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Research article

Effects of gene polymorphisms of *CYP1A1, CYP1B1, EPHX1*, *NQO1*, and *NAT2* on urinary 1-nitropyrene metabolite concentrations

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HIGHLIGHTS

- Compared with 1-AP, the urinary excretion of 1-NAAP is nearly three-fold greater.
- 1-NAAP showed a stronger correlation with 1-NP exposure than 1-AP and would be a better exposure biomarker.
- The relationship between 1-NP exposure and urinary 1-NAAP concentration depends on the SNP type of the metabolic enzymes.

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ABSTRACT

Nitropyrene (1-NP) is a specific indicator of exposure to diesel exhaust and is partly metabolized to 1-aminopyrene (1-AP) and *N*-acetyl-1-aminopyrene (1-NAAP), which are excreted in urine. This study was conducted to evaluate the effects of gene polymorphisms of metabolic enzymes for 1-NP on the urinary concentrations of 1-AP and 1-NAAP. The study participants were 70 South Koreans who were occupationally or environmentally exposed to diesel exhaust. To evaluate 1-NP exposure levels, we sampled airborne particulate matters with a personal air sampler and measured urinary 1-AP and 1-NAAP concentrations. The genetic polymorphisms of the 1-NP metabolic enzymes (CYP1A1, CYP1B1, EPHX1, NQO1, and NAT2) were determined by direct sequencing. The mean 1-NP exposure level was 20.40 pg/m^3, and the mean urinary concentrations of 1-AP and 1-NAAP were 0.074 nM and 0.213 nM, respectively. The correlation coefficient between the 1-NP exposure level and urinary 1-AP concentrations was 0.0138 and that between the 1-NP exposure level and urinary 1-NAAP concentrations was 0.1493, and neither correlation coefficient was statistically significant. The correlation coefficient between the 1-NP exposure level and urinary 1-AP concentrations showed statistically significant differences according to the CYP1A1 and CYP1B1 genotypes, and that between the 1-NP exposure level and urinary 1-NAAP concentrations was significantly different according to the CYP1A1, CYP1B1, and NAT2 genotypes. The urinary concentration of 1-NAAP is a better biomarker for exposure to 1-NP or DEPs because the former is higher, easier to measure, and more strongly correlated with 1-NP exposure levels than that of 1-AP. The relationship between 1-NP exposure and urinary 1-AP or 1-NAAP concentration depends on the single nucleotide polymorphism types of CYP1A1, CYP1B1, NQO1, and NAT2.

1. Introduction

Diesel engines are widely used in numerous industries including automobiles, heavy equipment, and ships and emit ultrafine dust, which causes cardiovascular and lung diseases. The International Agency for Research on Cancer defined particulate air pollutants as human carcinogens [1]. Diesel exhaust particles (DEPs) are respirable dust with small particle sizes and relatively large surface areas [2], thereby absorbing various substances such as polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs. In the diesel engine's combustion process, nitro-PAH compounds such as 1-nitropyrene (1-NP), 2-nitrofluorene, 9-nitrophenanthrene are formed [3, 4]. Of these, 1-NP accounts for the

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largest portion of nitro-PAHs emitted from light-duty diesel engines and can be used as an indicator of diesel engine combustion. The carcinogenicity and mutagenicity of 1-NP has been confirmed in previous studies [5, 6].

The concentration of 1-NP or its metabolites in blood or urine can be a good indicator for assessing environmental exposure to DEPs. A portion of absorbed 1-NP is metabolized to 1-aminopyrene (1-AP) and further metabolized to N-acetyl-1-aminopyrene (1-NAAP) by N-acetylation (Figure 1) [7]. Urinary 1-AP and 1-NAAP concentrations can therefore be employed as biomarkers for 1-NP and DEP exposure.

Cytochrome P450 enzymes, epoxide hydrolase (EPHX1), and the NAD(P)H quinone oxidoreductase (NQO1) enzymes are involved in the metabolism of 1-nitropyrene. Therefore, even when exposed to the same amount of 1-NP, the amount of 1-AP or 1-NAAP excreted in urine will change due to the difference in these enzymes' activity.

