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Effects on sister chromatid exchange frequency of aldehyde dehydrogenase 2 genotype and smoking in vinyl chloride workers

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Abstract

Vinyl chloride monomer (VCM) is a human carcinogen. However, the exact mechanism of carcinogenesis remains unclear. VCM may be metabolized by cytochrome *P*450 2E1 (CYP2E1), aldehyde dehydrogenase 2 (ALDH2) and glutathione *S*-transferases (GSTs). Thus workers with inherited variant metabolic enzyme activities may have an altered risk of genotoxicity. This study was designed to investigate which risk factors might affect sister chromatid exchange (SCE) frequency in polyvinyl chloride (PVC) workers. Study subjects were 44 male workers from three PVC factories. Questionnaires were administered to obtain detailed histories of cigarette smoking, alcohol consumption, occupations, and medications. SCE frequency in peripheral lymphocytes was determined using a standardized method, and CYP2E1, GSTM1, GSTT1 and ALDH2 genotypes were identified by the polymerase chain reaction (PCR). Analysis revealed that smoking status and exposure to VCM were significantly associated with increased SCE frequency. The presence of ALDH2 1–2/2–2 genotypes was also significantly associated with an elevation of SCE frequency (9.5 vs. 8.1, $p < 0.01$). However, CYP2E1, GSTM1 or GSTT1 genotypes were not significantly associated with SCE frequency. When various genotypes were considered together, combination of CYP2E1 c1c2/c2c2 with ALDH2 1–2/2–2 showed an additive effect on SCE frequency. Similar results were also found for the combination of smoking with CYP2E1, or smoking with ALDH2. These

Abbreviations: VCM, vinyl chloride monomer; CYP2E1, cytochrome *P*450 2E1; CEO, chloroethylene oxide; CAA, chloroacetaldehyde; ALDH2, aldehyde dehydrogenase class 2; GST, glutathione *S*-transferase; PVC, polyvinyl chloride; PCR, polymerase chain reaction; SCE, sister chromatid exchange; TWA, time weight average; dNTP, deoxynucleotide triphosphate

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results suggest that VCM workers with ALDH2 1–2/2–2 genotypes, who also smoke, may have increased risk of DNA damage.

Keywords: Sister chromatid exchange; Aldehyde dehydrogenase 2; Smoking; Vinyl chloride

1. Introduction

Vinyl chloride monomer (VCM; CAS No. 75-01-4) has been classified by the International Agency for Research on Cancer (IARC) as a Group I carcinogen [1]. In previous epidemiological studies, exposure to VCM has been associated with hepatoma [2] and angiosarcoma of the liver [3–7]. However, the majority of VCM workers do not develop hepatoma and angiosarcoma of the liver. Those with a higher risk of developing cancers may have susceptibility factors including inherited metabolic traits.

Animal studies have shown that VCM is metabolized by CYP2E1 to become active chloroethylene oxide (CEO) and chloroacetaldehyde (CAA) [8,9], both of which may bind covalently with macromolecules [10–12]. CYP2E1 has already been reported to be associated with lung cancer in smokers [13,14]. In a previous study we have shown that there was elevated ALT (alanine aminotransferase) abnormality in VCM workers who had c2 allele for CYP2E1 [15]. However, the modulatory effect of CYP2E1 polymorphism on mutagenesis has not yet been demonstrated in VCM workers. Furthermore, CEO may then be metabolized by glutathione *S*-transferases (GSTs), and CAA by aldehyde dehydrogenase (ALDH2). Thus, those with appropriate types of ALDH2 and GSTs may also have elevated reactive intermediates, which can lead to increased DNA damage.

Sister chromatid exchange (SCE) is one of the most sensitive markers of DNA damage and has been used previously to investigate the genotoxicity of a variety of chemicals [16]. Previous studies have shown that workers exposed to VCM have a higher SCE frequency than controls [17,18]. Additionally, cigarette smoke contains a variety of known carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aldehyde derivatives, and vinyl chloride. These carcinogens also require metabolic biotransformation to detoxify. Thus, workers with inherited variant metabolic enzyme activities might have an altered genotoxicity caused by vinyl chloride as well as cigarette smoke. In this study, we used SCE to determine the effects of CYP2E1, ALDH2, GSTM1 and GSTT1 metabolic traits and smoking on DNA damage in VCM workers.

