Journal of Agromedicine
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t792303961

Effects of Polymorphic Variations in Tumor Necrosis Factor Alpha and Occupational Exposure to Grain Dust on Longitudinal Decline in Pulmonary Function

Punam Pahwa[a]; Kazuko Nakagawa[b]; Niels Koehncke[a]; Helen H. McDuffie[a]
[a] Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, [b] Department of Pharmacology and Therapeutics, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Japan

To cite this Article Pahwa, Punam , Nakagawa, Kazuko , Koehncke, Niels and McDuffie, Helen H.(2009) 'Effects of Polymorphic Variations in Tumor Necrosis Factor Alpha and Occupational Exposure to Grain Dust on Longitudinal Decline in Pulmonary Function', Journal of Agromedicine, 14: 2, 215 — 221
To link to this Article: DOI: 10.1080/10599240902888518
URL: http://dx.doi.org/10.1080/10599240902888518

Journal of Agromedicine, 14:215–221, 2008

ISSN: 1059-924X print/1545-0813 online
DOI: 10.1080/10599240902888518

Taylor & Francis
Taylor & Francis Group

Effects of Polymorphic Variations in Tumor Necrosis Factor Alpha and Occupational Exposure to Grain Dust on Longitudinal Decline in Pulmonary Function

Punam Pahwa, PhD
Kazuko Nakagawa, MD, PhD
Niels Koehncke, MD
Helen H. McDuffie, PhD

ABSTRACT. *Background:* Longitudinal declines in pulmonary function are associated with individuals experiencing occupational exposure to organic dusts in combination with lifestyle factors such as cigarette smoking and with genetic factors, and interactions between these factors. *Objective:* To investigate the relationship between polymorphism of genes encoding Tumor Necrosis Factor Alpha (TNF-α) and longitudinal lung function decline in grain workers exposed to grain dust. *Method:* Male grain handlers who participated in the Saskatchewan Grain Workers Surveillance Program from 2002 through 2005 provided demographic, occupational, lifestyle, and respiratory symptoms information as well as pulmonary function measurements and DNA for genotyping. Marginal models using the generalized estimating equations approach were fitted by using a SAS PROC GENMOD to predict the annual decline in Forced Expired Volume in one second (FEV_1) and Forced Vital Capacity (FVC). *Results:* Smoking intensity contributed to the decline in FEV_1. Among *1/*1 homozygotes and *1/*2 heterozygotes, grain workers with <10 years in the grain industry had significantly lower FEV_1 declines compared to those of the other two exposure groups (>10 and ≤20 years, and >20 years in the grain industry). The annual declines in FEV_1 for grain workers who were either *1/*1 homozygote or *1/*2 heterozygote and had been in the grain industry for <10 years were lower by comparison to those of grain workers who were *2/*2 genotype and had been in the industry for <10 years. *Conclusion:* This research demonstrates that years in the grain industry is an effect modifier between TNF-α 308 genotype and longitudinal decline in FEV_1 in male subjects exposed to grain dust.

KEYWORDS. Grain dust, longitudinal decline, pulmonary function, TNF-α

INTRODUCTION

Longitudinal declines in pulmonary function among individuals are associated with occupational exposure to organic dusts,[1–5] lifestyle factors such as cigarette smoking,[6–10] genetic factors,[11–14] and the interactions between these factors.[15–17] Despite advancement in knowledge

Punam Pahwa, Niels Koehncke, and Helen H. McDuffie are affiliated with the Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan.

Kazuko Nakagawa is affiliated with the Department of Pharmacology and Therapeutics, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Japan.

Address correspondence to: Punam Pahwa, PhD, Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Royal University Hospital, 103 Hospital Drive, Saskatoon, SK S7N 0W8, Canada (E-mail: pup165@mail.usask.ca).

concerning the relationships between environmental/occupational exposures and disease, there remains an unanswered question: Why do certain people develop disease when challenged with environmental or occupational contaminants, while others who experience the same challenges remain disease-free?

The associations between long-term exposure to grain dust, decline in lung function, and increase in the prevalence of chronic respiratory symptoms have been investigated extensively.[1–5] Several other studies[9–17] have shown that genetic factors affect decline in pulmonary function, but limited research has been conducted to investigate the effects of either gene-gene or gene-environment interactions in studying longitudinal decline in lung function.