This study sought to determine the variation in the relationship between 1-NP exposure and urinary 1-AP excretion and between 1-NP exposure and urinary 1-NAAP excretion according to the single nucleotide polymorphism (SNP) type of the enzyme genes involved in 1-NP metabolism.

2. Materials and methods

2.1. Study participants

This study was conducted after the deliberation and approval of the Institutional Review Board of Chungbuk National University. The study participants consisted of 70 South Koreans; 17 were delivery and saleswomen for Yakult (a brand of yogurt), 8 were intercity bus terminal workers, 40 were street sweepers, and 5 were office workers. After obtaining detailed information about the study, subjects signed informed consent and provided blood and first morning urine of the day after an air sampling. A direct interview was conducted by an interviewer using a questionnaire on demographic characteristics, working environment, smoking habits, and diesel vehicle use.

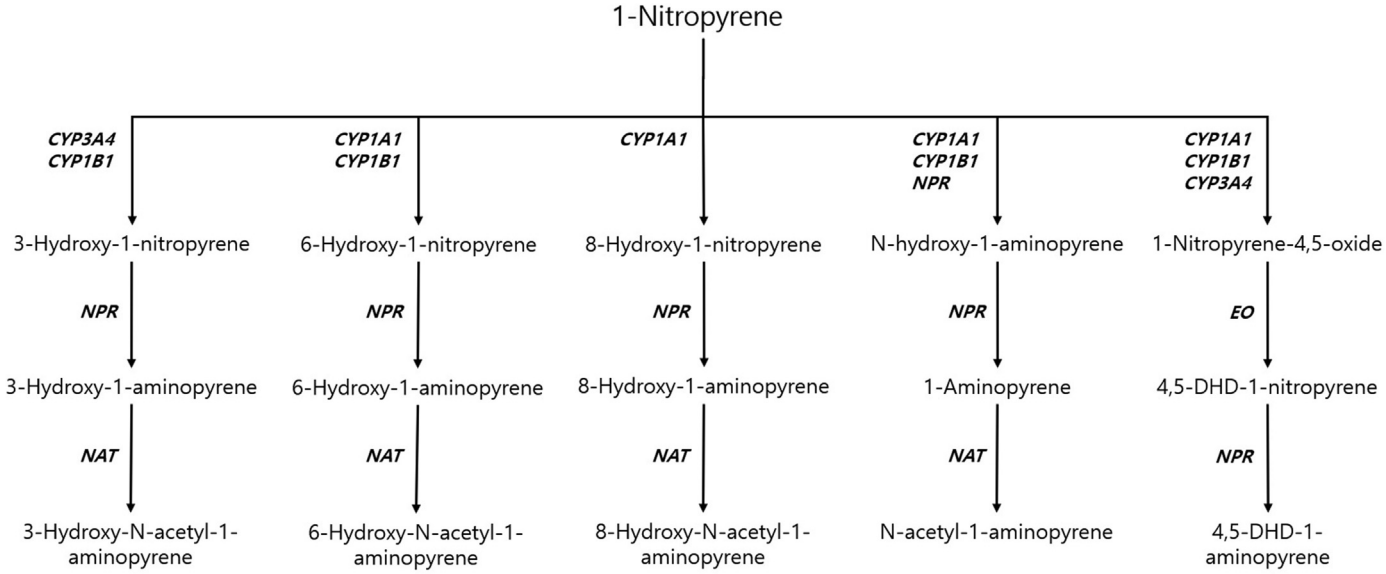
2.2. Measurement of 1-nitropyrene concentrations in the atmosphere

