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Abstract: Objectives: The object of this study is to assess the modulating effects of genetic polymorphisms of Glycine N-methyltransferase (GNMT) genotypes on 1-hydroxypyrene (1-OHP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine from coke-oven workers, consistently exposed to polycyclic aromatic hydrocarbons (PAHs).
Methods: The study participants included 289 coke oven workers from a steel company in Taiwan. Personal air samples, spot urine samples, peripheral blood samples and questionnaires were used to collect PAH exposure, oxidative DNA damage, genetic polymorphisms of GNMT, demographic data, and environmental pollutants.
Results: Urinary 1-OHP, GNMT STRP1 genotype, worksite were significant predictors of urinary 8-OHdG levels after adjustments are made for covariates.
Conclusions: This study suggests that GNMT STRP1 could modulate urinary 1-OHP and 8-OHdG levels in coke oven workers exposed to high levels of PAHs.

**Dear Editor,** **February 9, 2011**

On behalf of all co-authors, I am sending this manuscript entitled "Glycine N-methyltransferase Affects Urinary 1-Hydroxypyrene and 8-Hydroxy-2'-Deoxyguanosine Levels after PAH Exposure" for publication in **Journal of Occupational and Environmental Medicine**. This paper is co-authored by Marcelo Chen, MD; Chiao-Wei Ho, MSc; Yu-Chuen Huang, PhD; Kuen-Yuh Wu, PhD; Ming-Tseng Wu, ScD; Anna Jeng, ScD; Chiou-Jung Chen,PhD; Tung-Sheng Shih, ScD; Ching-Huang Lai, PhD; Chih-Hong Pan, PhD; and Yi-Ming Arthur Chen, ScD. Each of the authors has participated either in the conception and design of the work (Yi-Ming Arthur Chen, ScD; and Chih-Hong Pan, PhD ) or data collection and analysis activities (Marcelo Chen, MD; Chiao-Wei Ho, MSc; Yu-Chuen Huang, PhD; Kuen-Yuh Wu, PhD). The manuscript was mainly drafted by Chih-Hong Pan, PhD; and Yi-Ming Arthur Chen, ScD , and critically revised by Ming-Tseng Wu, ScD; Anna Jeng, ScD; Chiou-Jung Chen,PhD; Tung-Sheng Shih, ScD; Ching-Huang Lai, PhD to improve its intellectual content. This paper belongs to the research article of "**Genetic & Molecular Epidemiology**".

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# Glycine N-methyltransferase Affects Urinary 1-Hydroxypyrene and 8-Hydroxy-2'-Deoxyguanosine Levels after PAH Exposure

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**The authors declare they have no conflicting financial interests.**

**Running title:** GNMT affects on Urinary 1-OHP and 8-OHdG after PAH exposure

**Keywords:** glycine N-methyltransferase, coke-oven workers, polycyclic aromatic hydrocarbons, 1-hydroxypyrene, 8-hydroxy-2-deoxyguanosine, genetic polymorphisms

# Glycine N-methyltransferase Affects Urinary 1-Hydroxypyrene and 8-Hydroxy-2'-Deoxyguanosine Levels after PAH Exposure

**Objectives:** The object of this study is to assess the modulating effects of genetic polymorphisms of Glycine N-methyltransferase (GNMT) genotypes on 1-hydroxypyrene (1-OHP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine from coke-oven workers, consistently exposed to polycyclic aromatic hydrocarbons (PAHs).

**Methods:** The study participants included 289 coke oven workers from a steel company in Taiwan. Personal air samples, spot urine samples, peripheral blood samples and questionnaires were used to collect PAH exposure, oxidative DNA damage, genetic polymorphisms of GNMT, demographic data, and environmental pollutants.

**Results:** Urinary 1-OHP, GNMT STRP1 genotype, worksite were significant predictors of urinary 8-OHdG levels after adjustments are made for covariates.

**Conclusions:** This study suggests that GNMT STRP1 could modulate urinary 1-OHP and 8-OHdG levels in coke oven workers exposed to high levels of PAHs.

# Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of toxic and lipophilic compounds, are formed during incomplete combustion of organic material and are by-products of coal and coke gasification when coal is pyrolyzed into coke. Thus, coke oven workers (COWs) are likely exposed to PAHs from coke oven emissions (COEs). Long-term exposure to COEs with PAH concentrations has been associated with increased risks of cancer, especially the lung cancer.[1] For example, topside coke-oven workers with occupational exposure over 15 years had a 16-fold increase in risk of developing lung cancer as compared to the general population.[2]

Measurement of urinary metabolites, as a marker of internal dose, is an important way of assessing PAH exposure and its health consequences, since such an approach takes into account all absorption routes and metabolism pathways of exogenous compounds. Pyrene is a useful indicator of environmental and occupational exposure to PAHs since it is abundant in most of PAH mixtures. Pyrene is absorbed through the lung and/or skin and then metabolized to 1-hydroxypyrene (1-OHP), which is ultimately excreted in urine. Urinary 1-OHP has been widely used as a biomarker to reflect recent occupational exposure to PAHs in firefighters,[3] iron foundry workers,[4] coke oven workers[5,6] and restaurant workers.[7,8]

PAHs in biological system are capable of generating reactive oxygen species, which

may cause oxidative damage to nucleic acids, proteins and lipids. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most common DNA lesion that is induced by the reaction of hydroxyl radicals with guanosine at the C-8 position in DNA.[9] DNA damage may be repaired by the base excision repair pathway, and the resulting repair product, urinary 8-OHdG, is affected by neither diet nor cell turnover.[10] The measurement of urinary 8-OHdG is useful in evaluating risks of lung cancer[11] and other oxidative stress-related diseases.[12]

The multi-functional protein, glycine N-methyltransferase (GNMT, EC2.1.1.20, localized to chromosome 6p12), affects genetic stability by regulating the ratio of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocystine,[13] and interacting with environmental carcinogens, such as benzo(a)pyrene (BaP).[14] Our recent studies showed that GNMT may protect cells from attacks by PAHs, such as BaP, by inhibiting DNA-adduct formation[15] and altering detoxification pathway geneotype expression profiles.[16] We have recently identified three variants including a GA di-nucleotide short tandem repeat polymorphism (STRP1), one GAGT tetra-nucleotide insertion or deletion (INS/DEL) polymorphism, and a single nucleotide polymorphism (SNP1; rs10948059) in the promoter region of GNMT.[17] These three variants exhibited strong linkage disequilibrium between any two loci of the three polymorphic sites.[18] Also, they are related to the susceptibility of hepatocellular

carcinoma and prostate cancer.[17, 18] Furthermore, the luciferase reporter gene assay has shown that the three variants are related to GNMT expression.[17, 18] Therefore, genetic polymorphisms may affect GNMT expression, which, in turn, may vary the degree of cancer risk and susceptibility to environmental carcinogens.

