

## Letter to the Editor

**NOS3 polymorphism, lung function, and exposure in swine operations: Results of 2 studies**

To the Editor:

Previous studies have demonstrated that working in swine operations is associated with respiratory problems due to exposure to swine barn environment.<sup>1</sup> Increased levels of nitric oxide (NO) in exhaled air have been reported among swine operation workers and healthy adults after 3-hour exposure in pig confinement buildings, suggesting that this may be an important mediator of the observed health effects.<sup>1</sup>

Of the 3 human NOS genes (NOS1, NOS2, and NOS3), the NOS3 gene is likely more important than others for respiratory problems in swine operation workers because human airway epithelial cells express only NOS3.<sup>2</sup> The expression of the NOS1 gene in human airway epithelial cells is undetectable,<sup>3</sup> and the NOS2 gene is expressed only after exposure to LPS or cytokines.<sup>4</sup> The NOS3 gene also has a regulatory role in the activation and expression of NOS2.<sup>5</sup> However, to date, there is limited research on the relationship between NOS3 polymorphisms and lung function in humans.

This study aimed to investigate the effects of NOS3 polymorphisms on lung function among swine operation workers

and nonfarming rural dwellers in 2 independent studies from Saskatchewan, Canada—a cross-sectional study and a longitudinal study.

The cross-sectional study included 374 full-time workers (240 men and 134 women) and 411 nonfarming rural dwellers (184 men and 227 women). The longitudinal study had 302 male workers and 261 male nonfarming rural dwellers who completed the initial study in 1990-1991 and followed up in 1994-1995 and 2003-2004. Details of recruitment and data collection, lung function measurements, skin prick testing, and DNA genotyping are described in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

Multiple linear regression with and without generalized estimation equations were used to examine differences in lung function between genotypes in the longitudinal and cross-sectional studies respectively, after controlling for confounders and multiple comparisons by Bonferroni correction.

The characteristics of participants in the cross-sectional study and the longitudinal study at baseline are shown in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The NOS3-922A/G (rs1800779) was not considered because of its high linkage disequilibrium with NOS3-786T/C (rs2070744).

**TABLE I.** Differences in mean values of lung function parameters between the polymorphic and wild-type groups of NOS3 polymorphisms (-786 T/C and Glu298Asp) for swine operation workers and nonfarming rural dwellers in the cross-sectional study

| Lung function                | Polymorphism vs wild |                |                |                | Gene × Exposure interaction adjusted <i>P</i> value |
|------------------------------|----------------------|----------------|----------------|----------------|-----------------------------------------------------|
|                              | Workers              |                | Rural dwellers |                |                                                     |
|                              | Diff (SE)            | <i>P</i> value | Diff (SE)      | <i>P</i> value |                                                     |
| NOS3-786 T/C(rs2070744)*     |                      |                |                |                |                                                     |
|                              | (n = 278)            |                | (n = 316)      |                |                                                     |
| Observed†                    |                      |                |                |                |                                                     |
| FEV <sub>1</sub> (L)         | 0.21 (0.08)          | .009‡          | −0.17 (0.08)   | .03            | .001                                                |
| FVC (L)                      | 0.22 (0.10)          | .02‡           | −0.14 (0.09)   | .13            | .007                                                |
| FEV <sub>1</sub> /FVC (%)    | 0.64 (1.06)          | .55            | −2.02 (1.02)   | .05            | .07                                                 |
| FEF <sub>25%-75%</sub> (L/s) | 0.21 (0.17)          | .22            | −0.24 (0.17)   | .14            | .06                                                 |
| Percent predicted (%)§       |                      |                |                |                |                                                     |
| FEV <sub>1</sub>             | 5.89 (2.18)          | .007‡          | − 5.39 (2.08)  | .01‡           | .0002                                               |
| FVC                          | 5.59 (2.15)          | .009‡          | − 3.60 (2.05)  | .08            | .003                                                |
| FEV <sub>1</sub> /FVC        | 0.14 (0.44)          | .75            | − 0.43 (0.42)  | .31            | .35                                                 |
| FEF <sub>25%-75%</sub>       | 4.85 (4.49)          | .28            | − 6.92 (4.30)  | .11            | .06                                                 |
| NOS3 Glu298Asp(rs1799983)    |                      |                |                |                |                                                     |
|                              | (n = 274)            |                | (n = 314)      |                |                                                     |
| Observed†                    |                      |                |                |                |                                                     |
| FEV <sub>1</sub> (L)         | 0.17 (0.07)          | .02‡           | −0.05 (0.08)   | .54            | .04                                                 |
| FVC (L)                      | 0.22 (0.09)          | .01‡           | −0.05 (0.09)   | .63            | .04                                                 |
| FEV <sub>1</sub> /FVC (%)    | −0.31 (0.97)         | .25            | −0.51 (1.01)   | .07            | .04                                                 |
| FEF <sub>25%-75%</sub> (L/s) | 0.00 (0.16)          | .21            | −0.04 (0.17)   | .24            | .09                                                 |
| Percent predicted (%)§       |                      |                |                |                |                                                     |
| FEV <sub>1</sub>             | 3.93 (2.02)          | .05            | −1.66 (2.12)   | .43            | .06                                                 |
| FVC                          | 4.39 (1.98)          | .03            | −0.94 (2.07)   | .65            | .06                                                 |
| FEV <sub>1</sub> /FVC        | 0.16 (0.30)          | .60            | 0.33 (0.28)    | .23            | .67                                                 |
| FEF <sub>25%-75%</sub>       | 4.09 (3.08)          | .18            | −3.10 (2.82)   | .25            | .08                                                 |

AIC, Akaike Information Criterion; FEF<sub>25%-75%</sub>, forced expiratory flow between 25% and 75% of FVC.

