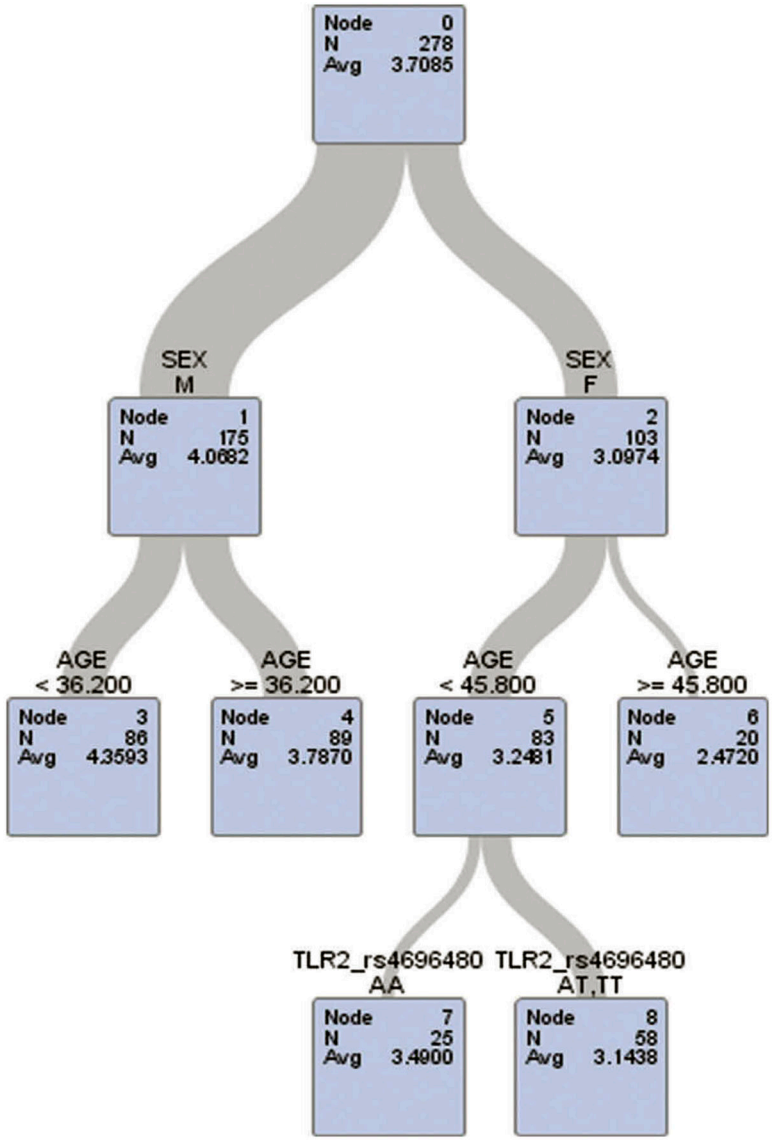

Figure 1.
Decision tree analysis of FEV1 with age, sex, and TLR2-rs4696480 in the model.