To our knowledge, no previous study has assessed the modulating effect of GNMT on urinary 1-OHP and 8-OHdG in PAH exposure workers. Therefore, we designed a cross-sectional study to investigate the modulating effect of polymorphisms at STRP1, INS/DEL and SNP rs10948059 of GNMT on the levels of urinary 1-OHP and 8-OHdG in coke-oven workers exposed to high levels of PAHs. We examined the correlation between GNMT polymorphisms and PAH exposure by comparing urinary biomarker levels and genotypic frequencies. Finally, we examined whether demographic parameters, such as cigarette smoking, alcohol consumption and vitamin supplement consumption, were predictors for modifying the effect of GNMT polymorphisms on exposure to PAHs.

## Materials and Methods

### Study Subjects

A total of 382 male coke-oven workers, employed for at least one year at two coke-oven plants in a steel company in Taiwan, have participated in an annual health

examination since 2005. These two coke-oven plants were beside each others and had the same manufacture process. During the health check-ups, trained interviewers collected a questionnaire survey from the participants. The survey included their socieo-demographics, work experience, cigarette smoking, alcohol consumption, and taking vitamin supplements. Cigarette smoking, alcohol consumption, and vitamin supplement consumption were deemed positive if any behavior occurred at least four days per week. Due to work commitments, 93 workers could not complete both questionnaire survey and health examination and were excluded from this study. That left a remaining total of 289 (response rate = 76%) coke oven workers for further examination of genetic polymorphism evaluation.

Based on job titles obtained from responses to the questionnaire survey, the 289 coke oven workers were classified into two groups to represent high and low exposure to PAHs. The topside-oven workers included, lidmen, tar chasers, and larry car operators, who were exposed to relatively high PAH levels in the coke oven plants. The side-oven workers included wharfmen, door repairmen, benchmen, coke side machine operators, quenchers, pushers, body repairmen, supervisors, heaters, and temperature controllers, who were exposed to low PAH levels in the coke oven plant [19]. The single blood and spot urine samples from the subjects were collected in post-work shifts during the weekend. All participants were asked to wash their hands before urine collection to prevent contamination. The Institutional Review Board of the National Yang-Ming University in Taiwan approved the study. Informed consent was obtained from all of the subjects.

**Exposure Measurement**

We quantified human subject exposure to PAHs by using a combination of personal dosimetry and biomonitoring of 1-OHP and 8-OHdG in urine.

**Particulate PAHs**

For personal dosimetry, personal breathing zone air samples were collected to determine external PAH exposure to coke-oven workers. This approach can accurately quantify PAH intake from inhalation and eliminate interference from the samplers themselves, which may have an impact on the workers' activities. The personal breathing zone samples were collected from 20 topside-oven workers and 20 side-oven workers. The sampling took place from 7:00 to 3:00 p.m. over two consecutive workdays. IOM (Institute of Occupational Medicine, England) samplers with glass fiber filters (diameter: 25mm, pore size: 0.7 μm) at a flow rate of 2.0 L/min were used for the particulate PAH sampling. Duplicate samples were obtained for each personal sampling. Total PAH exposure and exposure to 16 targeted PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene, benzo(a)anthracene, chrysene, Benzo(b)fluoranthene, benzo(k)fluoranthene, BaP, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene identified as priority pollutants by the U.S. Environmental Protection Agency (EPA) were analyzed using a gas chromatogram quadruple mass spectrometer (GC/MS) with an automatic sampler system.[5] The detection limits were determined by conducting seven repeated analyses of the lowest standards of each PAH species. The detection limits of the 16 PAHs ranged from 6.1 ng for dibenzo(a,h)anthracene to 9.0 ng for phenanthrene. The coefficient of variation among these repeated analyses was less than 2% for all 16 PAHs.

**Urinary 1-OHP and 8-OHdG**

As indicated earlier, spot urine samples were collected from human subjects in

post-work shifts during the week. Immediately after collection, samples were stored at -80$^{o}$C until analysis. Urinary 1-OHP was analyzed using HPLC with a fluorescence detector. The detection limit was about 0.1 μg/L, based on seven repeated analyses of 1-OHP at 15.0 μg/L, and the variation in the coefficients from the repeated analyses of urinary 1-OHP was less than 10%. Urinary 8-OHdG level was measured using an HPLC/MS/MS, as described elsewhere.[20] A detection limit of 5.7 ng/L was obtained using seven repeated analyses of deionized water. The coefficients of variation in inter-day and intra-day tests were less than 5%.

**DNA extraction from peripheral blood lymphocytes**

A 5-ml sample of peripheral blood, collected in 7.5% EDTA tubes, was obtained from each subject during the weekend. Peripheral blood lymphocytes (PBL) samples were prepared immediately and stored at -80°C until extraction of the genomic DNA. Genomic DNA was obtained by conventional phenol/chloroform extraction, followed by ethanol precipitation, and stored at -20°C until use for genotyping.