\*Recessive model (CC vs TC + TT) has the smallest AIC in the regression model of FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC, and FEF<sub>25%-75%</sub>.

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

‡Significant after applying Bonferroni correction for multiple comparisons (*P* < .025).

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

||Recessive model, polymorphism (TT) vs wild-type (TG + GG), has the smallest AIC in the regression model of FEV<sub>1</sub> and FVC; Dominant model, polymorphism (TT + TG) vs wild-type (GG), has the smallest AIC in the regression model of FEV<sub>1</sub>/FVC and FEF<sub>25%-75%</sub>.

**TABLE II.** Differences in annual lung function decline rates between genotypes of NOS3 polymorphisms (-786 T/C and Glu298Asp) for swine operation workers and nonfarming rural dwellers in the longitudinal study

| Annual lung function decline rate (per year) | Polymorphism vs wild |         |                |         |
|----------------------------------------------|----------------------|---------|----------------|---------|
|                                              | Workers              |         | Rural dwellers |         |
|                                              | Diff (SE)            | P value | Diff (SE)      | P value |
| NOS3-786 T/C(rs2070744)*                     |                      |         |                |         |
|                                              | (n = 143)            |         | (n = 83)       |         |
| Observed†                                    |                      |         |                |         |
| FEV <sub>1</sub> (L/y)                       | 0.026 (0.01)         | .004‡   | 0.002 (0.01)   | .80     |
| FVC (L/y)                                    | 0.029 (0.01)         | .017‡   | 0.014 (0.01)   | .08     |
| FEV <sub>1</sub> /FVC (%/y)                  | 0.003 (0.11)         | .98     | -0.153 (0.11)  | .16     |
| FEF <sub>25%-75%</sub> (L/s/y)               | 0.032 (0.02)         | .13     | -0.036 (0.02)  | .07     |
| Percent predicted (%)§                       |                      |         |                |         |
| FEV <sub>1</sub>                             | 0.85 (0.44)          | .05     | 0.06 (0.19)    | .74     |
| FVC                                          | 0.93 (0.52)          | .07     | 0.30 (0.18)    | .09     |
| FEV <sub>1</sub> /FVC                        | 0.00 (0.13)          | .99     | -0.19 (0.14)   | .16     |
| FEF <sub>25%-75%</sub>                       | 0.78 (0.70)          | .27     | -0.83 (0.51)   | .10     |
| NOS3 Glu298Asp(rs1799983)                    |                      |         |                |         |
|                                              | (n = 136)            |         | (n = 85)       |         |
| Observed†                                    |                      |         |                |         |
| FEV <sub>1</sub> (L/y)                       | 0.006 (0.01)         | .50     | 0.012 (0.01)   | .19     |
| FVC (L/y)                                    | 0.010 (0.01)         | .46     | 0.010 (0.01)   | .34     |
| FEV <sub>1</sub> /FVC (%/y)                  | -0.112 (0.09)        | .19     | 0.005 (0.08)   | .96     |
| FEF <sub>25%-75%</sub> (L/s/y)               | -0.017 (0.02)        | .29     | 0.023 (0.02)   | .41     |
| Percent predicted (%)§                       |                      |         |                |         |
| FEV <sub>1</sub>                             | 0.12 (0.24)          | .62     | 0.35 (0.28)    | .21     |
| FVC                                          | 0.19 (0.26)          | .46     | 0.27 (0.23)    | .23     |
| FEV <sub>1</sub> /FVC                        | -0.14 (0.09)         | .14     | -0.03 (0.11)   | .74     |
| FEF <sub>25%-75%</sub>                       | -0.60 (0.39)         | .12     | 0.09 (0.42)    | .82     |

FEF<sub>25%-75%</sub>, forced expiratory flow between 25% and 75% of FVC.

\*Polymorphism (CC) vs wild-type (TC + TT).

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

‡Significant after applying Bonferroni correction for multiple comparisons ( $P < .025$ ).

§P value from the multiple regression analysis after adjusting for smoking habit.

||Polymorphism (TT) vs wild-type (TG + GG): in the regression model of FEV<sub>1</sub> and FVC; polymorphism (TT + TG) vs wild-type (GG): in the regression model of FEV<sub>1</sub>/FVC and FEF<sub>25%-75%</sub>.