To measure atmospheric 1-nitropyrene concentrations, air was sampled at a flow rate of 3 L/min for 24 h using a personal air sampler (Apex standard, SN0376420 Casella CEL, Bedford, England) with a polytetrafluoroethylene filter of 2 μm pore size attached to the participant's collar. The filter was taken out and mixed with 2 mL of dichloromethane in a flask. The flask was shaken and sonicated to extract 1-NP. The extract was evaporated to dryness and the residue redissolved in acetonitrile. Aliquots of the solution were then injected into a two-dimensional high-performance liquid chromatography system with an on-line reduction column (NPpak-RS, 10 × 4.6 mm i. d. JASCO, Tokyo, Japan) and a fluorescence detector (RF-20Axs, Shimadzu, Japan) [8]. Nitro-PAH was reduced to amino derivatives by passing through the reduction column at 80 °C. Ethanol/acetate buffer (pH 5.5) (95/5, v/v) was the mobile phase of the reduction column, and the flow rate was 0.2 mL/min. The amino derivatives and unchanged PAH fractions eluted from the reduction column were mixed with 30 mM ascorbic acid at a flow rate of 1.6 mL/min, and then trapped in a concentration column (Spheri-5 RP-18, 30 × 4.6 mm i. d. 5 μm, PerkinElmer, MA, USA). The concentrated fractions were passed through a guard column (10 × 4.6 mm i. d.) and two separation columns in series (Inertsil ODS-P, 250 × 4.6 mm i. d. 5 μm, GL Sciences, Tokyo, Japan). The reduction column was maintained at 80 °C and all other columns at 20 °C. A programmed gradient elution of the separation column was performed using 10 mM imidazole buffer (pH 7.6) as eluent A and acetonitrile as eluent B. Finally, 1-NP was detected with a fluorescence detector.

2.3. Measurement of urinary 1-aminopyrene and N-acetyl-1-aminopyrene

All reagents were analytical grade. 1-AP, 1-NAAP and β-glucuronidase/aryl sulfatase (type H-2, from *Helix pomatia*: β-glucuronidase activity, 100,000 units/mL; and sulfatase activity, 7500 units/mL) were purchased from Sigma-Aldrich (St. Louis, MO). Blue rayon fiber (non-crystalline cellulose) was purchased from Funakoshi (Tokyo, Japan).

We measured urinary 1-AP and 1-NAAP levels as described by Bekkum et al. [9]. The limits of detection and quantitation of our 1-AP analysis were 3.6 and 11.9 fmol, respectively. The recovery rate was 79.5%. The limits of detection and quantitation of the 1-NAAP analysis method were 5.6 and 18.6 fmol, respectively. The average recovery rate of 1-NAAP was 104.0%. Two milliliters of sodium acetate buffer (pH 5.0), 200 μL of β-glucuronidase/aryl sulfatase and 80 mg of blue rayon were added to each 20-mL urine sample, and the urine was incubated at 37°C overnight. The blue rayon was passed through a glass wool filter and



- 4,5-DHD-1-aminopyrene, 1-aminopyrene-*trans*-4,5-dihydrodiol; 4,5-DHD-1-nitropyrene, 1-nitropyrene-*trans*-4,5-dihydrodiol; EO, epoxide hydrolase; NAT, N-acetyl transferase; NPR, NADPH-cytochrome P450 reductase

Figure 1. A schematic illustration of the 1-NP metabolism.

collected. The rayon was washed with 5 mL of water twice, air-dried, and put into 4 mL of methanol/ammonia solution (50/1, v/v). 1-AP and 1-NAAP were eluted by shaking for 30 min and sonicating for 30 min. After the eluate was dried with nitrogen gas, the residue was redissolved in 200 μL of methanol. Twenty microliter of the re-dissolved extract was injected into an HPLC system equipped with a fluorescence detector (Shimadzu, RF-20A, Kyoto, Japan). A reverse phase-amide column (Ascentis RP-Amide, 25 cm × 4.6 mm, 5 μm, Supelco, Bellefonte, PA, USA) was used as the analytical column, and methanol and 50 mM sodium acetate buffer (pH 7.2; 80:20, v/v) at a flow rate of 1.0 mL/min was as the mobile phase. The excitation/emission wavelengths were 254/425 nm for 1-AP (Figure S1), and 277/432 nm for 1-NAAP (Figure S2), respectively.