A polymorphism, a guanine (G)-to-adenine (A) substitution, at position −308 of Tumor Necrosis Factor Alpha-promoter region (TNF-α-308*1/2),[11,12] is known to be associated with alteration of TNF-α secretion.[12] The uncommon TNF-α-308*2 allele is associated with higher amounts of TNF-α production.[12] Previous studies have demonstrated a positive association between TNF-α-308 polymorphisms and a number of lung diseases including chronic obstructive pulmonary disease (COPD),[14] chronic bronchitis,[13] and asthma.[17] Also, it has been demonstrated that TNF-α is associated with cigarette smoke-induced inflammatory disease.[9] However, several other studies have shown no association between TNF-α polymorphisms and smoking-related COPD, and between TNF-α and COPD.

The present paper reports the relationship between polymorphism of gene encoding TNF-α and longitudinal lung function decline in grain workers exposed to grain dust.

MATERIALS AND METHODS

Subjects

The Canadian Centre for Health and Safety in Agriculture (CCHSA) [previously the Institute of Agricultural Rural and Environmental Health], University of Saskatchewan, offers an occupational health program to five of the six grain elevator companies in Saskatchewan. The program includes a mobile surveillance program for workers in the grain industry whereby workers were tested every three years. Each employee was administered a standard questionnaire that obtained information regarding demographic background, occupational history, respiratory symptoms, and smoking history. A SensorMedics volume displacement spirometer (SensorMedics; Anahein, California) that met the American Thoracic Society specifications (American Thoracic Society, 1987) was used for lung function test measurements. Pulmonary function measurements were performed in a mobile pulmonary unit operated by technical staff members of the CCHSA. The pulmonary test variables FVC (Forced Vital Capacity in liters) and FEV_1 (Forced Expiratory Volume in the first second in liters), the FEV_1/FVC ratio, and the MMFR (Midmaximal flow rates in liters per second). In 2002, the questionnaire information was augmented with genetic information. Grain handlers currently employed in Saskatchewan volunteered for genetic testing.

DNA Extraction and Genotyping

Details of the laboratory analyses are explained elsewhere.[15] Briefly, the buccal cells containing DNA were collected on sterile cotton swabs (Sanyo; Osaka, Japan) by rubbing on the inner cheek for 30 seconds. Genomic DNA was obtained using a protocol modified from Richards et al.[15]

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (as reported previously)[15] was used to determine polymorphism of the TNF promoter region at position −308 in 243 grain workers from 2002 through 2005. Results were linked with previous information collected from 1993 through 2001. Laboratory personnel were unaware of the smoking status and pulmonary function data of individuals prior to the compilation of genotype data.

Ethics

The research design, methodology, and all communications with the participants were

approved by the University of Saskatchewan Presidents' Committee for Biomedical Ethics. All participants gave written informed consent for their participation in the study.

Statistical Analysis

Means and standard deviations were used to describe the distribution of continuous variables. Frequencies and percentages were used to describe the distribution of categorical variables.

The longitudinal analyses for FEV_1 and FVC were conducted by means of a marginal model using the Generalized Estimating Equations (GEE) approach.[18] A series of marginal models were fitted using SAS (Statistical Analysis System)[19] procedure PROC GENMOD. The correlation matrix of repeated observations showed a first-order autoregressive pattern, which was then selected as a within-subject correlation structure. The main effects in the multivariable marginal models were selected based on standard model-building strategies;[20] potential interaction terms were included in the model one at a time; and a final model with significant main effects and interaction terms was used for predicting the annual declines in FEV_1 and FVC. Significant predictors were determined by using the Wald statistic.