In the cross-sectional study (Table I), the exposure group (workers vs nonfarming rural dwellers) was a significant effect modifier for the relationship between NOS3-786T/C and lung function (observed and percent predicted FEV<sub>1</sub> and forced vital capacity [FVC]). Workers with the CC genotype (polymorphic group) had significantly greater mean values of observed FEV<sub>1</sub> (3.75 vs 3.55 L,  $P = .009$ ) and FVC (4.79 vs 4.56 L,  $P = .02$ ), and percent predicted FEV<sub>1</sub> (102.0% vs 96.1%,  $P = .007$ ) and FVC (107.8% vs 102.2%,  $P = .009$ ) than did workers with the TC or TT genotypes (wild-type), whereas no significant associations were observed in nonfarming rural dwellers except a lower percent predicted FEV<sub>1</sub> in the polymorphic group.

As shown in Table I, the exposure group was also a significant effect modifier for the relationship between NOS3 Glu298Asp (rs1799983) and lung function (observed FEV<sub>1</sub>, FVC, and FEV<sub>1</sub>/FVC). Workers with the TT genotype (polymorphic group) had significantly greater mean values of observed FEV<sub>1</sub> (3.72 vs 3.55 L,  $P = .02$ ) and observed FVC (4.79 vs 4.56 L,  $P = .01$ ) than did workers with TG or GG genotypes (wild-type). Among nonfarming rural dwellers, these associations were not seen.

In the longitudinal study (Table II), workers with NOS3-786T/C polymorphism had significantly lower mean annual decline rates in observed FEV<sub>1</sub> (-30.2 vs -55.9 mL/y,  $P = .004$ ) and FVC (-16.8 vs -46.2 mL/y,  $P = .02$ ) than did workers with the wild type. No significant associations were observed in

nonfarming rural dwellers. No significant associations were observed between NOS3 Glu298Asp and lung function in either exposure group.

The results from multiple regression analyses remained substantially unchanged when the work status (quit or stayed) was included for the longitudinal data and atopic status was included for the cross-sectional data (results not shown). The NOS3 haplotype analysis showed a higher average lung function among workers with the haplotype of 2 minor alleles and a lower average lung function in workers with the haplotype of 2 major alleles in the cross-sectional study (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

To the best of our knowledge, this study is the first to report significant interactions between the NOS3 polymorphisms and environmental exposures on lung function in humans and to show protective effects of NOS3 polymorphisms on lung function in humans exposed to high respiratory hazards.

To date, few studies have examined the association between NOS3 polymorphisms and respiratory dysfunction, and the results are inconsistent.<sup>6,7</sup> These discrepancies may partly be explained by the significant interaction reported here between NOS3 polymorphisms and exposure. The protective effects of NOS3 polymorphisms on airways have mainly been investigated in experimental studies.<sup>8</sup> Our study extended these findings to humans exposed to a high concentration of respiratory hazards in the workplace.

We recently reported protective effects of TLR2 polymorphisms on lung function among swine operation workers.<sup>9</sup> Haplotype analysis of both the NOS3 and TLR2 genes showed that the 2 genes interacted with exposure independently and the interactions between the 2 genes were not statistically significant (data not shown).

The lack of objective measurement of exposures makes it difficult to identify a dose-response relationship. However, the nonfarming rural dwellers did provide a good comparison group for examining effects of NOS3 polymorphisms at a lower exposure level than what is typically found in intensive swine operations. Although information on race and ethnicity was not collected in our studies, a subsequent study conducted by our team on more than 8000 subjects in rural Saskatchewan demonstrated that 98.1% of the persons living on farms and 96.1% of the persons living in small towns self-listed themselves as of Caucasian origin (unpublished data). We do not expect that the percentages of Caucasians among the swine operation workers should be substantially different.

In conclusion, this study is the first to show variation in the association between NOS3 polymorphisms and lung function in humans exposed to high and low levels of respiratory hazards, demonstrating a possible gene-environment interaction. This study also extends our knowledge of the role of the NOS3 gene in responses to environmental exposures from experimental studies to humans, suggesting the relevance of the NOS3 gene in respiratory disease among populations with high levels of exposure to respiratory hazards.

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This study was supported by a grant from the Canadian Institutes of Health Research (CIHR) (grant no. MOP-57907). This research was funded by the CIHR Strategic Training Program in Public Health and the Agricultural Rural Ecosystem (PHARE) and CIHR partner institutes including the Institute of Health Services and Policy Research, the Institute of Circulatory and Respiratory Health, the Institute of Infection and Immunity, and the Institute of Population and Public Health.

Disclosure of potential conflict of interest: J. A. Dosman has received research support from the Canadian Institute of Health Research, is a board member for the Canadian Nuclear Safety Commission, has consultant arrangements with the International Labor Organization, and is employed by the University of Saskatchewan. D. A. Schwartz has received research support from the National Institutes of Health and the Veterans Administration; has provided expert testimony for Weitz and Luxenberg Law firm, Brayton Purcell Law firm, and Wallace and Graham Law firm; receives payment from several patents; and receives book royalties from Springer. I. V. Yang has received research support from the National Institutes of Health. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2014.04.006>

## METHODS

### The cross-sectional study

**Recruitment of workers in swine operations.** The study was conducted over 2 years from 2003 to 2004 in rural Saskatchewan by the Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Canada. The recruitment of workers in swine operations was conducted in collaboration with the Pork Producers' Association of Saskatchewan (SaskPork) and large swine production companies in Saskatchewan. A brief description of the study was posted in the SaskPork newsletter. Researchers from the study also made visits to the managements of large swine operations to encourage participation. The companies scheduled their workers into prearranged time slots so that they could have the opportunity to go to the evaluation clinics in nearby towns and villages.