**GNMT Genetic Polymorphism Determination**

**GeneScan Analysis**

Fragment analysis was used for detection of the STRP1 polymorphism. Primers for the STRP1 marker were forward: 5'-FAM-CAAGTTGGAAAGGAAGGAGGAGAG; and reverse: 5'-GCGAGCCAGCCAGCAGAAAGA. The forward primer was labeled with fluorescence. Polymerase chain reaction (PCR) amplification was carried out using 1.0 ng/μL PBL DNA, 1.0 unit of AmpliTaq Gold (Applied Biosystems, Foster City, CA), 0.5μM of each primer, 0.5 mM deoxynucleotides, and 2.5 mM $MgCl_2$ in a total reaction volume of 20μL. The PCR thermal profile was 93°C for 10 min to activate the AmpliTaq Gold, followed by 35 cycles consisting of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s. The final elongation was at 72°C for 60 min.

Primers used for genotyping of the INS/DEL marker were forward: 5'-HEX-GCACAAACAAAGCAAGAAAG; and reverse: 5'-ATGCCCGCCATTAATAAC. The forward primer was labeled with fluorescence. PCR amplification was carried out using 1.0 ng/μL PBL DNA, 1.0 unit of AmpliTaq Gold, 0.5μM of each primer, 0.5 mM deoxynucleotides, and 2.5 mM $MgCl_2$ in a total reaction volume of 20μL. The PCR thermal profile was 93°C for 10 min to activate the AmpliTaq Gold, followed by 10 cycles consisting of 94°C for 30 s, 62°C for 30 s,

and 72°C for 30 s, and 20 cycles consisting of 89°C for 30 s, 62°C for 30 s, and 72°C for 30 s. The final elongation was at 72°C for 60 min.

PCR for both STRP1 and INS/DEL was performed using a GenotypeAmp PCR 9700 thermocycler (Applied Biosystems, Foster City, CA). PCR product electrophoresis was performed with an ABI 377 PRISM sequencer (Applied Biosystems, Foster City, CA). Fluorescent signals from different size alleles were analyzed using GeneScan and Genotyper software (Applied Biosystems, Foster City, CA). In GNMT STRP1, the number of GA repeats ranged from 10 to 20, with the most common genotypes being 16 GA repeats. Therefore, a cutoff point of 16 GA repeats was used during genotyping.

**TaqMan assay**

The TaqMan Allelic Discrimination method was used for the detection of SNP1. The primers used for detecting SNP1 were forward: GCGCGCTCACCTGCTATT and reverse: GGAGCGGGTCCGGTACAC. The allelic-specific fluorogenic probes were VIC-TCCGCACTTAAAGCATAAGCACTGCT-TAMRA for C allele and 6-FAM-CTC6CCGCACTTAAAACATAAGCACT-TAMRA for T allele of SNP1 (antisense). Each PCR reaction mixture contained 2.5 µl 10x Buffer A, 3.5 µL 25 mM $MgCl_2$, 2 µL 200 µM dNTPs, 3 µL 2.5 µM primers, 1 µL 5 µM Probe 1, 1 µL Probe 2, 0.125 µL 5 units/µL TaqGold, 9.375 µL water, and 2.5µL 10 ng DNA. The thermal profile was 95°C for 5 min, followed by 40 cycles consisting of 95°C for 15 s and 64°C for 1 min. Plates were read in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA), and results analyzed using the Allelic

Discrimination software.

**Statistical Methods**

Personal intake PAHs, urinary 8-OHdG, and 1-OHP levels were first log-transformed to normalize their distributions before the Student *t* test, one-way analysis of variance (ANOVA) with the Scheffe mean comparison test or regression analysis was performed. The Student *t* test and Chi-Square statistics were used to compare the personal covariates. Non-parametric Mann-Whitney U tests were conducted to compare the PAH levels of the topside-oven workers and side-oven workers. Spearman correlation analysis was employed to evaluate the correlation between urinary 8-OHdG and 1-OHP levels.

Multiple linear regression models were conducted to assess the relationship among workers' urinary 1-OHP and 8-OHdG levels, and GNMT genetic polymorphisms. A level of $\alpha$=0.05 was considered for statistical significance in all tests. All statistical analyses were performed using the Statistical Package for the Social Sciences software package (version 12.0).

# Results

Table 1 summarizes the descriptive statistics of 289 coke oven workers by job title. These workers regularly work six days per week and eight hours per day. Smoking status and working years for side-oven workers and topside-oven workers significantly differed. In contrast, age, BMI, alcohol consumption, and taking vitamin supplements did not differ significantly between these two groups. The frequency of heterozygous genotypes at <16/<16, <16/≥16, and ≥16/≥16 GA repeats

were significantly different between these two groups. In GNMT INS/DEL, the alleles had either GAGT tetranucleotide insertion (INS) or deletion (DEL) genotype. The genotypic frequencies for DEL/DEL, INS/DEL and INS/INS were not significantly different between these two groups. The genotypic frequencies of GNMT SNP1 for C/C, C/T, and C/T were significantly different between side-oven workers and topside-oven workers. The geometric mean 1-OHP and 8-OHdG levels of topside-oven workers significantly exceeded those of side-oven workers using Student's *t* test, as shown in Table 1. After stratifying data with smoking status, urinary 8-OHdG and 1-OHP of topside-oven workers remained significantly different from those of side-oven workers.

The personal PAH exposure data are shown in Table 2. Workplace personal breathing samples were collected from 20 topside-oven workers and 20 side-oven workers. Each personal measurement was an average of duplicate samples collected on two consecutive working days. Due to the limited sample size, non-parametric Mann-Whitney U tests were used to compare the PAH exposure between these two groups. Of the 16 targeted PAHs, the air levels of 13 U.S. EPA-targeted PAH species and total PAHs were significantly higher in the topside-oven site than in the side-oven site.

Urinary 1-OHP is a metabolite of pyrene, which is one of the many chemicals in PAHs. Accordingly, the extent to which pyrene correlates with the other PAHs-related chemicals is important given these other chemicals are likely causes of most of the oxidative stress. Therefore, a Spearman correlation analysis was used to investigate correlations between

average pyrene levels and average levels of other PAHs. Pyrene levels were significantly correlated with acenaphthylene, phenanthrene, pyrene, benzo(a)anthracene, Benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene and total PAHs in coke oven workers' personal breathing air (Table 3).