2. Materials and methods

2.1. Study subjects

A total of 44 males, with 4 to 36 years of VCM exposure from three polyvinyl chloride (PVC) production plants, were included for analysis. Study subjects were selected from workers who had received annual medical surveillance based on predetermined VCM exposure level and smoking status. Information on smoking, alcohol consumption, medications and detailed occupational history were collected from interviewer administered questionnaires, after informed consent was obtained. Current smokers were those who had been smoking 6 months prior to the specimen collection.

VCM exposure levels for these study subjects were based on our previously published work [19]. Briefly, area and personal sampling and analysis were performed in accordance with the method recommended by the U.S. NIOSH [20]. Time weighted-average adjusted VCM exposure levels were assigned to each category of workers. In this study, subjects were divided into high and low VCM exposure groups. Individuals in the high exposure group had been exposed to a VCM concentration higher than 1 ppm. Those in the low exposure group had been exposed to a VCM concentration less than 1 ppm.

2.2. SCE assay

SCE was performed according to a modified cytogenetic method [21]. Venous blood was collected into heparinized tubes from all subjects. One ml of whole blood was mixed with 9 ml RPMI 1640 (Gibco) culture medium with L-glutamine containing

10% fetal bovine serum (Hyclone), penicillin (100 units/ml) and streptomycin (100 μg/ml). Phytohemagglutinin was added at a concentration of 2 μg/ml to stimulate the division of lymphocytes. Cells were then incubated in 5% CO_2 at 37°C for 72 h. 50 μM of 5-bromodeoxyuridine was added to the culture at 24 h to achieve differential staining. Colcemid (0.1 μg/ml) was added 1.5 h before harvesting. The lymphocytes were then harvested with 75 mM hypotonic KCl solution for 10 min. Subsequently, cells were washed twice and fixed in methanol: acetic acid (3:1; v/v). Slides were prepared by the air dry technique. The chromosomes were stained by a modification of the fluorescence-plus Giemsa technique. Each slide was mounted with Sorensen's solution (pH = 6.8), irradiated with black light from two 15-W lamps for 9 min and stained with 5% Giemsa stain for 5 min. For each subject, 50 cells at metaphase with 40 or more chromosomes were scored to determine the individual mean SCE frequency per cell. All slides were scored by one reader, who was blind to the status of the subjects.

2.3. CYP2E1-PstI polymorphism

For CYP450 2E1 gene analysis, Restriction fragment length polymorphism (RFLP) were detected by differences in *Pst*I sites in the 5′-flanking region after PCR amplification, using methods described by Hayashi et al. [22]. Primers were designed to hybridize the sequences 5′-CCA GTC GAG TCT ACA TTG TCA-3′ and 3′-TTC ATT CTG TCT GTC TTC TAA CTGG-5′. One half microliter of DNA was added to a PCR buffer containing 200 ng of primers, 1.25 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris–HCl (pH = 8.3) and 0.1% BSA in a final volume of 50 μl. Reaction mixtures were heated for 5 min at 95°C, and 2.5 units of Taq polymerase were then added at 80°C. Subsequently, 35 cycles of amplification were performed: denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The PCR products were digested with *Pst*I for 16 h at 37°C and analyzed on 2.2% agarose gel. Homozygous c1c1 individuals had a product fragment of 410 bp, whereas homozygous c2c2 individuals had 290 and 120 bp fragments, and heterozygous c1c2 individuals had all three fragments.

2.4. GSTM1 and GSTT1 genotypes

GSTM1 and GSTT1 genotypes were determined by co-amplification of two genes [23,24]. Primers used for the GSTM1 gene were 5′-CTG CCC TAC TTG ATT GAT GGG-3′ and 5′-CTG GAT TGT AGC AGA TCA TGC-3′. The primers used for the GSTT1 gene were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′. Amplification of human β-globin (110-bp) was also performed as a positive control in each reaction to confirm the presence of amplifiable DNA in the samples. The primers used for β-globin were 5′-ACA CAA CTG TGT TCA CTA GC-3′ and 5′-CAA CTT CAT CCA CGT TCA CC-3′. The amplification was carried out under conditions similar to those described previously for CYP2E1, except denaturing was conducted at 94°C for 1 min 30 s, annealing at 52°C for 1 min, and extension at 65°C for 1 min. The reaction product then underwent electrophoresis in a 2% agarose gel. Individuals with one or more GSTM1 alleles had a 273 bp fragment, and individuals with one and more GSTT1 alleles had a 480 bp fragment.