To participate in the study, workers in swine operations had to be 17 years or older and have worked in a confinement building for at least 4 days per week with a total work duration of 20 hours or more per week. A total of 374 workers from 38 large swine operations were included in this study. The number of workers from each operation varied from 3 to 37. The participation rate from the large swine operations was approximately 70%.

**Recruitment of nonfarming rural dwellers.** Nonfarming rural dwellers were recruited from all residents living in proximity (within 100 km radius) to a swine production site. Taxation lists provided by rural towns were used to locate the potential participants. A letter inviting them was mailed to all persons on the list. To participate in the study, they had to be older than 17 years. Nonfarming rural dwellers who were involved in mining, grain handling, metal, and auto-body work were excluded because of their potential exposure to respiratory hazards. A total of 411 nonfarming rural dwellers were included in this study.

**Data collection.** Person-to-person interviews and testing were conducted by trained technicians in hospitals and community centers near the swine operation sites and residence 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 participants before the interview.

The information of respiratory health and occupational history was collected from the participants using a detailed questionnaire, which had been well validated in previous studies, and included anthropomorphic data, respiratory symptoms, smoking history, past illnesses, and occupational history in swine operations.<sup>E1-E4</sup>

A current smoker was defined by the questionnaire as a person currently smoking cigarettes at an average of 1 or more per day. An ex-smoker was defined as a person who has smoked more than 400 cigarettes (or equivalent amount of tobacco) in his or her lifetime but was not currently smoking. A lifetime nonsmoker was defined as a person who had not smoked more than 400 cigarettes (or equivalent amount of tobacco) in his or her lifetime.

**Lung function measurements.** Lung function testing was obtained by trained technicians using a volume displacement spirometer (model 1022; SensorMedics, Yorba Linda, Calif) following the American Thoracic Society recommendations.<sup>E5</sup> These measurements include FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC ratio, and forced expiratory flow between 25% and 75% of FVC (FEF<sub>25%-75%</sub>). Percentage predicted values for FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC ratio, and FEF<sub>25%-75%</sub> were obtained using the equations developed by Crapo et al.<sup>E6</sup> Height (cm), weight (kg), and systolic and diastolic pressure (mmHg) measurements were obtained before the lung function testing.

### Skin prick tests

Solutions for allergens (*Alternaria* sp, swine, house-dust mite, cat dander, mixed grass allergens) for the allergy skin prick tests (SPTs) were from Western Allergy Services Ltd, Burnaby, BC, Canada, as described in another study reporting the reciprocal association between atopy and respiratory symptoms using the same cohort.<sup>E7</sup> Histamine and saline were also included in the SPT as positive and negative controls. The tests were conducted on the same occasion as the pulmonary function testing using methods in previous studies.<sup>E7</sup> The SPT was performed on the normal skin on each

subject's forearms, and the distance between 2 SPTs was 2 cm to avoid cross-contamination. Participants were considered positive if 1 or more SPT had a raised wheal of 3 mm or more in comparison to the saline control.<sup>E8</sup> The technicians conducting the SPT were blinded to the genotypes of each subject.

Blood samples from all subjects were also collected for genotyping, which was conducted at Dr David Schwartz's laboratory at the National Jewish Hospital in Denver, Colo. Blood samples were collected in Qiagen PAXgene tubes with blood cards spotted for each sample (S&S, catalog #10538414).<sup>E9</sup> The NOS3 gene was genotyped as a part of a 96-plex GoldenGate genotyping assay on Veracode beads. Assays were performed according to the manufacturer's protocols and scanned on a BeadXpress reader (Illumina, Inc, San Diego, Calif). Raw genotyping data were clustered and cleaned in the BeadStudio software (Illumina GenomeStudio).

### The longitudinal study

The longitudinal study consisted of workers in swine facilities and nonfarming rural dwellers who participated in the initial study in 1990-1991 (cycle 1), and were followed-up in 1994-1995 (cycle 2) and in 2003-2004 (cycle 3), respectively (Fig E1).

**Recruitment of male workers in swine facilities.** The longitudinal study included all swine operations with annual sales of at least 200 hogs in 1988 in the central part of the province of Saskatchewan (Lloydminster in the West, Carrot River in the East, Prince Albert in the North, and Moose Jaw in the South) between 1990 and 1991. The information on annual sales of swine operations was provided by the Saskatchewan Pork Producers Marketing Board. After excluding Hutterite colonies, swine operations with fewer than 200 pigs, and swine operations with a daily duration of work inside the barn of less than 2 hours, 412 swine producers were eligible to participate in the study. Of these, 331 agreed to participate in the study and 302 male workers had pulmonary function measurements and were included in the current study.