2.4. Genotyping of SNPs in CYP1A1, CYP1B1, EPHX1, NQO1

SNPs of CYP1A1, CYP1B1, EPHX1, and NQO1 related to activity change were identified. Only SNPs reported to have a frequency of mutant alleles ≥5% in the Korean population, such as CYP1A1 rs1048943, CYP1A1 rs4646903, CYP1B1 rs1056836, CYP1B1 rs10012, CYP1B1 rs1056827, EPHX1 rs1051740, EPHX1 rs2234922, and NQO1 rs1800566 were selected for analysis. DNA for SNP analysis was extracted from blood using the QIAamp DNA Blood Mini kit (Qiagen). Genomic DNA was amplified using an ABI 2720 Thermal Cycler (Applied Biosystems, USA). The amplified product was electrophoresed on 0.8% agarose gel, and the identified target DNA was purified through gel elution. Automatic sequencing of the purified polymerase chain reaction product was performed using an ABI 3730xl DNA sequencer (Applied Biosystems, USA).

2.5. Identifying NAT2 acetylation activity

NAT2 exon2 was amplified and sequenced to identify the number of rapid alleles (*NAT2*4, NAT2*11A, NAT2*12A, NAT2*12B, NAT2*12C, NAT2*13A, NAT2*18*). Individuals with two of the rapidly acetylated NAT2 alleles were classified as rapid acetylators, those with one of the rapid alleles were classified as intermediate acetylators, and those who lacked them were classified as slow acetylators [10, 11].

2.6. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 24.0 (IBM Corp. Armonk, NY, USA). The airborne nitro-PAH concentration and urinary 1-AP concentration were analyzed for outliers by creating a scatterplot. To prevent statistical significance distortion by extreme values, the airborne 1-nitropyrene concentration was checked with a scatterplot of log values, and outliers was replaced with a value corresponding to the geometric mean * [geometric standard deviation]^3). We evaluated whether the genotype distributions of CYP1A1, CYP1B1, EPHX1, NQO1, and NAT2 SNPs satisfied the Hardy-Weinberg equilibrium.

The difference between the two groups in the airborne nitro-PAH concentration and the urinary nitro-PAH metabolite concentration was compared using Student's t-test or the Wilcoxon rank-sum test. The relationship between urinary 1-AP concentrations and 1-NP exposure levels was statistically tested using Pearson's correlation analysis. When comparing the two correlation coefficients, the significance was tested through Fisher's Z transformation method. When the p-value was less than 0.05, statistical analyses were judged to be statistically significant.

3. Results

Table 1 shows the study participants' demographic characteristics.

The mean airborne 1-NP concentration was 20.40 pg/m^3, and the mean urinary concentrations of 1-AP and 1-NAAP were 0.074 nM and 0.213 nM, respectively. Urinary 1-AP and 1-NAAP concentrations were not statistically different according to sex, smoking habit, diesel vehicle ownership, and outdoor activity time (Table 1).

All genotype distributions of the SNPs satisfied the Hardy-Weinberg equilibrium. The GG type of CYP1B1 rs1056836, the CC type of CYP1B1 rs10012, and the AA type of CYP1B1 rs1056827 were not found in this study's participants (Table 2). Urinary 1-AP concentrations showed significant differences according to CYP1A1 rs1048943, CYP1A1 rs4646903, and NAT2 acetylation types, but there was no SNP with a significant difference in urinary 1-NAAP concentrations.

Table 3 shows the correlation coefficient between the airborne 1-NP concentration and the urinary 1-AP concentration and the correlation coefficient between the airborne 1-NP concentration and the urinary 1-NAAP concentration. The correlation coefficient between the airborne 1-NP concentration and the urinary 1-AP concentration was 0.0138, and the correlation coefficient between the airborne 1-NP concentration and the urinary 1-NAAP concentration was 0.1493. Neither correlation coefficient was statistically significant nor was the difference between the two correlation coefficients. The correlation coefficient between the airborne 1-NP concentration and urinary 1-AP concentration was significant in the AG + GG type of CYP1A1 rs1048943, the GG + GA type of CYP1A1 rs4646903, the GC type of CYP1B1 rs10012, and the CA type of CYP1B1 rs1056827 and was significantly different according to the

Table 1. General characteristics of study participants.