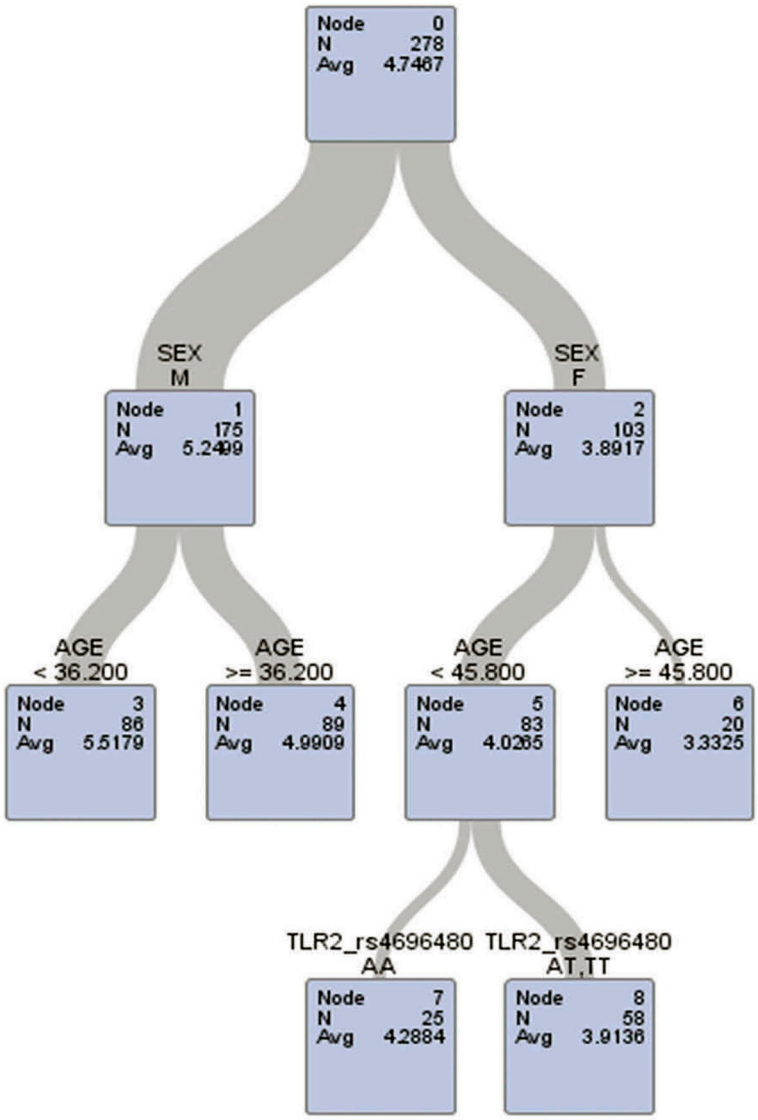

Figure 2.
Decision tree analysis of FVC with age, sex, and TLR2-rs4696480 in the model.

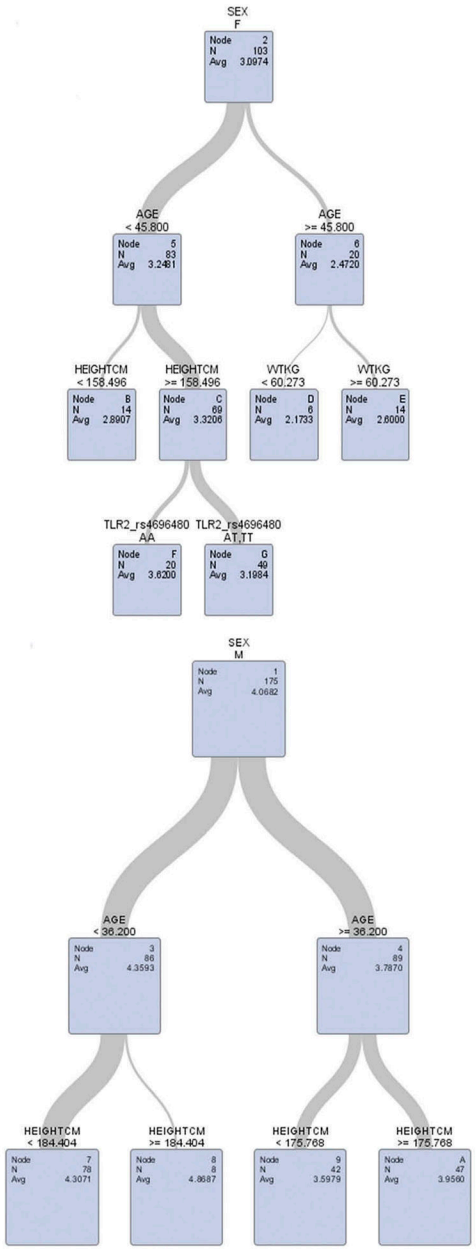

Figure 3.

Decision tree analysis of FEV1 with age, sex, TLR2-rs 4696480, smoke, weight, and height in the model.

