# SHORT COMMUNICATION

# Smoking-related DNA adducts and genetic polymorphism for metabolic enzymes in human lymphocytes

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**Smoking-related aromatic DNA adducts in lymphocytes were measured from smokers ($n$ = 76), ex-smokers ($n$ = 25) and non-smokers ($n$ = 56) by the $^{32}$P-postlabelling method, to clarify whether a genetic polymorphism for metabolic enzymes could explain the inter-individual variation of DNA adduct levels. Adduct levels were compared with respect to smoking status and polymorphic genotypes of cytochrome P4501A1 (CYP1A1) and glutathione S-transferase M1 (GTSM1). The mean adduct level (1.24 per $10^8$ nucleotides) in smokers was significantly higher than that (0.85 per $10^8$) in non-smokers. Although we expected higher adduct levels in the CYP1A1 variant or GSTM1 null subjects, the adduct level in 'GSTM1 nulls' was significantly lower than that in 'GSTM1 presents' among smokers. DNA adduct levels had significant positive correlations with smoking indices such as number of cigarettes or smoking years in all subjects. In smokers only, however, no correlation was found, because there were negative correlations between adduct levels and smoking dose in GSTM1 null genotypes. CYP1A1 genotypes had no effects on adduct levels.**

Keywords: *smoking, DNA adducts, lymphocyte, polymorphism.*

## Introduction

Environmental carcinogens are enzymatically activated to form intermediates that can react with cellular DNA and form DNA adducts. The measurement of DNA adducts is a useful indicator for environmental carcinogen exposure in humans (Hemminki 1995). Since smoking is a strong risk factor for cancer, several studies have examined smoking-related DNA adducts in peripheral blood samples by the $^{32}$P-postlabelling method (Holz *et al.* 1990, Jahnke *et al.* 1990, Phillips *et al.* 1990, Savela and Hemminki 1991, van Schooten *et al.* 1992, Popp *et al.* 1993) or the immunological method (Santella *et al.* 1992). However, most studies have shown a poor correlation between DNA adduct levels and smoking dose, because the inter-individual variation of adduct levels was very large. The genetic difference of metabolic enzymes occurring as genetic polymorphism has been thought to lead the individual variation of DNA adduct formation.

Polycyclic aromatic compounds, carcinogens contained in cigarette smoke, are activated by cytochrome P450 (CYP1A1) and detoxified by glutathione S-transferase (GSTM1) which are polymorphic enzymes. Increased cancer risk has been related to a polymorphism for CYP1A1 or GSTM1 (Hayashi *et al.* 1992, Rannug *et al.* 1995). The studies relating genetic polymorphism to cancer susceptibility are currently extended to DNA adduct analysis for lung tissue (Shields *et al.* 1993, Szyfter *et al.* 1994, Bartsch *et al.* 1995, Kato *et al.* 1995) or peripheral blood cells (Grinberg-Funes *et al.* 1994, Ichiba *et al.* 1994, Rothman *et al.* 1995). However the effects of genetic polymorphism on DNA adduct levels have not been consistent.

In this study, we measured smoking-related aromatic DNA adduct levels in lymphocytes from smokers, ex-smokers and non-smokers by the $^{32}$P-postlabelling method, to investigate whether genetic polymorphism for CYP1A1 or GSTM1 could explain inter-individual variations of smoking-related DNA adduct levels.

## SUBJECTS AND METHODS

Subjects were 157 healthy adult men. We asked them their smoking history (number of cigarettes per day, smoking years and brand names of cigarette). Heparinized venous blood and urine samples were obtained from them. Subjects were divided into three groups according to their smoking status; current smokers, ex-smokers (more than 1 year since giving up smoking) and non-smokers. Their daily tar and nicotine intake were calculated from the date of Tobacco catalog (Japan Tobacco Inc., August 1994). Urinary cotinine was determined according to the HPLC method (Yamano *et al.* 1990). Data obtained were adjusted with the urinary creatinine concentration. Creatinine was measured by the Jaffé method. Lymphocytes were separated by Ficoll-Paque (Pharmacia LKB Biotechnology). DNA was isolated from lymphocytes by the method using RNAase, protease digestion and ethanol precipitation (Kendall *et al.* 1991). DNA samples thus prepared were stored at –80 °C until analysis.