Variables		N (%)	Mean ± SD	1-aminopyrene, nM		N-acetyl-1-aminopyrene, nM	
				Mean ± SD	p	Mean ± SD	p
All participants		70 (100)		0.074 ± 0.227		0.213 ± 0.235	
Age, years			46.93 ± 9.02				
Sex	Males	50 (71.4)		0.077 ± 0.232	0.858	0.230 ± 0.269	0.183
	Females	20 (28.6)		0.066 ± 0.218		0.169 ± 0.110	
Smoking habit	Current smokers	18 (25.7)		0.059 ± 0.227	0.748	0.188 ± 0.151	0.515
	Non-smokers or ex-smokers	52 (74.3)		0.079 ± 0.228		0.221 ± 0.259	
Diesel car ownership	Yes	28 (40.0)		0.096 ± 0.253	0.513	0.275 ± 0.329	0.122
	No	42 (60.0)		0.060 ± 0.209		0.171 ± 0.131	
Outdoor activity time	<8 h	12 (17.1)		0.107 ± 0.279		0.287 ± 0.283	0.127*
	≥8 h	58 (82.9)		0.067 ± 0.217		0.197 ± 0.224	
Airborne 1-nitropyrene, pg/m^3			20.40 ± 42.82				
Urinary metabolites							
1-aminopyrene, nM			0.074 ± 0.227				
N-acetyl-1-aminopyrene, nM			0.213 ± 0.235				

* p-value by Wilcoxon rank-sum test.

Table 2. Urinary 1-aminopyrene and N-acetyl-1-aminopyrene concentrations according to *CYP1A1*, *CYP1B1*, *EPHX1*, *NQ O 1*, *NAT2* genetic polymorphisms.

Genotype	N (%)	1-aminopyrene, nM		N-acetyl-1-aminopyrene, nM	
		Mean ± SD	p	Mean ± SD	p
CYP1A1 rs1048943					
AA (wild type)	43 (61.4)	0.101 ± 0.283	0.038	0.225 ± 0.277	0.545
AG + GG	27 (38.6)	0.017 ± 0.033		0.193 ± 0.151	
CYP1A1 rs4646903					
AA (wild type)	31 (44.3)	0.142 ± 0.327	0.005	0.206 ± 0.238	0.825
GG + GA	39 (55.7)	0.020 ± 0.047		0.218 ± 0.236	
CYP1B1 rs1056836					
CC (wild type)	49 (70.0)	0.073 ± 0.234	0.945	0.199 ± 0.190	0.540
CG	21 (30.0)	0.077 ± 0.214		0.245 ± 0.320	
CYP1B1 rs10012					
GG (wild type)	54 (77.1)	0.090 ± 0.246	0.081	0.182 ± 0.194	0.140
GC	16 (22.9)	0.022 ± 0.061		0.315 ± 0.328	
CYP1B1 rs1056827					
CC (wild type)	55 (78.6)	0.088 ± 0.252	0.094	0.182 ± 0.192	0.131
CA	15 (21.4)	0.024 ± 0.062		0.326 ± 0.337	
EPHX1 rs1051740					
TT (wild type) + TC	49 (70.0)	0.083 ± 0.235	0.634	0.209 ± 0.232	0.834
CC	21 (30.0)	0.054 ± 0.210		0.222 ± 0.247	
EPHX1 rs2234922					
AA (wild type)	54 (77.1)	0.077 ± 0.225	0.876	0.232 ± 0.255	0.087
AG + GG	16 (22.9)	0.066 ± 0.241		0.147 ± 0.137	
NQ O 1 rs1800566					
GG (wild type)	25 (35.7)	0.098 ± 0.267	0.514	0.178 ± 0.217	0.368
GA + AA	45 (64.3)	0.061 ± 0.203		0.232 ± 0.245	
NAT2 N-acetylation					
Rapid (wild type)	36 (51.4)	0.137 ± 0.304	0.015	0.200 ± 0.240	0.638
Intermediate + slow	34 (48.6)	0.007 ± 0.015		0.226 ± 0.233	

genotype of SNPs such as CYP1A1 rs1048943, CYP1A1 rs4646903, CYP1B1 rs10012, and CYP1B1 rs1056827.