RESULTS

The number of grain workers in the province of Saskatchewan who participated in the Medical Surveillance Program during 1993–2005 is given in Table 1. There were 243 grain workers who contributed to 499 observations during Cycle VI through Cycle IX, 1993–2005. There were 11 grain workers who participated in all four cycles and contributed 44 observations; 79 grain workers who participated in any three cycles and contributed 237 observations; 65 grain workers who participated in any two cycles and contributed 130 observations; and 88 grain workers who participated in any one cycle and contributed 88 observations. It was reported earlier that allele frequencies of TNF-308*1 and TNF-308*2 were 79.3% and 20.7%, respectively, and genotype frequencies satisfy Hardy-Weinberg test for equilibrium.[15]

Table 1 displays the means and their standard errors (SE) for the demographic variables (age, height, and weight) and years in the grain industry, as well as count (percentage) for smoking status. Current smokers percentage decreased from Cycle VI (1993–1996) to Cycle IX (2002–2005). Table 2 shows the results from the multivariable model. Height, weight, cycle, and smoking intensity as well as interaction term (years in grain industry and TNF-α genotype) all had significant effect in the prediction of FEV_1. For FVC, all main effects significant for the prediction of FEV_1 were significant; however, years in grain industry was not an effect modifier in the relationship between TNF-α and FVC. Rather, smoking intensity was an effect modifier in the relationship between TNF-α and FVC. All variables and interaction

TABLE 1. Descriptive Statistics of Demographic Variables, Years in the Industry, Lung Function Values, and Smoking Status Stratified by Cycle

	Cycle VI (n = Mean ± S.D)	Cycle VII (n = Mean ± S.D)	Cycle VIII (n = Mean ± S.D)	Cycle IX (n = Mean ± S.D)
Age, yrs	34.6 ± 7.3	35.9 ± 8.2	35.9 ± 9.3	37.0 ± 9.3
Height, cm	177.1 ± 6.9	175.5 ± 7.3	176.9 ± 6.4	177.0 ± 6.8
Weight, Kg	87.4 ± 13.9	89.4 ± 15.1	90.1 ± 16.3	92.6 ± 15.7
Yrs. in grain industry	11.6 ± 6.8	12.7 ± 8.1	12.1 ± 8.7	13.2 ± 9.1
FEV1, litres	4.4 ± 0.7	4.4 ± 0.7	4.3 ± 0.7	4.3 ± 0.7
FVC, litres	5.5 ± 0.9	5.5 ± 0.9	5.5 ± 0.8	5.5 ± 0.9
Smoking Status	n (%)	n (%)	n (%)	n (%)
Current-smokers	38 (40%)	42 (32.8%)	38 (29.5%)	41 (28.1%)
Ex-smokers	19 (20%)	32 (25.0%)	30 (23.3%)	35 (24.0%)
Non smokers	38 (40%)	54 (42.2%)	61 (47.2%)	70 (47.9%)

TABLE 2. Estimates (± SE) of Regression Coefficients Based on Multivariable Regression Analysis of the Lung Function Values

	FEV_1 (mL) $\hat{\beta}(S.E.(\hat{\beta}))$	FVC (mL) $\hat{\beta}(S.E.(\hat{\beta}))$
Constant	–1.74 (1.16)	–2.28 (1.47)
<10 years	Ref	Ref
10–20 years	–0.16 (0.09)	–0.25 (0.06)****
≥ 20 years	–0.27(0.13)*	–0.35 (0.07)****
Height (/cm)	0.04 (0.01)****	0.05 (0.01)****
Weight (/kg)	–0.01 (0.002)***	–0.01 (0.002)***
TNF-α (*2/*2)	Ref	Ref
TNF-α (*1/*1)	0.31 (0.12)**	0.002 (0.15)
TNF-α (*1/*2)	0.49 (0.14)***	0.19 (0.15)
Non-smokers	Ref	Ref
Yrs of smoking: 0–10	0.01 (0.09)	–0.38 (0.21)
Yrs of smoking 10–20	–0.06 (0.08)	–0.34 (0.18)
Yrs of smoking > 20	–0.17 (0.08)*	–0.59 (0.19)**
Cycle VI	Ref	Ref
Cycle VII	0.05 (0.04)	0.11 (0.04)**
Cycle VIII	–0.11 (0.04)*	0.02 (0.05)
Cycle IX	–0.09 (0.05)	0.05 (0.06)
TNF-α (*1/*1)*(10–20 years)	–0.32 (0.18)	–
TNF-α (*1/*1)*(≥20 years)	–0.04 (0.14)	–
TNF-α (*1/*2)*(10–20 years)	–0.31 (0.14)*	–
TNF-α (*1/*2)*(≥20 years)	–0.04 (0.10)	–
TNF-α (*2/*2)* (< 10 years)	Ref	–
TNF-α (*1/*1)* yrs smoking 0–10	–	0.44 (0.24)*
TNF-α (*1/*1)*yrs smoking 10–20	–	0.40 (0.21)
TNF-α (*1/*1)*yrs smoking > 20	–	0.52 (0.22)
TNF-α (*1/*2)* yrs smoking 0–10	–	0.33 (0.27)
TNF-α (*1/*2)* yrs smoking 10–20	–	0.16 (0.26)
TNF-α (*1/*2)* yrs smoking > 20 yrs	–	0.22 (0.23)
TNF-α (*2/*2)* (Non-smokers*)	–	Ref