**Recruitment of male nonfarming rural dwellers.** On the basis of age distribution and residences of male workers in swine operations, 270 male nonfarming rural dwellers were selected from the Saskatchewan hospital services plan lists, school board lists, and bank and post office employees. Of these, 261 nonfarming rural dwellers had pulmonary function measurements and were included in the current study.

**Follow-up.** Of the 302 workers and 261 nonfarming rural dwellers studied in cycle 1, 217 workers and 171 nonfarming rural dwellers participated in cycle 2, which resulted in a 4-year follow-up of 71.9% in the workers and 65.5% in the nonfarming rural dwellers (Fig E1). An effort was made to contact all subjects previously studied. Of the 217 workers and 171 nonfarming rural dwellers who participated in cycle 2, 163 workers and 118 nonfarming rural dwellers participated in cycle 3, which resulted in a follow-up of 75% in the workers and 69% in the nonfarming rural dwellers (Fig E1).

**Data collection.** A technician-administered questionnaire was used to collect information on respiratory and general health status. The same questionnaire was used on all 3 cycles, with the exception that additional questions were added in cycle 2 and cycle 3 to elicit changes in health status and employment status that might have occurred since the previous observations. Respiratory symptoms were obtained using the questions in the Standard American Thoracic Society questionnaire.<sup>E10</sup> The study was approved by the Biomedical Research Ethics Board of the University of Saskatchewan. Informed written consent was obtained from all participants before the interview.

A current smoker was defined by the questionnaire as a person currently smoking cigarettes at an average of 1 or more per day. An ex-smoker was defined as a person who had smoked more than 400 cigarettes (or equivalent amount of tobacco) in his or her lifetime but was not currently smoking. A lifetime nonsmoker was defined as a person who had not smoked more than 400 cigarettes (or equivalent amount of tobacco) in his or her lifetime.

Years of swine work during the study period was determined by the time intervals between the cycles. For workers who reported that they quit swine farming in response to a question "Have you quit hog farming since the last

study?" in cycle 3, their years of swine work were adjusted by the response to a subsequent question "if yes, in what year did you quit pig farming?"

**Lung function measurements.** Lung function testing was performed using a volume-displacement spirometer (model 1022; SensorMedics). Measurements include FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC ratio, and FEF<sub>25%-75%</sub>. These measurements were obtained by trained technicians following the American Thoracic Society recommendations.<sup>E5</sup>

In cycle 3, blood samples from all subjects were collected for genotyping, which was conducted at Dr David Schwartz's laboratory at the National Jewish Hospital in Denver, Colo. Blood samples were collected in Qiagen PAXgene tubes with blood cards spotted for each sample (S&S, catalog #10538414).<sup>E9</sup> The NOS3 gene was 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). Raw genotyping data were clustered and cleaned in the BeadStudio software (Illumina GenomeStudio).

### Selection of single nucleotide polymorphisms

The single nucleotide polymorphisms (SNPs) for the NOS3 gene were selected on the basis of the following criteria:

1. The association between SNPs with 1 or more disease phenotypes was confirmed in several studies.
2. Tagging SNPs for the NOS3 gene from high-density SNP maps were generated by using the HapMap project. SNP selector, a Web-based tool for SNP selection, was used to select tagging SNPs for the NOS3 gene.<sup>E11</sup> The tagging SNPs were those in high linkage disequilibrium ( $r^2 \geq 0.8$ ). SNPs with low minor allele frequency (<10%) were to be excluded and not considered as tagging SNPs.

### Ethnicity of the participants

Although information on race and ethnicity was not collected in our studies, a subsequent study conducted by our team on more than 8000 subjects in rural Saskatchewan demonstrated that 98.1% of the persons living on farms and 96.1% of the persons living in small towns self-listed themselves as of Caucasian origin (unpublished data).<sup>E12</sup> We do not expect that the percentages of Caucasians among the swine operation workers should be substantially different.

**Results.** The characteristics of workers in swine operations and nonfarming rural dwellers in the cross-sectional study and at baseline of the longitudinal study are listed in Table E1. Workers in swine operations were significantly younger in the 2 studies and taller than nonfarming rural dwellers in the cross-sectional study. The average weight between the 2 groups was similar in the 2 studies. There were more men and smokers among the workers than among the rural dwellers in the cross-sectional study. However, smoking was more prevalent among the rural dwellers than among the workers at baseline of the longitudinal study. The proportion of subjects with positive skin prick test results was greater

in nonfarming rural dwellers than in workers in the cross-sectional study, but the difference was not statistically significant ( $P = .06$ ). Mean values of FEV<sub>1</sub> and FVC were significantly greater in the workers than in the rural dwellers in the cross-sectional study.

As shown in Table E1, the genotype distributions of the 3 NOS3 SNPs were similar between the workers and nonfarming rural dwellers. Hardy-Weinberg equilibrium was followed by the 3 SNPs in both exposure groups. NOS3-786T/C and -922A/G polymorphisms were in high linkage disequilibrium, and the latter was not considered further in the study.