2.5. ALDH2-Mbo II polymorphism

ALDH2-Mbo II polymorphism was determined by a modification of the methods developed by Harada and Zhang [25]. Primers were synthesized from the 5′ region of exon 12 (5′-CAA ATT ACA GGG TCA ACT GCT ATG-3′) and the 3′ region of exon 12 (3′-CCA CAC TCA CAG TTT TCT CTT-5′). Amplification was carried out under conditions similar to those described for the GSTs. The PCR products were digested with Mbo II for 8 h at 37°C and analyzed with 6% polyacrylamide gel electrophoresis (PAGE). Homozygous 2–2 individuals had a single product fragment of 135 bp, whereas homozygous 1–1 individuals had 125 and 10 bp fragments, and heterozygous 1–2 individuals had all three fragments.

2.6. Statistical analysis

Data analyses were performed using the SAS 6.11 software package [26]. Comparison between the low and high VCM exposure groups for age, duration of

employment, smoking, alcohol consumption, and the prevalence of genotypes of CYP2E1, ALDH2, GSTM1 and GSTT1 were made using a *t*-test for continuous variables and a χ^2-test for discrete variables. Subsequent, crude SCE frequency was evaluated using stratified analysis by smoking and various factors. *t*-test was used to test the association of different metabolic traits and SCE frequency. Association of these variables with SCE frequency was assessed using general linear model (GLM). Furthermore, least-squares mean was performed to predict the adjusted SCE frequency for individuals with different metabolic traits. In addition, GLM was also used to test for any trends.

3. Results

Forty-four male subjects were included in the analysis. The demographic characteristics of the study subjects are summarized in Table 1. The mean age of the subjects was 45.1 ± 1.4 (SE) years. 55.6% of the subjects were current smokers. The mean age, proportion of current smokers and duration of employment at PVC plants were not significantly different between the high and low exposure groups (*t*-test or χ^2-test, $p < 0.05$).

The genotype prevalence of CYP2E1, ALDH2, GSTM1 and GSTT1 in the study subjects is shown in Table 2. The frequencies of the c1 and c2 allele of CYP2E1 were 80.5 and 19.5%, respectively. Whereas the frequency of the ALDH2[1] and ALDH2[2] allele were 73.8 and 26.2%, respectively. A slightly higher prevalence of the GSTM1 null-type was observed in individuals in the high VCM exposure group ($p = 0.05$, Mantel–Haenszel χ^2-test).

The overall mean SCE frequency per cell was 8.6 ± 0.3. Those who smoked equal or more than one pack each day had 9.1 SCE/cell, those who smoked less than one pack each day had 8.7 SCE/cell, whereas those who never smoked had 8.4 SCE/cell. Further analysis on the association of SCE frequency with various factors in individuals by different smoking status was performed (Table 3). Since workers with at least one CYP2E1 c2 allele have higher enzyme activity than CYP2E1 c1c1 [22], those with at least one CYP2E1 c2 allele were grouped together as CYP2E1 variants because of small number. Similarly, since workers with at least one ALDH2[2] allele have lower enzyme activity than ALDH2 1–1 [27], those with at least one ALDH2[2] allele were grouped as ALDH2 variants. SCE frequencies were found to be higher for individuals with CYP2E1 c1c2/c2c2 genotypes than those with CYP2E1 c1c1 genotype (9.0 vs. 8.5). When stratified by smoking status, CYP2E1 c1c2/c2c2 genotypes had higher SCE frequencies than CYP2E1 c1c1 genotype in different smoking status, although this

Table 1
Demographic characteristics of VCM (vinyl chloride monomer) workers stratified by different intensities of exposure

Variables	VCM exposure		
	Low	High	All
Number	16	28	44
Age (year)	48.4 ± 1.9	43.3 ± 1.9	45.1 ± 1.4[a]
Duration of employment (year)	23.0 ± 1.8	18.2 ± 1.8	19.8 ± 1.4
Smoking			
Current smoker (%)	7(43.8%)	18(62.1%)	25(55.6%)[b]
Cigarette/day	10.4 ± 3.3	10.3 ± 1.9	10.4 ± 1.7
Pack-year	9.9 ± 2.9	8.6 ± 2.0	9.1 ± 1.7
Alcohol consumption (g/week)	55.7 ± 42.8	31.7 ± 23.3	41.1 ± 21.7

[a] Mean $\pm$ SE.
[b] *N* (%).