The nuclease P1 modification of the $^{32}$P-postlabelling method (Reddy and Randerath 1986, Ichiba *et al.* 1994) was used for the measurement of aromatic DNA adducts. For this measurement, 5 μg of DNA was digested with micrococcal nuclease, spleen phosphodiesterase and nuclease P1. The digested material was dried and taken up in a total of 2 μl of T4 polynucleotide kinase labelling mixture containing [$\gamma$-$^{32}$P]ATP. The labelled samples were spotted and developed on polyethyleneimine–cellulose thin layer chromatography (TLC) plates (Macherry-Nagel, Germany) using three solvent systems: D1, 1 M sodium phosphate, pH 6.0; D3, 3.6 M lithium formate, 8.5 M urea, pH 3.5; D4, 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0. DNA adducts were detected using Bio-Image Analyzer (BAS2000; Fuji Photo Film Co., Tokyo, Japan) after exposing TLC plates to the Fuji imaging plate. A diagonal radioactive area on the TLC plates was counted and a background level obtained from the same plate was subtracted. The measurements were performed in duplicate or triplicate for each sample. The results were given as a total number of adducts per $10^8$ normal nucleotides.

The analysis of Ile-Val polymorphism in the CYP1A1 gene (exon 7) was performed by the method of Oyama *et al.* (1995). The Ile-Val polymorphism results

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in population subgroups corresponding to genotype Ile/Ile, Ile/Val and Val/Val. The analysis of the GSTM1 gene was performed by the method of Groppi *et al.* (1991). Polymorphism results in population subgroups corresponding to genotype GSTM1 present (+) and null (–).

Differences in frequency for genetic polymorphism were assessed by $\chi^2$ test. DNA adducts data were log transformed before statistical analysis. Differences in adduct levels were assessed by ANOVA among three groups and Student's or Welch's t-test for two groups. Pearson's correlation coefficient and partial correlation coefficient were calculated.

## Results

Table 1 shows questionnaire data and analytical results. The mean level of urinary cotinine in smokers was significantly elevated compared with those in ex-smokers and non-smokers. The DNA adduct levels of smokers were significantly higher than those in non-smokers ($p = 0.015$). The distribution frequencies of metabolic enzyme genotypes for CYP1A1 and GSTM1 were not different among smokers, ex-smokers and non-smokers.

The adduct levels were compared in relation to smoking status and metabolic enzyme genotypes (Table 2). On CYP1A1 genotype, all subjects were divided into two groups, CYP1A1 wild (Ile/Ile) and CYP1A1 variant (Ile/Val, Val/Val), because the number of Val/Val is too small. Adduct levels of GSTM1(+) were higher than those of GSTM1 (–) in smokers. Significant differences were found among CYP1A1 Ile/Ile types ($p = 0.004$) and all smokers ($p = 0.02$). Mean numbers of cigarettes per day were not significantly different between GSTM1(+) and (–) in smokers. Among ex-smokers and non-smokers, there was no difference in adducts between GSTM1 (+) and (–). No differences in adduct levels were found between the CYP1A1 wild and CYP1A1 variant, irrespective of smoking status and GSTM1 genotype.