Haplotypes of the NOS3 gene from the cross-sectional study were derived, and the results of NOS3 haplotype analysis are given in Table E2. As shown in Table E2, workers with the haplotype of C-T (2 minor alleles) under the recessive model had significantly higher mean FEV<sub>1</sub> than did workers with other haplotypes; workers with haplotypes of T-G (2 major alleles) under the dominant model had significantly lower mean lung function in FEV<sub>1</sub>, FVC, and predicted FEV<sub>1</sub> and FVC than did workers with other haplotypes.

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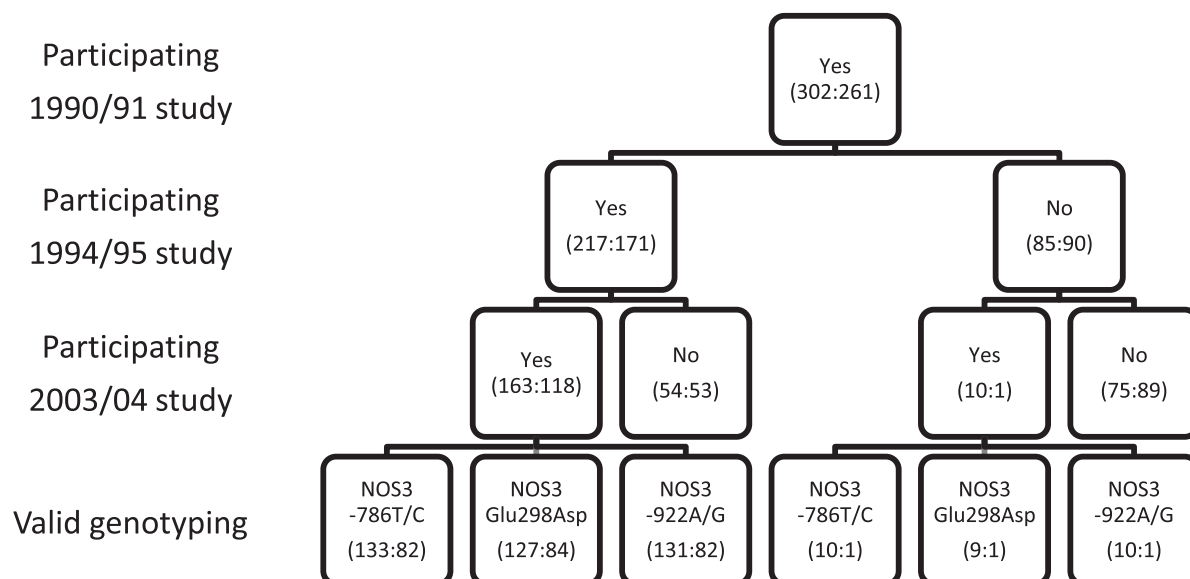


**FIG E1.** The follow-up of the workers and nonfarming rural dwellers from 1990-1991 to 2003-2004 (number of workers: number of rural dwellers).

**TABLE E1.** Characteristics of workers in swine operations and nonfarming rural dwellers

| Characteristic                | Cross-sectional study |                  | Longitudinal study (at baseline) |                 |
|-------------------------------|-----------------------|------------------|----------------------------------|-----------------|
|                               | Workers               | Rural dwellers   | Workers                          | Rural dwellers  |
| N                             | 374                   | 411              | 173                              | 119             |
| Age (y), mean $\pm$ SD        | 35.8 $\pm$ 11.5       | 39.3 $\pm$ 13.0* | 37.5 $\pm$ 10.8                  | 41.6 $\pm$ 9.9* |
| Height (cm), mean $\pm$ SD    | 172.3 $\pm$ 8.4       | 170.8 $\pm$ 9.5* | 177.1 $\pm$ 6.1                  | 177.3 $\pm$ 6.8 |
| Weight (kg), mean $\pm$ SD    | 83.6 $\pm$ 17.1       | 82.2 $\pm$ 18.8  | 84.3 $\pm$ 13.7                  | 84.4 $\pm$ 15.2 |
| Male, n (%), mean $\pm$ SD    | 240 $\pm$ 64.2        | 184 $\pm$ 44.8*  | 173 (100)                        | 119 (100)       |
| Smoking, n (%)                |                       |                  |                                  |                 |
| Current smoker                | 117 (31.3)            | 74 (18.0)*       | 20 (11.6)                        | 29 (24.4)*      |
| Ex-smoker                     | 101 (27.0)            | 115 (28.0)*      | 44 (25.4)                        | 39 (32.8)*      |
| Nonsmoker                     | 156 (41.7)            | 222 (54.0)*      | 109 (63.0)                       | 51 (31.9)*      |
| Atopy, n (%)                  | 123 (35.3)            | 169 (42.1)       | NA                               | NA              |
| Lung function tests, n (%)    |                       |                  |                                  |                 |
| FEV <sub>1</sub>              | 3.7 (0.8)             | 3.6 (0.9)*       | 4.4 (0.7)                        | 4.3 (0.6)       |
| FVC                           | 4.8 (1.0)             | 4.5 (1.1)*       | 5.5 (0.8)                        | 5.4 (0.8)       |
| FEV <sub>1</sub> /FVC         | 78.6 (6.6)            | 79.1 (6.4)       | 79.4 (6.7)                       | 80.3 (5.6)      |
| FEF <sub>25%-75%</sub>        | 3.6 (1.2)             | 3.4 (1.2)        | 4.3 (1.3)                        | 4.3 (1.2)       |
| NOS3, n (%)                   |                       |                  |                                  |                 |
| -786 T/C† (rs2070744)         |                       |                  |                                  |                 |
| TT                            | 105 (37.8)            | 132 (41.8)       | 57 (39.9)                        | 31 (37.4)       |
| TC                            | 138 (49.6)            | 146 (46.2)       | 72 (50.4)                        | 38 (45.8)       |
| CC                            | 35 (12.6)             | 38 (12.0)        | 14 (9.8)                         | 14 (16.9)       |
| Glu298Asp† (rs1799983)        |                       |                  |                                  |                 |
| GG                            | 110 (40.2)            | 142 (45.2)       | 65 (47.8)                        | 40 (47.1)       |
| TG                            | 119 (43.4)            | 134 (42.7)       | 55 (40.4)                        | 40 (47.1)       |
| TT                            | 45 (16.4)             | 38 (12.1)        | 16 (11.8)                        | 5 (5.9)         |
| -922 A/G† (rs1800779)         |                       |                  |                                  |                 |
| AA                            | 109 (39.2)            | 133 (42.1)       | 59 (41.8)                        | 31 (37.4)       |
| AG                            | 134 (48.2)            | 145 (45.9)       | 68 (48.2)                        | 38 (45.8)       |
| GG                            | 35 (12.6)             | 38 (12.0)        | 14 (9.9)                         | 14 (16.9)       |
| Linkage disequilibrium, n (%) |                       |                  |                                  |                 |
| -786T/C vs Glu298Asp          | 0.34                  | 0.46             | 0.48                             | 0.48            |
| -786T/C vs -922A/G            | 0.94                  | 0.98             | 0.98                             | 1.00            |
| Glu298Asp vs -922A/G          | 0.35                  | 0.46             | 0.49                             | 0.48            |