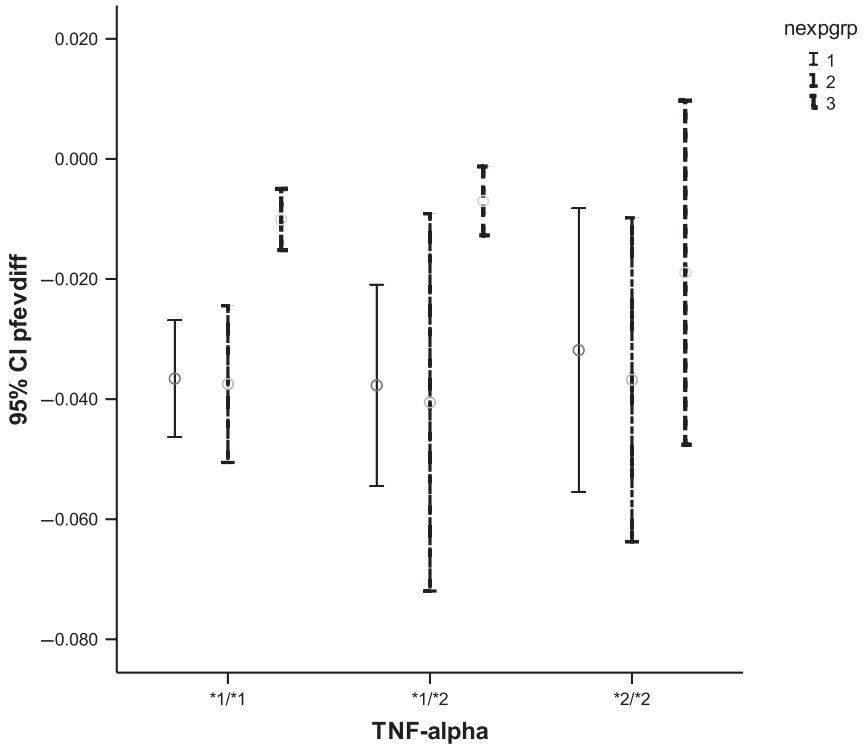
*<0.05; **<0.01; ***<0.001; ****<0.0001.

terms significant at p-value <.05 were retained in the final model and the final model was used to predict the annual decline. Based on the significant interaction terms, these annual declines were plotted to investigate how the annul decline changes for different levels of factors/variables involved in the interaction terms. From Figure 1, the relationship between years in grain industry and annual decline in predicted FEV_1 clearly is inconsistent across wild (*1/*1) and polymorphic (*1/*2 and *2/*2) categories of TNF-α. Grain workers with TNF-α *1/*1 genotype or *1/*2 genotype and are in the grain industry for less than 10 years have significantly lower decline compared to those who were in the industry for more than 10 years. In contrast, no differences were observed among grain workers with *2/*2 genotype based on years in the grain industry.

DISCUSSION

The authors investigated the potential relationships between longitudinal declines in pulmonary function measurements (FVC and FEV_1) and factors related to occupation (grain dust), lifestyle (cigarette smoking), inherited susceptibility/resistance (TNF-308 alpha genotype), and interactions between the aforementioned factors based on longitudinally collected data on currently employed male grain handlers. The important findings of this report are: (1) Among *1/*1 homozygotes and *1/*2 heterozygotes, grain workers with <10 years in the grain industry had significantly lower FEV_1 decline compared to that of the other two exposure groups (>10 and ≤20 years, and >20 years in the grain industry); (2) In contrast, among the *2/*2 genotype group, no difference was