Table 4 compares urinary levels of 8-OHdG and 1-OHP by GNMT polymorphism using ANOVA with the Scheffe mean comparison test. Overall, the geometric mean 1-OHP and 8-OHdG levels of workers with STRP1 ≥16/≥16 GA repeats genotype were significantly higher than those workers with STRP1 <16/<16 GA repeats genotype ($P$<0.001). The urinary 8-OHdG levels of workers with STRP1 <16/≥16GA repeats genotype were significantly higher than those workers with STRP1 <16/<16 GA repeats genotype ($P$<0.01). The urinary 1-OHP levels of side-oven workers with STRP1 <16/≥16GA repeats genotype were also significantly higher than side-oven workers with STRP1 <16/<16 GA repeats genotype (P<0.001). However, there was no significant difference in urinary 1-OHP levels between side-oven workers with STRP1 ≥16/≥16 GA repeats genotype and with STRP1 <16/≥16GA repeats genotype ($P$ > 0.05). The urinary 1-OHP levels of topside-oven workers with STRP1 ≥ 16/≥16GA repeats genotype were significantly higher than those workers with STRP1 <16/≥16 GA repeats genotype ($P$<0.001). No statistically significant differences in 1-OHP levels were found among workers with INS/DEL genotypes or SNP1 genotypes ($P$ > 0.05). No statistically significant differences for 8-OHdG levels were also found among workers with INS/DEL genotypes or SNP1 genotypes ($P$ > 0.05).

Table 5 presents the results of multiple linear regression models for predictors of urinary 1-OHP and 8-OHdG levels in coke oven workers. The urinary 1-OHP levels in coke-oven workers with both the STRP1 <16/≥16 GA repeats genotype and ≥16/≥16

GA repeats genotype significantly exceeded those of workers with the STRP1 <16/<16 GA repeats genotype. However, smoking status, alcohol consumption, taking vitamin supplements, working years, age and BMI were not significant predictors of urinary 1-OHP levels in the coke-oven workers. Urinary 1-OHP, GNMT STRP1 genotype, and worksite were significant predictors of urinary 8-OHdG levels in the models adjusted for other personal covariates. The urinary 8-OHdG levels in coke-oven workers with both the STRP1 <16/≥16 GA repeats genotype and ≥16/≥16 GA repeats genotype significantly exceeded those of workers with the STRP1 <16/<16 GA repeats genotype. The urinary 8-OHdG levels of the topside-oven workers significantly exceeded those of the side-oven workers ($p < 0.001$). The increase in urinary 1-OHP was significantly related to the increase in urinary 8-OHdG. Working at a topside-oven still had an effect after controlling for the effect of urinary 1-OHP in our models. However, smoking status, alcohol consumption, taking vitamin supplements, working years, age and BMI were not significant predictors of urinary 8-OHdG levels in the coke-oven workers.

## Discussion

Our results showed that personal uptake of BaP, total PAH, and urinary 1-OHP levels significantly and positively correlated with pyrene levels. Both Student's t tests and multiple linear regression models indicated that the urinary 1-OHP level of topside-oven workers was significantly higher than that of side-oven workers. This could be because topside oven workers had been exposed to significantly higher levels of particulate PAHs than side oven workers using a personal air-sampling device. These findings indicate that urinary 1-OHP is a sensitive internal dose of exposure to PAHs among coke oven workers.

Even though one cigarette contains about 50-200 ng of pyrene,[21] cigarette smoke did not significantly influence urinary 1-OHP levels in this study. The similar finding was observed in a previous study on urinary 1-OHP of coke oven workers.[6] Conversely, a significantly higher level of urinary 1-OHP was found among smoking workers (n=44) compared to nonsmokers (n=44) at a steel plant.[21] Because the relationship between cigarette smoking and urinary excretion of 1-OHP in occupational studies has been inconsistent, further studies are needed to clarify the relationships among smoking, occupational PAH exposure, and urinary levels of 1-OHP.

Workers with GNMT STRP1 ≥16/≥16 GA repeats genotype had higher urinary 1-OHP levels than those workers with STRP1 <16/<16 GA repeats genotype after adjustments were made for other covariates. This suggests urinary 1-OHP levels could be modulated by GNMT STRP1. Furthermore, smoking, alcohol consumption, taking vitamin supplements, working years, age and BMI were not significant predictors of urinary 1-OHP. This finding is consistent with a previous study of urinary 1-OHP in male restaurant workers .[8]

Multiple regression analysis revealed that urinary 1-OHP, GNMT STRP1 genotype and worksite were significantly correlated with urinary 8-OHdG levels, such as, urinary 1-OHP and work at topside-oven may be served as good indicators of exposure to PAHs for predicting oxidative stress in workers at coke oven plants. It has been indicated that PAHs probably exert their biological effects through the generation of reactive oxygen species.[22] These reactive oxygen species can lead to the formation of oxidative damage to DNA.[22] Among the most abundant oxidatively

damaged DNA is 8-OHdG which was found to induce mutation through G to T transversion.[23] In this study, we found that coke-oven workers subjected to higher PAH exposure had increased oxidative damage as reflected by higher 8-OHdG levels. This finding is consistent with several occupationally related epidemiological studies in workers exposed to PAHs from a variety sources such as cooking oil fumes,[7] asbestos[24], fire,[25] and benzene.[26]

Urinary 8-OHdG is considered to arise from three sources: repair products of oxidized DNA, removal of oxidized dG in the neucleotide pool, and cell turnover. This reveals that urinary 8-OHdG represents average oxidative DNA damage level throughout the body.[27] In this study, we found that adverse oxidative responses to PAH exposure, reflected in elevation of urinary 8-OHdG levels, were aggravated in coke oven workers with GNMT STRP1 ≥16/≥16 GA repeats genotype or STRP1 <16/≥16 GA repeats genotype. These findings support the concept that STRP1 ≥16/≥16 GA repeats genotype and STRP1 <16/≥16 GA repeats genotype may impart greater susceptibility to PAH-associated oxidative stress effects.