NA, Not available.

\*Significantly different between workers and rural dwellers ( $P < .05$ ).

†Hardy-Weinberg equilibrium was followed in both workers and rural dwellers.

**TABLE E2.** Haplotype analysis of the NOS3 gene (-786T/C and Glu298Asp) in the multiple regression analysis of lung function in the cross-sectional study\*

| Cross-sectional study                                                 |                   |                             |                           |              |                        |                             |              |              |                        |
|-----------------------------------------------------------------------|-------------------|-----------------------------|---------------------------|--------------|------------------------|-----------------------------|--------------|--------------|------------------------|
| Haplotype                                                             | Inheritance model | Workers in swine operations |                           |              |                        | Nonfarming control subjects |              |              |                        |
|                                                                       |                   | FEV <sub>1</sub>            | FVC                       | Ratio        | FEF <sub>25%-75%</sub> | FEV <sub>1</sub>            | FVC          | Ratio        | FEF <sub>25%-75%</sub> |
| Difference in the mean values (SE) of observed lung function          |                   |                             |                           |              |                        |                             |              |              |                        |
| C-T                                                                   | Recessive         | 0.25 (0.12) <sup>†</sup>    | 0.25 (0.15)               | 1.08 (1.60)  | 0.22 (0.26)            | -0.24 (0.12) <sup>†</sup>   | -0.22 (0.15) | -1.66 (1.60) | -0.40 (0.26)           |
|                                                                       | Dominant          | 0.05 (0.05)                 | 0.00 (0.07)               | 0.82 (0.71)  | 0.13 (0.12)            | -0.01 (0.05)                | 0.06 (0.06)  | -1.18 (0.67) | -0.16 (0.11)           |
|                                                                       | Additive          | 0.07 (0.05)                 | 0.04 (0.05)               | 0.72 (0.60)  | 0.12 (0.10)            | -0.04 (0.04)                | 0.01 (0.05)  | -1.06 (0.57) | -0.17 (0.09)           |
| T-T                                                                   | Recessive         | 0.29 (0.17)                 | 0.64 (0.21) <sup>‡</sup>  | -4.43 (2.27) | -0.40 (0.37)           | 0.23 (0.32)                 | 0.07 (0.38)  | 5.25 (4.15)  | 0.99 (0.68)            |
|                                                                       | Dominant          | 0.01 (0.06)                 | 0.01 (0.08)               | -0.11 (0.83) | -0.03 (0.14)           | 0.06 (0.06)                 | 0.08 (0.08)  | -0.12 (0.84) | 0.10 (0.14)            |
|                                                                       | Additive          | 0.04 (0.05)                 | 0.07 (0.07)               | -0.53 (0.72) | -0.06 (0.12)           | 0.07 (0.06)                 | 0.07 (0.07)  | 0.09 (0.80)  | 0.12 (0.13)            |
| C-G                                                                   | Recessive         | 0.20 (0.18)                 | 0.22 (0.22)               | 0.69 (2.42)  | 0.24 (0.39)            | -0.14 (0.16)                | -0.26 (0.19) | 0.54 (2.12)  | 0.23 (0.35)            |
|                                                                       | Dominant          | -0.03 (0.06)                | 0.04 (0.08)               | -1.52 (0.84) | -0.15 (0.14)           | -0.08 (0.06)                | -0.10 (0.07) | -0.24 (0.81) | -0.04 (0.13)           |
|                                                                       | Additive          | -0.01 (0.06)                | 0.05 (0.07)               | -1.09 (0.74) | -0.09 (0.12)           | -0.08 (0.05)                | -0.10 (0.06) | -0.11 (0.69) | -0.00 (0.11)           |
| T-G                                                                   | Recessive         | 0.03 (0.07)                 | 0.02 (0.08)               | 0.52 (0.87)  | 0.02 (0.14)            | 0.02 (0.06)                 | -0.02 (0.07) | 0.85 (0.73)  | 0.11 (0.12)            |