Table 2
Prevalence of genotypes of CYP2E1, ALDH2, GSTM1 and GSTT1, among VCM workers stratified by intensity of VCM exposure

Genotype		VCM exposure		
		Low	High	All
Number		16	28	44 (100%)[a]
CYP2E1	c1c1	11	18	29 (65.9%)
	c1c2	4	9	13 (29.5%)
	c2c2	1	1	2 (4.4%)
ALDH2	1–1	10	16	26 (59.1%)
	1–2	6	7	13 (29.5%)
	2–2	0	5	5 (11.4%)
GSTM1	Null	6	19[b]	25 (56.8%)
	Non-null	10	9	19 (43.2%)
GSTT1	Null	9	16	25 (56.8%)
	Non-null	7	12	19 (43.2%)

[a] *N* (%).
[b] $p = 0.05$, Mantel–Haenszel χ^2-test.

Table 3
SCE (sister chromatid exchange) frequencies stratified by smoking status and various factors

Variable	Smoking status					
	Non-smoker		Smoker		All	
	N	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE
All	19	8.4 ± 0.3	25	8.9 ± 0.4	44	8.6 ± 0.3
VCM exposure						
High	11	8.6 ± 0.3	18	9.0 ± 0.3	29	8.9 ± 0.3
Low	8	8.0 ± 0.4	7	8.6 ± 0.2	15	8.3 ± 0.3
Age						
≥ 45	11	8.5 ± 0.3	11	8.9 ± 0.3	22	8.7 ± 0.3
< 45	8	8.2 ± 0.3	14	8.8 ± 0.3	22	8.6 ± 0.3
CYP2E1						
c1c2/c2c2	9	8.6 ± 0.4	6	9.4 ± 0.4	15	9.0 ± 0.4
c1c1	10	8.2 ± 0.3	19	8.7 ± 0.2[†]	29	8.5 ± 0.3
ALDH2						
1–2/2–2	7	8.8 ± 0.3	11	9.7 ± 0.3	18	9.4 ± 0.3
1–1	12	8.1 ± 0.3	14	8.3 ± 0.3**	26	8.2 ± 0.3**
GSTM1						
Null	11	8.3 ± 0.3	14	8.9 ± 0.3	25	8.6 ± 0.3
Non null	8	8.5 ± 0.3	11	9.0 ± 0.3	19	8.9 ± 0.3
GSTT1						
Null	13	8.5 ± 0.2	12	8.9 ± 0.3	25	8.7 ± 0.3
Non null	6	8.2 ± 0.3	13	8.9 ± 0.4	19	8.7 ± 0.3

** $p < 0.01$ between ALDH2 1–2/2–2 and 1–1 genotypes, *t*-test.
[†] $p = 0.07$ between CYP2E1 c1c2/c2c2 and c1c1 genotypes, *t*-test.

did not reach statistical significance. Interestingly, smokers with CYP2E1 c1c2/c2c2 genotypes had the highest SCE frequencies, followed by nonsmoker with CYP2E1 c1c2/c2c2 genotypes and smokers with CYP2E1 c1c1. Nonsmokers with CYP2E1 c1c1 had the lowest SCE frequencies. This trend in decreasing frequencies was shown to be statistically significant (GLM, $p < 0.01$). In addition, difference on SCE frequency between smokers with CYP2E1 c1c2/c2c2 genotypes (9.4 ± 0.4) and nonsmokers with CYP2E1 c1c1 genotype (8.2 ± 0.3), equal to the summation of differences among smokers with CYP2E1 c1c1 genotype (8.7 ± 0.2), nonsmokers with CYP2E1 c1c2/c2c2 genotypes (8.6 ± 0.4), and nonsmokers with CYP2E1 c1c1 genotype (8.2 ± 0.3). Moreover, ALDH2 1–2/2–2 genotypes had significantly higher SCE frequencies than ALDH2 1–1 genotype (9.4 vs. 8.2, *t*-test, $p < 0.01$). A trend similar to that observed for CYP2E1 was also observed for ALDH2 (GLM, $p = 0.02$). Similarly, differences on SCE frequency among individuals with different smoking status and ALDH2 genotypes also revealed an additive effect as CYP2E1 genotypes. However, there were no similar results for SCE frequency in GSTM1 and GSTT1 genotypes.