BaP is one of many PAHs that have been identified as major factors for developing cancers. A previous study found a positive association between BaP levels and urinary 8-OHdG in restaurant workers.[28] Epidemiologic studies indicate an increase in incidence of cancer among workers exposed to PAHs,[29] even when the exposure to

BaP was below the permissible exposure limit of US Occupational Safety and Health Administration (OSHA) Standards: 0.2 mg/m$^3$.[30] For example, a significant positive correlation exists between DNA adducts in lymphocytes and BaP (from 2 to 62,107 ng/m$^3$) in the inhaled air of coke oven workers.[29] Furthermore, Nilsson noted increased urinary 8-OHdG in engine room personnel exposed to PAHs.[31] Moreover, a previous study demonstrated that GNMT may protect cells from attacks by environmental carcinogens such as BaP through direct interaction.[14] Another previous study demonstrated that GNMT sequestered BaP, diminished BaP's effects to the liver detoxicication pathway and prevented subsequent cytotoxicity.[15] In this study, we found that GNMT STRP1 <16/<16 GA repeats genotype could reduce levels of urinary 1-OHP and 8-OHdG. The fact that urinary 1-OHP is a metabolite of PAHs and is significantly and positively correlated with BaP, and 1-OHP has a significant positive correlation with 8-OHdG, suggests that GNMT STRP1 <16/<16 GA repeats genotype may reduce the effects of exposure to PAH in coke oven workers. According to our previous report, a GNMT promoter with shorter than 16 GA repeats has shown higher expression levels than a GNMT promoter with ≥ 16 GA repeats.[17] Higher GNMT promoter activity was correlated with lower urinary 1-OHP and 8-OHdG levels. It provides the evidence that GNMT may play an important role in cells to defend against the PAHs attacks.

The use of worksite as an independent predictor of 8-OHdG in multiple linear regression models reveals that coke oven workers may be exposed to other unmeasured hazards, such as benzene,[26] and phenol.[32] Our findings suggest that topside-oven workers are more likely to have oxidative stress than side-oven workers, which can be attributed to factors other than their urinary 1-OHP levels. Conversely, smoking, alcohol consumption, taking vitamin supplements, working years, age and BMI were not significant predictors of urinary 8-OHdG levels in the coke oven workers. This finding is consistent with a previous study of urinary 8-OHdG in restaurant workers.[7]

There were inconsistent findings regarding the influence of smoking status on 8-OHdG. Loft et al., showed that smokers excrete about 30-50% more 8-OHdG than nonsmokers.[24,33] However, this study found that smoking did not significantly affect urinary 8-OHdG levels. This finding is consistent with a previous study of urinary 8-OHdG levels in coke oven workers.[6]

Multiple vitamins contain antioxidants, such as vitamin C, that can protect DNA from the damage of oxidative stress.[34] Cooke et al. examined 8-OHdG in mononuclear cell DNA, serum and urine from subjects undergoing supplementation with 500 mg/day of vitamin C. They found that significant decreases in DNA levels of 8-OHdG correlated strongly with increases in plasma vitamin C levels.[34] However, this study found that coke oven workers who took vitamin supplements did not reduce urinary excretion of 8-OHdG. This finding is consistent with a previous study of urinary 8-OHdG levels in 116 non-smoking coke-oven workers.[6]

Some studies have shown an inverse relationship between urinary 8-OHdG levels and both age and BMI, possibly because older or leaner individuals have higher metabolic rates than younger or obese individuals.[23,35] However, this study did not determine that age or BMI influenced urinary 8-OHdG levels. The results herein are consistent with an earlier study of firefighters.[25]

This study has certain limitations. First, other unmeasured data concerning coke oven emissions, such as levels of benzene,[24] and phenol[32] were lacking, possibly confounding the results concerning oxidative stress. The other limitation was the lack of data on the exposure to PAHs outside occupational settings, such as from vehicle traffic emissions. However, the coke oven workers herein spent about eight hours daily at coke oven plants, including work and rest periods, but less than one hour daily in traffic. The contribution of traffic sources to PAH exposure of coke oven workers is thus assumed to be limited. Regardless of this limitation, this study concluded that urinary 1-OHP and 8-OHdG in coke oven workers could be modulated by GNMT STRP1.

An earlier animal study suggested that BaP may affect DNA methylation via interaction with DNA methyltranferase and GNMT and thus contribute to a carcinogenic pathway.[14] Another study demonstrated that GNMT may have a protective effect against the exposure to carcinogens by decreasing DNA adduct

formation.[36] This study found that GNMT STRP1 ≥16/≥16 GA repeats genotype and STRP1 <16/≥16 GA repeats genotype may increase susceptibility to PAH-associated oxidative stress effects. Conversely, urinary 8-OHdG could be reduced in coke-oven workers with STRP1 <16/<16 GA repeats genotype. Furthermore, urinary levels of 8-OHdG have been used to evaluate oxidative stress status in cancer patients with lung, bladder, prostate, and breast cancers showed higher urinary levels of 8-OHdG.[37, 38] Thus, this study provides evidence that genetic polymorphisms of GNMT may affect its expression and modulate PAH-associated oxidative stress regarding cancer risk in coke oven workers.

## Conclusion

In conclusion, this study proves that urinary 1-OHP and 8-OHdG in coke oven workers could be modulated by GNMT STRP1 genotype. This study also provides evidence that genetic polymorphisms of GNMT may affect its expression and modulate PAH-associated oxidative stress regarding cancer risk in coke oven workers.

