Ann Allergy Asthma Immunol 110 (2013) 44–50

Contents lists available at SciVerse ScienceDirect

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

Zhiwei Gao, MD [*]; James A. Dosman, MD [†]; Donna C. Rennie, PhD [‡]; David A. Schwartz, MD [‡]; Ivana V. Yang, PhD [‡]; Jeremy Beach, MD [§]; and Ambikaipakan Senthilselvan, PhD [*]

[*] Department of Public Health Sciences, School of Public Health, University of Alberta, Edmonton, Alberta, Canada
[†] Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[‡] Department of Medicine, University of Colorado, Denver
[§] Division of Preventive Medicine, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

ARTICLE INFO

Article history:
Received for publication July 12, 2012.
Received in revised form October 16, 2012.
Accepted for publication November 5, 2012.

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.

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,

Reprints: Ambikaipakan Senthilselvan, PhD, School of Public Health, University of Alberta, 3-349 Edmonton Clinic Health Academy, 11405 87 Ave, Edmonton, AB, Canada T6G 1C9; E-mail: sentil@ualberta.ca.

Disclosures: Authors have nothing to disclose.

Funding sources: The study was supported by grant MOP-57907 from the Canadian Institutes of Health Research (CIHR), the CIHR Strategic Training Program in Public Health and the Agricultural Rural Ecosystem, and the CIHR Partner Institutes, including the Institute of Health Services and Policy Research, Institute of Circulatory and Respiratory Health, Institute of Infection and Immunity, and Institute of Population and Public Health.

1081-1206/12/$36.00 - see front matter
http://dx.doi.org/10.1016/j.anai.2012.11.003

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 (FEV_1), forced vital capacity (FVC), FEV_1/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; SensorMedics, 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 FEV_1, FVC, FEV_1/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 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

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%; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; LD, linkage disequilibrium.

[a]Data are presented as mean ± SD unless otherwise indicated.

[b]Hardy-Weinberg equilibrium was followed in both workers in swine operations and nonfarming rural dwellers.

[c]Fisher exact test.

[d]LD between the 2 single-nucleotide polymorphisms in the *TLR4* and *TLR2* genes, respectively.