A multiple regression model for SCE frequency as function of age, smoking, VCM exposure and genotypes of CYP2E1, ALDH2, GSTM1, GSTT1 is shown in Table 4 (GLM). SCE frequency increased with smoking status, and VCM exposure. High VCM exposure group had higher SCE frequency than low VCM exposure group ($p = 0.06$). Those who have smoked also had a significantly higher SCE frequency than those who never smoked ($p < 0.01$). In addition, workers with CYP2E1 c1c2/c2c2 genotypes also had a higher SCE frequency than those with CYP2E1 c1c1 genotype, though they did not reach statistic significance ($p = 0.14$). Interestingly, workers with ALDH2 1–2/2–2 genotypes also had significantly higher SCE frequency than those with ALDH2 1–1 genotype ($p = 0.05$). However, no significant results for SCE frequency in GSTM1 and GSTT1 genotypes were revealed.

Subsequently, least-square mean was performed to assess the SCE frequency for VCM workers with the combination of CYP2E1 and ALDH2 genotypes adjusted for VCM exposure, smoking and age. Individuals with both CYP2E1 c1c1 genotype and ALDH2 1–1 genotype had the lowest SCE frequen-

Table 4
Multiple regression model for SCE

Variables	Regression coefficient	SE	*p*-Value
Intercept	7.19	0.46	< 0.01
Age: ≥ 45 vs. < 45	0.10	0.31	0.75
VCM exposure group: high vs. low	0.64	0.34	0.06
Smoking status: yes vs. none	0.85	0.31	< 0.01
Genotyping			
CYP2E1: c1c2/c2c2 vs. c1c1	0.50	0.33	0.14
ALDH2: 1–2/2–2 vs. 1–1	0.63	0.31	0.05
GSTM1: non-null vs. null	−0.41	0.31	0.19
GSTT1: non-null vs. null	0.27	0.30	0.38

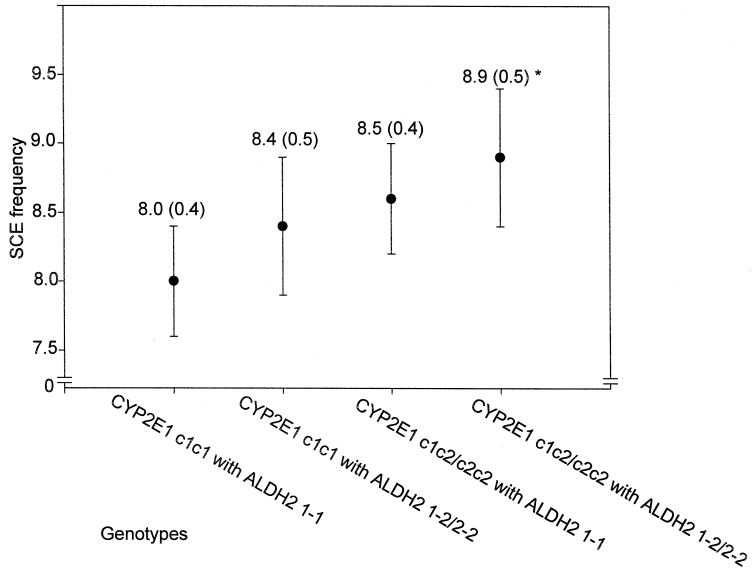

Fig. 1. Adjusted SCE frequency by CYP2E1 and ALDH2 genotypes. ∗Mean (SE).

cies, 8.0 ± 0.4 ($n = 17$, Fig. 1), whereas individuals with both CYP2E1 c1c2/c2c2 genotypes and ALDH2 1–2/2–2 genotypes had the highest frequency of SCE, 8.9 ± 0.6 ($n = 6$). Those with both CYP2E1 c1c2/c2c2 genotypes and ALDH2 1–1 genotype ($n = 9$), and those with both CYP2E1 c1c1 genotype and ALDH2 1–2/2–2 genotypes ($n = 12$) had a moderate increase in SCE frequency. This trend in SCE frequency was shown to be statistically significant with $p < 0.01$ (GLM). Further, SCE frequency difference between combined wild-types (CYP2E1 c1c1 and ALDH2 1–1) and combined variants (CYP2E1 c1c2/c2c2 and ALDH2 1–2/2–2) approximates the summation of differences between one wild-type with one variant (CYP2E1 c1c1 and ALDH2 1–2/2–2; CYP2E1 c1c2/c2c2 and ALDH2 1–1) and both wild-types (CYP2E1 c1c1 and ALDH2 1–1).