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# References


1. Bertrand JP, Chau H, Patris A, et al. Mortality due to respiratory cancers in the coke oven plants of the Lorraine coalmining industry. *Br J Ind Med* 1987;44:559-65.
2. International Agency for Research on Cancer (IARC). Polynuclear aromatic compounds. Industrial exposures Part 3 Vol. 34, *IARC*, Lyon, France; 1984.
3. Caux C, O'Brien C, Viau C. Determination of firefighter exposure to polycyclic aromatic hydrocarbons and benzene during fire fighting using measurement of biological indicators. *Appl Occup Environ* Hyg 2002;17:379-86.
4. Hansen, AM, Omland O, Poulsen OM, et al. Correlation between work process-related exposure to polycyclic aromatic hydrocarbons and urinary levels of α-naphthol β- naphthylamine and 1- hydroxypyrene in iron foundry workers. *Int Arch Occup Environ Health*. 1994; 65:385-94.
5. Lin YC, Pan CH, Chen CJ, et al. Associations between exposure to polycyclic aromatic hydrocarbons and temporal change of urinary 1-hydroxypyrene levels in Taiwanese coke-oven workers. *J Occup Environ Med*. 2006;48:930-6.
6. Wu MT, Pan CH, Huang YL, Tsai PJ, Chen CJ, Wu TN. Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. *Environ Mol Mutagen*. 2003; 42:98-105.

7. Pan CH, Chan CC, Wu, KY. Effects on Chinese Restaurant Workers of Exposure to cooking oil fumes: a Cautionary Note on Urinary 8-Hydroxy-2'-Deoxyguanosine. *Cancer Epidemiol Biomarkers Prev.* 2008;17: 3351-7.
8. Pan CH, Chan CC, Huang YL, Wu, KY. Urinary 1-hydroxypyrene and malondialdehyde in male workers in Chinese restaurants. *Occup Environ Med.* 2008;65:732-5.
9. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis.* 1986;7:1849-51.
10. Cooke MS, Evans MD, Dove R, et al. DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. *Mutat Res* 2005;574:58-66.
11. Erhola M, Toyokuni S, Okada K, et al. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett*. 1997;409:287-91.
12. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta*. 2004;33:1-9.
13. Kerr SJ. Competing methyltransferase systems. *J Biol Chem.* 1972; 247:4248-52.
14. Chen SY, Lin JR, Darbha R, Lin P, Liu TY, Chen YM. Glycine

N-methyltransferase tumor susceptibility genotype in the benzo(a)pyrene-detoxification pathway. *Cancer Res.* 2004; 64:3617-23.

15. Lee CM, Chen SY, Lee YC, Huang CY, Chen YM. Benzo[a]pyrene and glycine N-methyltransferse interactions: gene expression profiles of the liver detoxification pathway. *Toxicol Appl Pharmacol*. 2006; 214:126-35.
16. Yen CH, Hung JH, Ueng YF, et al. Glycine N-methyltransferase affects the metabolism of aflatoxin B1 and blocks its carcinogenic effect. T*oxicol Appl Pharmacol*. 2009; 235:296-304.
17. Tseng TL, Shih YP, Huang YC, et al. Genotypic and phenotypic characterization of a putative tumor susceptibility genotype, GNMT, in liver cancer. *Cancer Res.* 2003; 63:647-54.
18. Huang YC, Lee CM, Chen M, et al. Haplotypes, loss of heterozygosity, and expression levels of glycine N-methyltransferase in prostate cancer. *Clin Cancer Res*. 2007; 13:1412-20
19. Wu MT. Assessment of the effectiveness of respirator usage in coke oven workers. *Am Ind Hyg Assoc J*. 2002;63:72-5.
20. Hu CW, Wang CJ, Chang LW, Chao MR. Clinical-scale high-throughput analysis of urinary 8-oxo-7,8-dihydro-2’-deoxyguanosine by isotope-dilution liquid chromatography-tandem mass spectrometry with on-line solid phase extraction.

*Clin Chem*. 2006;52:1381-1388.

21. Kang D, Rothman N, Cho SH, et al. Association of exposure to polycyclic aromatic hydrocarbons (estimated from job category) with concentration of 1-hydroxypyrene glucuronide in urine from workers at a steel plant. *Occup Environ Med*. 1995 Sep;52:593-9.
22. Chao MR, Wang CJ, Wu MT, et al. Repeated measurements of urinary methylated/oxidative DNA lesions, acute toxicity and mutagenicity in coke oven workers. *Cancer Epidemiol Biomarkers Prev*. 2008;17:3381-9
23. Toraason M, Hayden C, Marlow D, et al. DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. *Int Arch Occup Environ Health*. 2001;75:279.
24. Tagesson C, Chabiuk D, Axelson O, Barański B, Palus J, Wyszyńska K. Increased urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industries. *Pol J Occup Med Environ Health*. 1993;6:357-68
25. Hong YC, Park HS, Ha EH. Influence of genetic susceptibility on the urinary excretion of 8-hydroxydeoxyguanosine of firefighters. *Occup Environ Med*. 2000;57:370-5.
26. Lagorio S, Tagesson C, Forastiere F, Iavarone I, Axelson O, Carere A. Exposure to

benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occup Environ Med*. 1994;51:739-43.

27. Loft S, Poulsen HE. Antioxidant intervention studies related to DNA damage, DNA repair and gene expression. *Free Radic Res*. 2000;33 (suppl): S67-S83.
28. Pan CH, Shih TS, Chen CJ, et al. Reduction of cooking oil Fume exposure by engineering intervention in Chinese restaurants. *Occup Environ Med*. 2011;68:10-5.
29. Binková B, Topinka J, Mracková G, et al. Coke oven workers study: the effect of exposure and GSTM1 and NAT2 genotypes on DNA adduct levels in white blood cells and lymphocytes as determined by 32P-postlabelling. *Mutat Res*. 1998;416:67-84.
30. US Occupational Safety and Health Administration. Standard. 1983;29 CFR 1910.1000.
31. Nilsson R, Nordlinder R, Moen BE, et al. Increased urinary excretion of 8-hydroxydeoxyguanosine in engine room personnel exposed to polycyclic aromatic hydrocarbons. *Occup Environ Med*. 2004;61:692-6.
32. Robinson FP, Patterson CC. 1985; Changes in liver function test after propofo ('Diprivan'). *Postgrad Med J*. 61 (Suppl):160-1.
33. Loft S, Fischer-Nielsen A, Jeding IB, et al. 8-Hydroxydeoxyguanosine as a urinary

biomarker of oxidative DNA damage. *J Toxicol Environ Health*. 1993;40:391-404.