The correlation coefficient between airborne 1-NP concentrations and urinary 1-NAAP concentrations was significant in the GG + GA type of CYP1A1 rs4646903, the CG type of CYP1B1 rs1056836, the GA + AA type of NQO1 rs1800566, and the NAT2 N-acetylation intermediate + slow type. When two correlation coefficients were tested using Fisher's Z transformation method, they were significantly different according to CYP1A1 rs4646903, CYP1B1 rs1056836, and NAT2 N-acetylation SNPs.

4. Discussion

As biomarkers for 1-NP exposure, urinary 1-AP, 6-hydroxy-1-nitropyrene and 8-hydroxy-1-nitropyrene have been studied. In our past study, we reported that there was a significant relationship between urinary 1-AP concentrations and airborne nitro-PAH levels. However, Toriba et al. [12] reported that there was no association between 1-NP exposure and 1-AP emissions. Studies on the relationship between 6-hydroxy-1-nitropyrene or 8-hydroxy-1-nitropyrene and 1-NP also showed inconsistent results [13, 14, 15]. Further studies are therefore needed on the urinary metabolites as exposure biomarkers for 1-NP.

Only a small portion of 1-NP is excreted in urine as 1-AP, which is somewhat chemically unstable; it is therefore difficult to measure 1-NP with an HPLC with a fluorescence detector. Compared with 1-AP, the urinary excretion of 1-NAAP is nearly three-fold greater; 1-NAAP is also chemically more stable and has a stronger fluorescence signal. All of the study's participants had 1-NAAP detected in their urine. To the best of our knowledge, no studies have yet established a relationship between urinary 1-NAAP concentrations and 1-NP exposure.

There was a significant or borderline significant difference in urinary 1-AP concentrations according to the CYP1A1 and CYP1B1 genotypes and to the acetylation type, which might be because these enzymes catalyze the metabolic process from 1-NP to 1-AP. These results are consistent with the study that showed that CYP1A1 and CYP1B1 mRNA expression increased after exposure of A549 cells to 1-NP [16]. Variant alleles of the CYP1A1 and CYP1B1 SNPs investigated in this study showed greater enzymatic activity; however, urinary 1-AP concentrations tended to be higher in the wild type, which might be because CYP1A1 and CYP1B1 are enzymes that metabolize 1-NP to 3-hydroxy-1-NP, 6-hydroxy-1-NP, or 8-hydroxy-1-NP. Therefore, in individuals with those mutant alleles, most of the 1-NP would be metabolized to those hydroxy-1-NPs, and only a small fraction would be metabolized to 1-AP.

Given that the NQO1 enzyme acts on the metabolism of N-hydroxy-1-aminopyrene to 1-aminopyrene, N-hydroxy-1-aminopyrene is converted more to 1-NAAP than to 1-AP in individuals with the mutant allele of low enzymatic activity, which is consistent with the results of our study.

When tested using Fisher's Z transformation method, the correlation coefficient between airborne 1-NP concentrations and urinary 1-AP concentrations and that between airborne 1-NP concentrations and urinary 1-NAAP concentrations were different according to the SNP types of CYP1A1 and CYP1B1. Those two correlations were significant in the participants with variant alleles of CYP1A1 or CYP1B1, which are more active than the wild types. When the activity of CYP1A1 or CYP1B1 is high, only a small portion of absorbed 1-NP is metabolized to N-hydroxy-1-aminopyrene; however, CYP1A1 and CYP1B1 also act during the metabolism of N-hydroxy-1-aminopyrene to 1-aminopyrene. Therefore, when the enzymatic activity of CYP1A1 or CYP1B1 is high, the amount of 1-AP production will be proportional to the 1-NP concentration. The correlation between airborne 1-NP concentrations and urinary 1-NAAP concentrations was significant in the GA + AA type of

Table 3. Correlation coefficient of airborne 1-nitropyrene level with urinary 1-aminopyrene and N-acetyl-1-aminopyrene.