|                                                                       | Dominant          | -0.20 (0.06) <sup>‡</sup>   | -0.26 (0.08) <sup>‡</sup> | 0.22 (0.85)  | -0.07 (0.14)           | 0.08 (0.06)                 | 0.06 (0.08)  | 1.25 (0.83)  | 0.08 (0.14)            |
|                                                                       | Additive          | -0.07 (0.04)                | -0.10 (0.05) <sup>†</sup> | 0.28 (0.54)  | -0.02 (0.09)           | 0.04 (0.04)                 | 0.01 (0.04)  | 0.78 (0.48)  | 0.07 (0.08)            |
| Difference in the mean values (SE) of percent predicted lung function |                   |                             |                           |              |                        |                             |              |              |                        |
| C-T                                                                   | Recessive         | 6.12 (3.35)                 | 5.20 (3.27)               | -0.39 (0.67) | 5.13 (6.84)            | -6.67 (3.34) <sup>†</sup>   | -5.02 (3.26) | 0.35 (0.67)  | -9.64 (6.82)           |
|                                                                       | Dominant          | 1.34 (1.50)                 | 0.37 (1.47)               | 0.04 (0.30)  | 3.45 (3.05)            | -0.69 (1.40)                | 1.03 (1.37)  | 0.02 (0.28)  | -4.55 (2.85)           |
|                                                                       | Additive          | 1.75 (1.26)                 | 1.00 (1.23)               | -0.06 (0.25) | 3.02 (2.56)            | -1.23 (1.20)                | 0.16 (1.17)  | 0.06 (0.24)  | -4.29 (2.43)           |
| T-T                                                                   | Recessive         | 7.90 (4.74)                 | 13.5 (4.61) <sup>‡</sup>  | 1.45 (0.95)  | -6.24 (9.65)           | 8.28 (8.69)                 | 2.66 (8.44)  | -0.00 (1.73) | 31.65 (17.68)          |
|                                                                       | Dominant          | -0.09 (1.73)                | -0.28 (1.69)              | -0.10 (0.35) | -0.59 (3.53)           | 2.38 (1.74)                 | 2.73 (1.70)  | 0.47 (0.35)  | 2.33 (3.55)            |
|                                                                       | Additive          | 0.69 (1.50)                 | 1.30 (1.45)               | -0.09 (0.30) | -1.23 (3.04)           | 2.31 (1.67)                 | 2.39 (1.62)  | 0.51 (0.33)  | 3.09 (3.39)            |
| C-G                                                                   | Recessive         | 5.15 (5.06)                 | 4.95 (4.93)               | 0.81 (1.01)  | 3.36 (10.32)           | -5.17 (4.41)                | -6.95 (4.30) | -1.39 (0.88) | 3.78 (8.99)            |
|                                                                       | Dominant          | -0.78 (1.77)                | 1.18 (1.73)               | -0.03 (0.35) | -3.99 (3.61)           | -2.06 (1.70)                | -2.04 (1.66) | -0.12 (0.34) | -1.73 (3.46)           |
|                                                                       | Additive          | 0.07 (1.56)                 | 1.44 (1.51)               | 0.05 (0.31)  | -2.37 (3.17)           | -2.29 (1.45)                | -2.34 (1.41) | -0.22 (0.29) | -1.30 (2.95)           |
| T-G                                                                   | Recessive         | 0.88 (1.81)                 | 0.54 (1.77)               | -0.19 (0.36) | 0.49 (3.69)            | 0.15 (1.53)                 | -1.19 (1.49) | -0.39 (0.30) | 3.38 (3.11)            |
|                                                                       | Dominant          | -5.36 (1.76) <sup>‡</sup>   | -5.92 (1.72) <sup>‡</sup> | 0.07 (0.36)  | -1.72 (3.63)           | 2.97 (1.73)                 | 1.75 (1.68)  | 0.27 (0.35)  | 2.87 (3.55)            |
|                                                                       | Additive          | -1.80 (1.12)                | -2.25 (1.08) <sup>†</sup> | 0.07 (0.22)  | -0.46 (2.27)           | 1.12 (1.00)                 | 0.14 (0.98)  | -0.12 (0.20) | 2.52 (2.05)            |

\*Adjusted for age, sex, height, weight, and smoking status in the regression analysis of observed lung function; adjusted for smoking status in the regression analysis of percent predicted lung function.

<sup>†</sup> $P < .05$ .

<sup>‡</sup> $P < .01$ .