34. Cooke MS, Herbert KE, Butler PC, et al. Further evidence for a possible role of conformation in the immunogenicity and antigenicity of the oxidative DNA lesion, 8-oxo-2'deoxyguanosine. *Free Radic Res*. 1998;28:459-69.
35. Loft S, Vistisen K, Ewertz M, et al. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenotypesis*. 1992;13:2241–7.
36. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull*. 1999;55:578-92.
37. Miyake H, Hara I, Kamidono S, et al. Oxidative DNA damage in patients with prostate cancer and its response to treatment. *J Urol*. 2004;171:1533-6.
38. Wu LL, Chiou CC, Chang PY, et al. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta*. 2004;339:1-9.

Table 1

**TABLE 1**
Descriptive Statistics for 289 Coke Oven Workers by Job Title

| Personal characteristics, Mean ± SD | Side-oven workers (n=181) | Topside-oven workers (n=108) | *P* value |
|---|---|---|---|
| Age (years) | 44.8 ± 9.5 | 44.5 ± 8.3 | 0.788 |
| BMI ($kg/m^2$) | 24.9 ± 9.8 | 24.3 ± 3.7 | 0.500 |
| Work duration (years) | 17.5 ± 12.4 | 13.0 ± 7.1 | 0.001 |
| Health behaviors, n (%) | | | |
| Smoking | 80 (44.2%) | 65 (60.7%) | 0.007 |
| Alcohol consumption | 38 (21.0%) | 18 (16.7%) | 0.368 |
| Taking vitamin supplements | 68 (37.8%) | 29 (26.9%) | 0.058 |
| *GNMT* genotypic frequencies | | | |
| STRP1 | | | <0.001 |
| <16GA/<16GA | 53(29.3%) | 23 (21.3%) | |
| <16GA/≥16GA | 75 (41.4%) | 26 (24.1%) | |
| ≥16GA/≥16GA | 53 (29.3%) | 59 (54.6%) | |
| INS/DEL | | | 0.219 |
| DEL/DEL | 74(41.1%) | 55(50.9%) | |
| INS/DEL | 79(43.9%) | 42(38.9%) | |
| INS/INS | 27(15.0) | 11(10.2%) | |
| SNP1 | | | 0.018 |
| C/C | 92(50.8%) | 68(63.0%) | |
| C/T | 71(39.2%) | 25(23.1%) | |
| T/T | 18(10.0%) | 15(13.9%) | |
| Urinary1-OHP, GM (GSD) | 9.3(3.5) | 66.6(3.5) | <0.001 |
| Urinary 8-OHdG, GM (GSD) | 5.5 ( 3.1) | 16.4 ( 2.4) | <0.001 |

GM: geometric mean, GSD: geometric standard deviation.

**TABLE 2**

Comparisons of PAH Levels Between Side-oven Workers and Topside-oven Workers

| PAH (ng/m3) | Side-oven workers (n=20) | | Topside-oven workers (n=20) | | *P* value* |
|---|---|---|---|---|---|
| | Median | GM (GSD) | Median | GM (GSD) | |
| Naphthalene | 593.7 | 401.8 (2.5) | 724.7 | 505.3 (3.0) | 0.477 |
| Acenaphthylene | 158.0 | 144.0 (1.7) | 227.7 | 209.0 (2.0) | 0.065 |
| Acenaphthene | 52.9 | 53.9 (1.3) | 71.0 | 64.5 (1.3) | 0.038 |
| Fluorene | 225.3 | 232.9 (1.9) | 222.9 | 300.5 (1.9) | 0.212 |
| Phenanthrene | 15.7 | 23.9 (4.8) | 112.7 | 148.1 (8.2) | 0.003 |
| Anthracene | 211.6 | 139.5 (4.8) | 410.6 | 406.1 (1.9) | 0.001 |
| Fluoranthene | 94.2 | 97.8 (2.8) | 323.7 | 308.5 (2.0 ) | <0.001 |
| Pyrene | 227.0 | 178.9 (2.7) | 1220.0 | 543.4 (2.1 ) | <0.001 |
| Benzo(a)anthracene | 2103.2 | 1568.6 (2.0 ) | 3019.6 | 2707.6 (1.5 ) | 0.005 |
| Chrysene | 241.8 | 159.5 (2.6 ) | 446.0 | 419.6 (1.4 ) | <0.001 |
| Benzo(b)fluoranthene | 56.9 | 56.8 (5.0 ) | 313.0 | 260.4 (1.2 ) | 0.002 |
| Benzo(k)fluoranthene | 139.1 | 130.7 (1.7 ) | 260.6 | 213.0 (2.1 ) | 0.021 |
| Benzo(a)pyrene | 247.2 | 222.2 (2.2 ) | 577.3 | 487.4 (1.7 ) | 0.001 |
| Indeno(1,2,3-cd)pyrene | 42.8 | 27.5 (7.2 ) | 311.3 | 194.2 (3.4 ) | 0.001 |
| Dibenzo(a,h)anthracene | 72.7 | 24.9 (6.7 ) | 216.2 | 196.0 (1.3 ) | <0.001 |
| Benzo(ghi)perylene | 3.3 | 8.6 (4.6 ) | 119.5 | 47.9 (5.4 ) | 0.002 |
| Total PAHs | 4942.8 | 4210.4 (1.6) | 9210.9 | 8621.5 (1.5 ) | <0.001 |

GM: geometric mean, GSD: geometric standard deviation.

* Mann-Whitney U tests.