Genotype	N (%)	With urinary 1-aminopyrene, nM		With urinary N-acetyl-1-aminopyrene, nM	
		Correlation coefficient	p	Correlation coefficient	p
All participants	70 (100)	0.0138	0.910	0.1493	0.218
CYP1A1 rs1048943					
AA (wild type)	43 (61.4)	−0.027	0.863	0.175	0.262
AG + GG	27 (38.6)	0.531	0.004	−0.280	0.157
*Z**		−2.395	0.008	1.798	0.964
CYP1A1 rs4646903					
AA (wild type)	31 (44.3)	−0.075	0.687	−0.095	0.610
GG + GA	39 (55.7)	0.636	<.0001	0.346	0.031
*Z**		−3.284	0.001	−1.812	0.035
CYP1B1 rs1056836					
CC (wild type)	49 (70.0)	0.019	0.895	−0.076	0.604
CG	21 (30.0)	-0.001	0.995	0.451	0.040
*Z**		0.074	0.530	−2.020	0.022
CYP1B1 rs10012					
GG (wild type)	54 (77.1)	0.004	0.977	−0.001	0.997
GC	16 (22.9)	0.545	0.029	0.116	0.668
*Z**		−1.955	0.025	−0.378	0.353
CYP1B1 rs1056827					
CC (wild type)	55 (78.6)	0.006	0.965	0.000	0.999
CA	15 (21.4)	0.539	0.038	0.099	0.725
*Z**		−1.865	0.031	−0.310	0.378
EPHX1 rs1051740					
TT (wild type) + TC	49 (70.0)	0.103	0.480	0.016	0.912
CC	21 (30.0)	−0.121	0.600	0.344	0.127
*Z**		0.812	0.792	−1.231	0.109
EPHX1 rs2234922					
AA (wild type)	54 (77.1)	0.050	0.719	0.231	0.092
AG + GG	16 (22.9)	−0.078	0.773	−0.193	0.475
*Z**		0.414	0.661	1.386	0.917
NQ O 1 rs1800566					
GG (wild type)	25 (35.7)	0.001	0.998	−0.038	0.856
GA + AA	45 (64.3)	0.007	0.966	0.369	0.013
*Z**		−0.023	0.491	−1.616	0.053
NAT2 N-acetylation					
Rapid (wild type)	36 (51.4)	0.005	0.790	−0.037	0.831
Intermediate + slow	34 (48.6)	0.069	0.699	0.569	0.004
*Z**		−0.256	0.399	−2.731	0.003

* by Fisher's Z transformation.

NQO1 rs1800566. These results suggest that, in individuals who have a variant allele of low NQO1 enzyme activity, N-hydroxy-1-aminopyrene will be metabolized more to 1-NAAP than to 1-AP, and the amount of 1-NAAP produced will be proportional to that of N-hydroxy-1-aminopyrene.

The limitations of this study are the small number of participants included in this study, and the facts that the time of first morning urination differed for each participant, and that the half-life of 1-NP metabolites was reported to be more than 10 h; therefore, the concentration of the metabolites in the morning urine after air sampling may be affected by 1-NP exposure levels in the days before urine sampling.

Another limitation is that we did not correct the urinary 1-AP and 1-NAAP concentration by urinary creatinine level or specific gravity. However, because the first urine in the morning is concentrated overnight, the effect of the hydration state would not be very significant.

In summary, 1-NAAP showed a stronger correlation with 1-NP exposure than 1-AP and would therefore be a better exposure biomarker for 1-NP and DEP exposure. The relationship between 1-NP exposure and urinary 1-NAAP concentration depends on the SNP type of the metabolic enzymes such as CYP1A1, CPY1B1, NQO1, and NAT2.

5. Conclusions

The urinary concentration of 1-NAAP is a better biomarker for exposure to 1-NP or DEPs because the former is higher, easier to measure, and more strongly correlated with 1-NP exposure levels than that of 1-AP. The relationship between 1-NP exposure and urinary 1-AP or 1-NAAP concentration depends on the single nucleotide polymorphism types of CYP1A1, CPY1B1, NQO1, and NAT2.

Declarations

Author contribution statement

Jung-Kuk Yun: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sang-Yong Eom: Conceived and designed the experiments.

Yong-Dae Kim: Conceived and designed the experiments; Analyzed and interpreted the data.

Heon Kim: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bolormaa Ochirpurev: Performed the experiments; Analyzed and interpreted the data.

Akira Toriba: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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