4. Discussion

In this study, the metabolic trait of ALDH2, smoking and VCM exposure were significantly associated with SCE frequency.

The 19.5% prevalence of the CYP2E1 c2 allele found in this study was similar to that found in a previous study of individuals of Taiwanese decent (20.3%) [28]. The frequency of ALDH2[1] allele (73.8%) was also comparable to that for the control group of other alcoholism studies on Taiwanese population (68–76%) [29,30]. The prevalence of GSTM1 null-type of 56.8% was likewise consistent with previous studies (53–62%) [31–33]. Prevalence of GSTT1 null-type (56.8%) was also similar to those found in previous studies—64.4% for Chinese [34] and 51.4% for Taiwanese [33]. These finding to some extent validate the technique of our genotyping.

Previous animal studies have shown that VCM is metabolized by CYP2E1 to become CEO [8,9], a reactive intermediate which may bind with macromolecules [10–12]. Those with CYP2E1 c1c2 and CYP2E1 c2c2 had higher metabolic activity than those with CYP2E1 c1c1 [22]. CEO may spontaneously transform into CAA, which may be subsequently metabolized by ALDH2. Investigation on the correlation of genotypes with phenotypes of ALDH2 have shown that inactive ALDH2[2] allele was dominant [27]. Although individuals with variant ALDH2[2] allele have a lower enzyme activity and polymorphism of ALDH2 has been reported to be associated with alcoholism [29,30], the role of ALDH2 in the metabolism of VCM has not been assessed in VCM

workers. In this study, we first demonstrated that ALDH2 may play an important role in the metabolic pathway of VCM in exposed workers. However, those with at least one CYP2E1 c2 allele had higher SCE frequency than those with CYP2E1 c1c1. Interestingly, further analysis of CYP2E1 and ALDH2 together revealed an additive effect for these two genotypes on VCM-induced SCE. This implies that CYP2E1 c1c2/c2c2 may also play a role in VCM metabolism, thus VCM workers with more than one susceptible genotype may have a greater DNA damage.

Many previous studies have shown that GSTs may act as detoxication enzymes by reacting with the epoxide product of many different chemicals [34–38]. However, we did not observe any significant association of increased SCE frequency with GSTT1 or GSTM1 alone in exposed workers. In addition to CYP2E1 and ALDH2 enzymes, other enzymes including alcohol dehydrogenase (ADH) and epoxide hydratase may also play a role in the metabolism of VCM [8,9]. Future studies, in which more subjects are tested for SCE frequency among these additional genotypes at various levels of VCM exposure, may shed more light on the metabolism of VCM.

Previous reports have shown that smoking and age are associated with an increase in the SCE frequency [21,39]. We also found cigarette smoking was associated with increased SCE frequency in this study. While age was only slightly associated with increase SCE because the range of age in our subjects was small. In addition to age and smoking, alkylating agents are also associated with SCE. VCM exposure has been previously reported to be associated with increased SCE frequency [16,17]. Again, we also found similar result in the present study.

In our study, both analysis on smoking status and CYP2E1 together, and analysis on smoking status and ALDH2 together revealed an additive effect for these two genotypes on cigarette smoke-induced SCE. This implies that chemicals within cigarette smoke also require metabolic transformation to become electrophilic prior to forming a covalent bond with DNA. Previous studies have reported that cigarette smoke contains aldehyde derivatives and vinyl chloride which may be metabolized through CYP2E1 and ALDH2. Thus, individuals with inherited variant CYP2E1 and ALDH2 enzyme activities could have an altered risk of cigarette smoke-induced DNA damage. Again, these results still need more studies to confirm.

The small number of subjects in our study has precluded us from drawing firm conclusions. It is difficult for us to include adequate controls without VCM exposure in this study. In addition, our reference group (office workers in the same facility) was previously shown to have some potential VCM exposure based on environmental monitoring in PVC factories [19]. However, our multi-variate adjustment corroborates the possible mechanism of VCM metabolism and carcinogenesis. In conclusion, VCM workers with ALDH2 1–2/2–2 genotypes as well as smoking, may have a higher risk of DNA damage.

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