Table 3

**TABLE 3**

Correlation Between Urinary 1-Hydroxypyrene (1-OHP) and Polycyclic Aromatic Hydrocarbons (PAHs) in Coke Oven Workers (n=40)

| | 1-OHP | |
|---|---|---|
| PAHs | r | *P* value* |
| Naphthalene | 0.098 | 0.546 |
| Acenaphthylene | 0.541 | <0.001 |
| Acenaphthene | 0.312 | 0.050 |
| Fluorene | 0.012 | 0.942 |
| Phenanthrene | 0.592 | <0.001 |
| Anthracene | 0.189 | 0.244 |
| Fluoranthene | 0.306 | 0.055 |
| Pyrene | 0.330 | 0.038 |
| Benzo(a)anthracene | 0.354 | 0.025 |
| Chrysene | 0.126 | 0.438 |
| Benzo(b)fluoranthene | 0.314 | 0.048 |
| Benzo(k)fluoranthene | 0.063 | 0.701 |
| Benzo(a)pyrene | 0.414 | 0.008 |
| Indeno(1,2,3-cd)pyrene | 0.160 | 0.323 |
| Dibenzo(a,h)anthracene | 0.320 | 0.044 |
| Benzo(ghi)perylene | 0.343 | 0.030 |
| Total PAHs | 0.452 | 0.003 |

* *P* value calculated using Spearman correlation analysis.

**TABLE 4**

Comparison by GNMT Genotypic Polymorphism of Urinary 1-Hydroxypyrene (1-OHP) and 8-Hydroxy-2'-deoxyguanosine (8-OHdG) **L**evels (μg/L) in 289 Coke Oven Workers at Coke Oven Plants

| | | GNMT genotypic polymorphism | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | STRP1 | | | INS/DEL | | | SNP1 | | |
| Markers | Work site | <16GA/<16GA | <16GA/≥16GA | ≥16GA/≥16GA | DEL/DEL | INS/DEL | INS/INS | C/C | C/T | T/T |
| 1-OHP, GM(GSD) | Side-oven workers (n=181) | 5.2 (3.7) | 10.2 (2.9 )* | 14.7 (3.4)* | 10.7 (3.7) | 7.8 (3.3) | 10.6 (3.6) | 9.1 (3.8) | 9.7 (3.3) | 9.0 (2.6) |
| | Topside-oven workers (n=108) | 30.1 (1.6) | 43.2 (2.0) | 109.7 (1.5) *# | 72.7 (1.6) | 57.0 (1.8) | 77.6 (2.0) | 69.9 (1.6) | 71.5 (2.1) | 47.4 (1.6) |
| | All (n=289) | 8.8 (4.4) | 14.8 (3.9) | 42.4 (4.4) *# | 24.0 (4.7) | 15.6 (4.8) | 18.9 (5.2) | 21.7 (5.0) | 16.3 (4.9) | 19.2 (3.7) |
| 8-OHdG, GM(GSD) | Side-oven workers (n=181) | 3.2 (3.7) | 6.2 (2.8) † | 7.5 (2.5)† | 5.8 (2.7) | 5.2 (3.3) | 5.7 (3.6) | 5.4 (3.1) | 5.6 (3.1) | 5.9 (3.3) |
| | Topside-oven workers (n=108) | 8.2 (1.7) | 15.8 (1.3) † | 21.7 (1.3)† | 17.0 (1.4) | 16.0 (1.6) | 14.5 (1.7) | 16.4 (1.5) | 14.7 (1.5) | 19.2 (1.3) |
| | All (n=289) | 4.5 (3.9) | 7.9 (2.8) † | 13.1 (2.6)†♣ | 9.2 (2.8) | 7.7 (3.5) | 7.4 (3.7) | 8.7 (3.2) | 7.2 (3.2) | 10.1 (3.0) |

* Urinary 1-OHP levels in workers with STRP1 at ≥16GA/≥16G repeats genotype significantly exceeded those with <16GA/≥16GA repeats genotype, or those with <16GA/<16GA repeats genotype.

# Urinary1-OHP levels in workers with STRP1 at ≥16GA/≥16G significantly exceeded those with <16GA/ ≥16GA repeats genotype.

† Urinary 8-OHdG levels in workers with STRP1 at ≥16GA/≥16G or at<16GA/ ≥16GA repeats genotype significantly exceeded those with <16GA/<16GA repeats genotype.

♣ Urinary 8-OHdG levels in workers with STRP1 at ≥16GA/≥16G significantly exceeded those with <16GA/ ≥16GA repeats genotype.

GM: geometric mean, GSD: geometric standard deviation.

**TABLE 5**

Multiple Linear Regression Analysis: Predictors of Urinary 1-hydroxypyrene (1-OHP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in 289 Coke Oven Workers at Coke Oven Plants

| Predictors | $Log_{10}$ 1-OHP (μg/L) | $Log_{10}$ 8-OHdG (μg/L) |
|---|---|---|
| | Regression coefficient (95% Confidence interval) | Regression coefficient (95% Confidence interval) |
| STRP1 genotype | | |
| <16GA/≥16GA vs <16GA/<16GA | 0.252 (0.097 to 0.407)* | 0.189 (0.060 to 0.317)** |
| ≥16GA/≥16GA vs <16GA/<16GA | 0.525 (0.369 to 0.681 )** | 0.232 (0.096 to 0.368)** |
| Work site (Topside-oven workers vs. side-oven workers) | 0.736 (0.603 to 0.869 )*** | 0.238 (0.109 to 0.367)*** |
| Smoking (Yes vs. no) | 0.058 (-0.066 to 0.182 ) | 0.066 (-0.035 to 0.167) |
| Alcohol consumption (Yes vs. no) | 0.020 (-0.138 to 0.178 ) | 0.052 (-0.077 to 0.180) |
| Taking vitamin supplements (Yes vs. no) | -0.056 (-0.183 to 0.072) | -0.014 (-0.118 to 0.089) |
| Working years (years) | 0.003 (-0.002 to 0.009) | 0.001 (-0.004 to 0.005) |
| Age (years) | -0.003 (-0.010 to 0.004) | 0.001(-0.005 to 0.006) |
| BMI ($kg/m^2$) | -0.001 (-0.009 to 0.006) | -0.003 (-0.009 to 0.003) |
| $Log_{10}$ 1-OHP (μg/L) | — | 0.264 (0.168 to 0.360)*** |

* $P<0.05$

** $P<0.01$

*** $P<0.001$