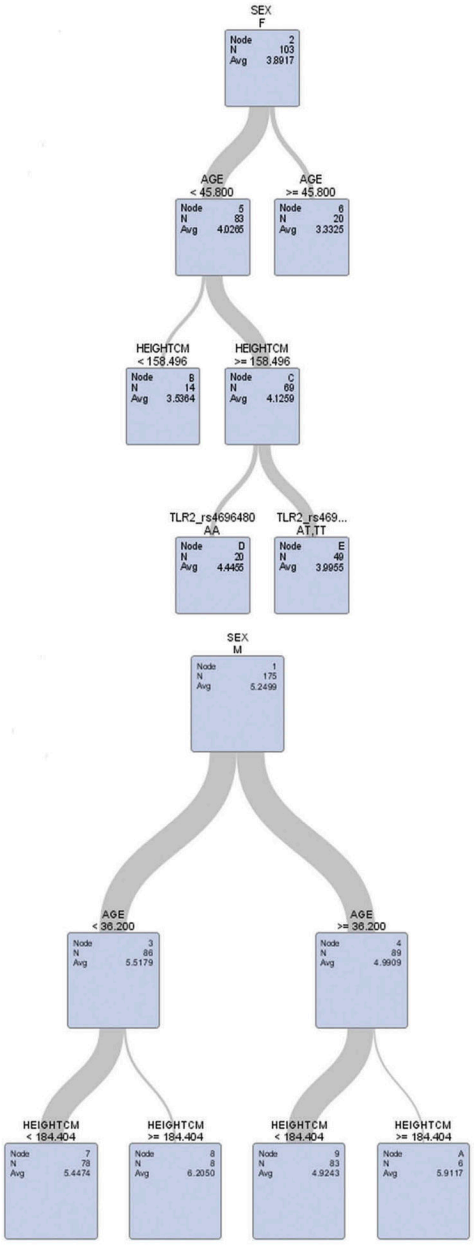

Figure 4.
Decision analysis of FVC with age, sex, TLR2-rs 4696480, smoke, weight, and height in the model.

Table 1.

Comparison of characteristics between male and female workers*.

	Workers in swine operations	
Characteristic	**Males (N = 240)**	**Females (N = 134)**
Age, year	36.2 ±11.9	34.9 ± 10.7
Weight, kg	88.1 ± 16.6	75.5 ± 15.0#
Height, cm	170.8 ± 6.1	164.4 ± 5.8#
Smoking Status, N (%)		
Current smoker	73 (30.4)	44 (32.8)
Former smoker	68 (28.3)	33 (24.6)
Non smoker	99 (41.3)	57 (42.5)
Atopy, N (%)		
Yes	86 (38.7)	37 (29.4)
No	136 (61.3)	89 (70.6)
Lung function		
Observed		
FEV_1, L	4.1 ± 0.7	3.1 ± 0.6#
FVC, L	5.3 ± 0.8	3.9 ± 0.6#
FEV1/FVC, %	77.8 ± 6.6	78.0 ± 6.2#
$FEF_{25\%-75\%}$, L	3.8 ± 1.2	3.2 ± 1.0#
TLR1, N (%)		
RS5743551		
AA	78 (44.6)	58 (56.3)
AG	78 (44.6)	38 (36.9)
GG	19 (10.9)	7 (6.8)
MAF(G)	116 (33.1)	52 (25.2)
TLR2, N (%)		
RS4696480		
AA	40 (22.9)	28 (27.2)
AT	89 (50.9)	55 (53.4)
TT	46 (26.3)	20 (19.4)
MAF(A)	181 (51.7)	95 (46.1)
RS5743708		
AG	9 (5.2)	9 (8.8)
GG	164 (94.8)	93 (91.2)
MAF(A)	9 (2.6)	9 (4.4)
LD (D′)	0.5523	
TLR3, N (%)		
RS3775291		
AA	27 (15.3)	9 (8.8)
AG	72 (40.9)	45 (44.1)

	Workers in swine operations	
Characteristic	**Males (N = 240)**	**Females (N = 134)**
GG	77 (43.8)	48 (47.1)
MAF(A)	126 (35.8)	63 (30.9)
TLR4, N (%)		
RS4986790		
AA	150 (86.7)	88 (88.0)
AG	23 (13.3)	12 (12.0)
MAF(G)	23 (6.7)	12 (6.0)
RS4986791		
CT	20 (11.6)	13 (13.0)
TT	153 (88.4)	87 (87.0)
MAF(C)	20 (5.8)	13 (6.5)
LD (D′)	0.9652	
TLR5, N (%)		
RS5744168		
AG	9 (5.1)	16 (15.8)#
GG	169 (94.9)	85 (84.2)
MAF(A)	9 (2.5)	16 (7.9)
TLR6, N (%)		
RS5743810		
AA	21 (12.0)	17 (16.7)
AG	80 (45.7)	43 (42.2)
GG	74 (42.3)	42 (41.2)
MAF(A)	122 (34.9)	77 (37.8)
TLR9, N (%)		
RS187084		
AA	66 (37.7)	27 (26.5)
AG	76 (43.4)	54 (52.9)
GG	33 (18.9)	21 (20.6)
MAF(G)	142 (40.6)	96 (47.1)
TLR10, N (%)		
RS4129009		
AA	97 (55.4)	74 (72.6)#
AG	64 (36.6)	23 (22.6)
GG	14 (8.0)	5 (4.9)
MAF(G)	92 (26.3)	33 (16.2)

*Data are presented as mean ±SD unless otherwise indicated.

The polymorphisms of rs2302267 in the *TLR7* gene were excluded from our study due to the violation of the HWE in female workers.

The polymorphism of rs5743708 in the *TLR2* gene and the polymorphisms of rs5744168 in the *TLR5* gene were excluded due to their minor allele frequency (MAF) less than 5%.