Table 3 shows simple correlation coefficients between log transformed DNA adduct levels and smoking indices in all subjects and in only smokers. Significant correlations were found between DNA adducts and age or smoking indices (cigarettes per day, smoking years) in all subjects. In all smokers, however, we could find no significant correlation. When subjects were divided according to genotype, we found weakly negative correlations in CYP1A1 variant and GSTM1(–) between adduct levels and smoking status. In addition a significant correlation was found between adducts and urinary continine ($p = 0.035$), this significance was not changed after adjustment for age ($r = -0.43$, $p = 0.013$).

| | Smoker | Ex-smoker | Non-smoker |
|---|---|---|---|
| Number | 76 | 25 | 56 |
| Age (years) | 40 ± 14 (18–58) | 47 ± 10 (20–58) | 38 ± 14 (19–59) |
| Cigarettes per day | 20 ± 9 (2–50) | 23 ± 9[a] (10–50) | |
| Smoking years | 18 ± 13 (1–36) | 16 ± 9[a] (1–31) | |
| Tar per day (mg) | 226 ± 121 (18–600) | | |
| Nicotine per day (mg) | 18 ± 10 (1.6–48) | | |
| Cotinine ($mg^{-1}$ gCr) | 916 ± 959** (0–5387) | 12 ±24 (0–107) | 20 ±40 (0–227) |
| DNA adducts (per $10^8$ nucleotides) | 1.24 ± 0.78* (0.10–4.0) | 0.92 ± 0.28 (0.38–1.5) | 0.85 ± 0.35 (0.32–1.8) |
| CYP1A1 Ile/Ile | 46 (60%) | 15 (60%) | 31 (56%)[b] |
| Ile/Val | 27 (36%) | 9 (36%) | 23 (42%) |
| Val/Val | 3 (4%) | 1 (4%) | 1 (2%) |
| GSTM1 + | 48 (63%) | 13 (52%) | 33 (59%) |
| – | 28 (37%) | 12 (48%) | 23 (41%) |

**Table 1.** Questionnaire data and analytical results.

Mean ± SD (range).

[a] Data before giving up smoking.

[b] CYP1A1 genotype was unknown for one subject.

** $p < 0.01$, comparison with ex-smoker and non-smoker.

* $p < 0.05$, comparison with non-smoker.

## Discussion

Though there have been some papers about the effect of CYP1A1 and GSTM1 genotypes on DNA adduct levels, the method for measuring adducts or the cell types used have been different. In lung tissues, Shields *et al.* (1993) and Kato *et al.* (1995) found a positive association for PAH-dGMP levels with GSTM1 null genotype by the $^{32}$P-postlabelling combined HPLC method. On the other, Szyfter *et al.* (1994) reported that GSTM1 was not the proper candidate to explain differences in DNA adduct formation. Bartsch *et al.* (1995) found the amount of DNA adducts in the lung of smoking lung cancer patients was slightly higher in GSTM1 null genotypes than in those with the wild type gene, but this was not significant. In peripheral blood cells, previously we analysed aromatic DNA adducts in total WBCs of chimney sweeps by the $^{32}$P-postlabelling method. There was an association of the CYP1A1 genotype ml/ml, lacking a Msp1 restriction site at the 3' end of the gene, with high adduct levels (1994). Grinberg-Funes *et al.* (1994) did not find a significant difference for adduct levels between GSTM1 present and null genotype in total WBCs by the ELISA method. Rothman *et al.* (1995) reported that PAH–DNA adduct levels were non-significantly lower in subjects with GSTM1 null or CYP1A1 variant in WBCs measured by ELISA among non-smokers. As mentioned above, the effects of genetic polymorphism on DNA adduct levels have not been consistent.

In this study, we measured smoking-related aromatic DNA adducts in lymphocytes from smokers, ex-smokers and non-smokers by the $^{32}$P-postlabelling method. Though we could expect smoking-related higher adduct levels in CYP1A1 variant or GSTM1(–) genotypes theoretically, we found that GSTM1(–) has lower adduct levels in all subjects and CYP1A1 wild types among smokers. Rothman *et al.* (1995) also reported similar results but in non-smokers. In addition we found a weakly negative correlation between adduct levels and urinary cotinine levels in GSTM1(–) in smokers. Though the mechanism of this negative correlation was unknown, it was thought to be one reason for a poor correlation between