FIGURE 1. Longitudinal decline in FEV1 during 1993–2005 stratified by TNF-α and years in grain industry.



observed in annual decline for the three exposure groups; (3) The annual decline in FEV_1 for grain workers who were either *1/*1 homozygote or *1/*2 heterozygote and had been in the grain industry for <10 years was lower compared to that of those grain workers who were *2/*2 genotype and had been in the industry for <10 years; and (4) Smoking intensity contributed to the decline in FEV_1. Smoking was not an effect modifier for the relationship between FEV_1 and TNF-α genotype. This finding was consistent with the results of Sandford et al.,[12] who genotyped both continuing smokers who experienced rapid declines in FEV_1 (decline in FEV_1 = −15.4 ± 3 ml/yr) and nondecliners (decline in FEV_1 = +15 ±2 ml/yr) over a five-year period. Sandford et al.[12] did not find an association between rapid declines in FEV_1 and any of the TNF-α genotypes. Irrespective of the smoking intensity category, grain workers with genotype *2/*2 had slightly higher declines in FVC compared to those of grain workers with *1/*1 and *1/*2 genotypes (data not shown). Based on a cross-sectional study, the influence of TNF-α 308 genotype and smoking status interaction on pulmonary function of physically active grain handlers has been examined.[15]

Subjects who participated in the current study had handled a variety of types of grains (listed in descending order of frequency of handling these included barley, oats, canola, field peas, wheat, flax, rye, canary seed, mustard seed, lentils, soy beans, beans, and sunflower seeds). The respiratory health of Canadian grain elevator workers has been extensively investigated.[1–5] Grain dust remediation measures gradually have been implemented into grain-handling facilities, and these measures have been shown to decrease both the longitudinal declines in pulmonary function and prevalence rates of respiratory diseases among grain workers.[5] Percentage of current smokers decreased from Cycle VI (1993–1996) to Cycle IX (2002–2005) as a consequence of "quit

smoking" educational programs implemented for grain elevator workers under the Grain Dust Medical Surveillance Program, which commenced in 1978 and was conducted until 1993 at a national level.

Most of the studies that cite genetic information based on inherited resistance/susceptibility to grain dust exposure have focused on alpha$_1$ antitrypsin genotypes, atopy, asthma, and bronchial hyperreactivity as factors influencing individuals to leave the industry early or to avoid it altogether as a result of healthy worker effect which has been repeatedly demonstrated by several authors.[15]

When analyses were conducted for ratio FEV$_1$/FVC as an outcome variable, the main effects were not significant; however, even with small cell numbers there was a significant three-way interaction between smoking, years in the grain industry, and TNF-α . Due to small numbers after stratification into smoking intensity, years in the grain industry, and TNF- genotype, the results for FEV$_1$/FVC ratio are not reported in this manuscript. As reported earlier, the only two participants who met the clinical definition of COPD had the variant *2 allele.[15] Pathophysicological roles of TNF-308 genotypes in bronchial inflammation may differ depending on disease stage with almost normal lung function, and with severity of COPD.[12]

Stayed-in grain workers' baseline demographic characteristics, smoking status, lung function values (FEV$_1$, FVC, FEV$_1$/FVC ratio), and chronic respiratory symptoms were compared with those of workers who dropped out from the program during the study period. Compared to the stayed-in grain workers, dropouts were younger and had fewer years in the industry, lower FEV$_1$/FVC ratios, and higher proportions of current smoking status.

This study has several strengths. The compliance with the genetic component among eligible male grain workers was 79% and the success rate for genotyping TNF-α was 100%.[15] The pulmonary function tests were conducted by a highly trained nurse using techniques that conformed to American Thoracic Society standards. The pulmonary function tests were reviewed by an occupational health physician. The study participants had similarities in occupational exposures to a variety of organic dusts and all were currently employed in the grain industry. The limitation of this study was that women were excluded from these analyses as a result of small numbers employed in that industry and also because their job descriptions and working conditions were not similar to those of men working in the grain industry.

This research has demonstrated that years in the grain industry is an effect modifier between TNF-α 308 genotype and longitudinal decline in FEV$_1$ in male subjects exposed to grain dust.