#Significant from males $p < 0.05$

Table 2.

Gender-specific effects of polymorphisms of the *TLR* genes on lung function in the workers in multivariate analysis*.

		Male workers			Female workers		
Gene	**Lung function**	**Polymorphism mean (SE)**	**Wild-type mean (SE)**	**Difference (SE)**	**Polymorphism mean (SE)**	**Wild-type mean (SE)**	**Difference (SE)**
TLR1							
RS5743551	FEV_1, L	3.84 (0.06)	3.86 (0.06)	−0.03 (0.07)^d	3.22 (0.07)	3.22 (0.07)	−0.01 (0.07)^d
	FVC, L	4.92 (0.07)	4.98 (0.09)	−0.06 (0.09)^d	4.17 (0.09)	4.24 (0.08)	−0.07 (0.09)^d
	FEV_1/FVC, %	78.35 (1.55)	77.67 (0.66)	0.68 (1.55)^r	76.82 (0.99)	75.76 (0.94)	1.06(0.98)^d
	$FEF_{25\%–75\%}$, L	3.70 (0.27)	3.62 (0.11)	0.09 (0.27)^r	2.84 (0.28)	2.94 (0.13)	−0.10 (0.29)^r
TLR2							
RS4696480	FEV_1, L	3.93 (0.08)	3.81 (0.05)	0.12 (0.09)^r	3.39 (0.08)	3.14 (0.06)	0.25 (0.08)^{r‡}
	FVC, L	5.01 (0.09)	4.92 (0.06)	0.08 (0.10)^r	4.38 (0.09)	4.11 (0.08)	0.27 (0.09)^{r‡}
	FEV_1/FVC, %	78.41 (1.06)	77.25 (0.72)	1.16 (1.19)^r	76.82 (1.12)	75.92 (0.91)	0.90 (1.13)^r
	$FEF_{25\%–75\%}$, L	3.88 (0.18)	3.48 (0.12)	0.40 (0.21)^r	3.16 (0.16)	2.82 (0.13)	0.34 (0.16)^{r‡}
TLR3							
RS3775291	FEV_1, L	4.02 (0.09)	3.80 (0.05)	0.21 (0.10)^{r†}	3.15 (0.13)	3.26 (0.06)	−0.11 (0.12)^r
	FVC, L	5.16 (0.12)	4.89 (0.06)	0.27 (0.12)^{r†}	4.20 (0.15)	4.23 (0.08)	−0.04 (0.15)^r
	FEV_1/FVC, %	77.31 (0.75)	78.20 (0.87)	−0.89 (0.99)^d	74.91 (1.75)	76.64 (0.87)	−1.73 (1.71)^r
	$FEF_{25\%–75\%}$, L	3.84 (0.22)	3.56 (0.12)	0.28 (0.24)^r	2.66 (0.25)	3.02 (0.13)	−0.36 (0.25)^r
TLR4							
RS4986790	FEV_1, L	3.91 (0.11)	3.87 (0.05)	0.04 (0.12)^d	3.17 (0.11)	3.29 (0.07)	−0.12 (0.12)^d
	FVC, L	5.13 (0.14)	4.97 (0.06)	0.16 (0.14)^d	4.05 (0.12)	4.23 (0.08)	−0.18 (0.13)^d
	FEV_1/FVC, %	76.31 (1.44)	77.82 (0.66)	−1.50 (1.45)^d	77.65 (1.39)	77.59 (0.92)	0.06 (1.51)^d
	$FEF_{25\%–75\%}$, L	3.58 (0.25)	3.65 (0.11)	−0.06 (0.25)^d	3.01 (0.22)	3.19 (0.14)	−0.18 (0.23)^d
TLR6							
RS5743810	FEV_1, L	4.03 (0.11)	3.82 (0.05)	0.21 (0.11)^r	3.23 (0.07)	3.21 (0.08)	0.02 (0.07)^d