| | | CYP1A1 Ile/Ile | Ile/Val, Val/Val | All | p[a] |
|---|---|---|---|---|---|
| Smoker | GSTM1+ | 1.44 ± 0.77 (n = 27) | 1.32 ± 0.93 (n = 21) | 1.39 ± 0.84 (n = 48) | 0.27 |
| | GSTM1– | 0.91 ± 0.55 (n = 19) | 1.15 ± 0.73 (n = 9) | 0.99 ± 0.61 (n = 28) | 0.44 |
| | All | 1.22 ± 0.73 (n = 46) | 1.27 ± 0.87 (n = 30) | 1.24 ± 0.78 (n = 76) | > 0.5 |
| | p[b] | 0.004 | > 0.5 | 0.02 | |
| Ex-smoker | GSTM1+ | 0.86 ± 0.34 (n = 7) | 0.93 ± 0.34 (n = 6) | 0.89 ± 0.33 (n = 13) | > 0.5 |
| | GSTM1– | 0.96 ± 0.26 (n = 8) | 0.94 ± 0.20 (n = 4) | 0.95 ± 0.23 (n = 12) | > 0.5 |
| | All | 0.92 ± 0.29 (n = 15) | 0.93 ± 0.28 (n = 10) | 0.92 ± 0.28 (n = 25) | > 0.5 |
| | p[b] | 0.42 | > 0.5 | 0.43 | |
| Non-smoker | GSTM1+ | 0.86 ± 0.28 (n = 21) | 0.92 ± 0.47 (n = 11) | 0.86 ± 36 (n = 33) | > 0.5 |
| | GSTM1– | 0.93 ± 0.24 (n = 10) | 0.74 ± 0.40 (n = 13) | 0.82 ± 0.35 (n = 23) | 0.09 |
| | All | 0.88 ± 0.26 (n = 31) | 0.82 ± 0.43 (n = 24) | 0.85 ± 0.35 (n = 56) | 0.21 |
| | p[b] | 0.44 | 0.39 | > 0.5 | |

**Table 2.** Aromatic DNA addition (per $10^8$ nucleotides, mean ± SD) with respect to smoking status and genotype.

[a] Comparison between Ile/Ile and Ile/Val, Val/Val base on log transformed data by *t*-test.

[b] Comparison between GST+ and GST– base on log transformed data by *t*-test.

| | All subjects (n = 157) | Smokers | | | | |
|---|---|---|---|---|---|---|
| | | All (n = 76) | CYPIle/Ile (n = 46) | Ile/Val, Val/Val (n = 30) | GSTM1+ (n = 48) | GSTM1– (n = 28) |
| Age | 0.200* | 0.192 | 0.167 | 0.231 | 0.251 | 0.057 |
| Cigarettes per day | 0.180* | 0.007 | 0.043 | –0.068 | 0.110 | –0.325 |
| Smoking years | 0.190* | 0.087 | 0.175 | –0.036 | 0.112 | 0.048 |
| Tar per day | 0.142 | –0.053 | 0.000 | –0.156 | 0.070 | –0.353 |
| Nicotine per day | 0.136 | –0.062 | –0.040 | –0.104 | 0.078 | –0.315 |
| Cotinine | 0.149 | 0.052 | 0.130 | –0.036 | 0.148 | –0.399* |

**Table 3.** Correlation coefficients between aromatic DNA adducts[a] and smoking indices in all subjects and in smokers with respect to genotype.

[a] Log transformed data.

[b] $p < 0.05$, Pearson's correlation coefficient.

smoking dose and DNA adduct levels among all smokers in this study and other previous studies. Gineste *et al.* (1991) and Bartsch *et al.* (1995) have found a positive correlation between DNA adducts and aryl hydrocarbon hydroxylase activity measured as CYP1A1 activity in lung. So genetic polymorphisms may be less important than induced enzyme activity. For other reasons, since CYP1A1 or GSTM1 catalyse one of several steps in the metabolism of polycyclic aromatic compounds, it may be difficult to explain individual variation with only CYP1A1 and GSTM1 enzymes. Adduct levels appear to be influenced by factors other than those which have not been considered in this and previous studies. DNA repair may be one such important factor (Harris 1989). Further studies are needed to explain inter-individual variation of DNA adduct formation.



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