REFERENCES

1. Moira CY, Enarson DA, Kennedy SM. The Impact of grain dust on respiratory health. *Am Rev Respir Dis.* 1992;145(2 Pt 1):476–487.

2. Pahwa P, Senthilselvan A, McDuffie HH, Dosman JA. Longitudinal estimates of pulmonary function decline in grain workers. *Am J Respir Crit Care Med.* 1994; 150(3):656–662.

3. Pahwa P, Senthilselvan A, McDuffie HH, Dosman JA. Longitudinal decline in lung function measurements among Saskatchewan grain workers. *Can Respir J.* 2003; 10(3):135–141.

4. Pahwa P, McDuffie HH, Dosman JA. Longitudinal changes in prevalence of respiratory symptoms among Canadian grain elevator workers. *Chest.* 2006;129(6):1605–1613.

5. Pahwa P, Dosman JA, McDuffie HH. Effects of grain dust on lungs prior to and following dust remediation. *J Occup Environ Med.*. 2008;50(12):1394–1400.

6. Cotton DJ, Graham BL, Li KY, Froh F, Barnett GD, Dosman JA. Effects of smoking and occupational exposure on peripheral airway function in young cereal grain workers. *Am Rev Respir Dis.* 1982;126(4): 660–665.

7. Cotton DJ, Graham BL, Li KY, Froh F, Barnett GD, Dosman JA. Effects of grain dust exposure and smoking on respiratory symptoms and lung function. *J Occup Med.* 1983;25(2):131–141.

8. Dosman JA, Cotton DJ, Graham BL, Li KY, Froh F, Barnett GD. Chronic bronchitis and decreased forced expiratory flow rates in lifetime nonsmoking grain workers. *Am Rev Respir Dis.* 1980;121(1):11–16.

9. Churg A, Dai J, Tai H, Xie C, Wright JL. Tumor necrosis factor-alpha is central to acute cigarette smoke-induced inflammation and connective tissue breakdown. *Am J Respir Crit Care Med.* 2002;166(6):849–854.

10. Keatings VM, Cave SJ, Henry MJ, Morgan K, O'Connor CM, FitzGerald MX, Kalsheker N. A polymorphism in the tumor necrosis factor-alpha gene promoter region may predispose to a poor prognosis in COPD. Chest. 2000;118(4):971–975.

11. Higham MA, Pride NB, Alikhan A, Morrell NW. Tumour necrosis factor-alpha gene promoter polymorphism in chronic obstructive pulmonary disease. *Eur Respir J*. 2000;15(2):281–284.

12. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD. Susceptibility genes for rapid decline of lung function in the Lung Health Study. *Am J Respir Crit Care Med.* 2001;163(2):469–473.

13. Huang SL, Su CH, Chang SC. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med.* 1997;156(5):1436–1439.

14. Sakao S, Tatsumi K, Igari H, Shino Y, Shirasawa H, Kuriyama T. Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2001;163(2):420–422.

15. McDuffie HH, Nakagawa K, Pahwa P, Shindo J, Hashimoto M, Nakada N, Ghosh S, Kirychuk SP, Hucl P. Tumor necrosis factor alpha and pulmonary function in Saskatchewan grain handlers. *J Occup Environ Med.* 2006;48(5):505–512.

16. Takayuki S, Pahwa P, McDuffie HH, Shindo J, Goto S, Hashimoto M, Ghosh S, Nakagawa K. Association between paraoxonase-1 Q192R polymorphism and lung function among Saskatchewan grain handlers. *Pharmacogenomics*. 2007;8(8):901–908.

17. Sandford AJ, Chan HW, Wong GW, Lai CK, Chan-Yeung M. Candidate genetic polymorphisms for asthma in Chinese schoolchildren from Hong Kong. *Int J Tuberc Lung Dis.* 2004;8(5):519–527.

18. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika.* 1986;73(1):13–22.

19. PROC GENMOD Statement. Available from: http://support.sas.com/documentation/onlinedoc/91pdf/sasdoc_91/stat_ug_7313.pdf [accessed 7 Jan 2009].

20. Hosmer DW, Lemshow S. Applied Logistic Regression. A Wiley-Interscience Publication, John Wiley and Sons Inc., Canada, 1989; 82–134.