		Male workers			Female workers		
Gene	**Lung function**	**Polymorphism mean (SE)**	**Wild-type mean (SE)**	**Difference (SE)**	**Polymorphism mean (SE)**	**Wild-type mean (SE)**	**Difference (SE)**
	FVC, L	5.02 (0.14)	4.93 (0.06)	0.09 (0.14)^{r}	4.23 (0.08)	4.18 (0.09)	0.05 (0.09)^{d}
	FEV_1/FVC, %	80.40 (1.49)	77.34 (0.66)	3.06 (1.50)^{r†}	77.34 (1.37)	76.01 (0.86)	1.33 (1.29)^{r}
	$FEF_{25\%–75\%}$, L	4.14 (0.26)	3.55 (0.11)	0.59 (0.26)^{r†}	3.07 (0.20)	2.91 (0.13)	0.16 (0.19)^{r}
TLR9							
RS187084	FEV_1, L	3.74 (0.09)	3.88 (0.05)	−0.14 (0.09)^{r}	3.25 (0.09)	3.21 (0.07)	0.03 (0.09)^{r}
	FVC, L	4.68 (0.10)	5.02 (0.06)	−0.34 (0.11)^{r‡}	4.30 (0.11)	4.17 (0.08)	0.13 (0.10)^{r}
	FEV_1/FVC, %	79.59 (1.16)	77.09 (0.69)	2.51 (1.24)^{r†}	75.01 (1.20)	76.67 (0.89)	−1.66 (1.19)^{r}
	$FEF_{25\%–75\%}$, L	3.79 (0.20)	3.56 (0.12)	0.24 (0.22)^{r}	2.98 (0.13)	2.82 (0.17)	0.16 (0.16)^{d}
TLR10							
RS4129009	FEV_1, L	3.94 (0.13)	3.84 (0.05)	0.11 (0.13)^{r}	3.18 (0.17)	3.23 (0.06)	−0.04 (0.17)^{r}
	FVC, L	5.04 (0.16)	4.94 (0.06)	0.10 (0.16)^{r}	4.13 (0.20)	4.21 (0.08)	−0.08 (0.20)^{r}
	FEV_1/FVC, %	77.83 (0.83)	77.51 (0.79)	0.32 (0.98)^{d}	76.55 (1.17)	76.11 (0.89)	0.45 (1.09)^{d}
	$FEF_{25\%–75\%}$, L	3.57 (0.14)	3.65 (0.14)	−0.09 (0.17)^{d}	2.76 (0.34)	2.95 (0.13)	−0.19 (0.34)^{r}

*: Only those SNPs which follow HWE and are common variances (MAF>5.0%) were selected into the multivariate models adjusting for age, height, weight, and smoking habit.

†: Significant at $\alpha = 0.05$ level.

‡: Significant after adjusting for multiple comparison at $\alpha = 0.007$ (0.05/7).

^{d}: (dominant inheritance has the best-fitted model).

^{r}: (recessive inheritance has the best-fitted model).

Table 3.

Modification effects of age on the female-specific associations between the *TLR2*-rs4696480 polymorphisms and lung function (N = 103)[*].

	***TLR2*-RS4696480 polymorphisms**		
	Polymorphism mean (STE)	**Wild-type mean (STE)**	**Comparison[†] difference (STE)**
	Female workers aged <45.8 years		
	(N = 83, Mean = 31, range:18–45)		
Lung function			
FEV1, L	3.68 (0.09)	3.39 (0.08)	0.30 (0.09)[‡]
FVC, L	4.59 (0.09)	4.28 (0.08)	0.30 (0.10)[‡]
	Female workers aged ≥45.8 years		
	(N = 20, Mean = 50 Range:46–57)		
FEV1, L	2.66 (0.25)	2.56 (0.15)	0.11 (0.27)
FVC, L	3.76 (0.34)	3.79 (0.21)	−0.03 (0.38)

[*]: The cut-point of the age variable at 45.8 years was provided by the decision tree models.

[†]: p values were from multivariate models adjusting for height, weight and smoking habit.

[‡]: Significant after adjusting for multiple comparison at $\alpha = 0.007$ (0.05/7).

Table 4.

Female-specific associations between the *TLR2*-rs 696480 polymorphisms and lung function in different age groups using age cut-offs at 40, 45 and 50 years (N = 103).

	Comparison in female workers* (Polymorphisms - Wild-type)	
Lung Function	**Difference (STE)**	**Difference (STE)**
	Age cut-off at 40 years	
	<40 years	≥40 years
	(N = 67)	(N = 36)
FEV1, L	3.71 (0.08)†	−0.31 (0.26)
FVC, L	0.30 (0.10)†	0.40 (0.28)
	Age cut-off at 45 years	
	<45 years	≥45 years
	(N = 82)	(N = 21)
FEV1, L	0.30 (0.09)†	0.10 (0.28)
FVC, L	0.30 (0.10)†	−0.04 (0.38)
	Age cut-off at 50 years	
	<50 years	≥50 years
	(N = 94)	(N = 9)
FEV1, L	0.30 (0.10)†	N/A
FVC, L	0.30 (0.10)†	N/A

*: p values were from multivariate models adjusting for height, weight and smoking habit.

†: Significant after adjusting for multiple comparison at $\alpha = 0.007$ (0.05/7).