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METABOLIC ACTIVATION/INACTIVATION

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# *XRCC1*, *CYP2E1* and *ALDH2* genetic polymorphisms and sister chromatid exchange frequency alterations amongst vinyl chloride monomer-exposed polyvinyl chloride workers

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**Abstract** Vinyl chloride monomer (VCM) is a known human carcinogen, which may be metabolized by cytochrome P450 2E1 (CYP2E1), aldehyde dehydrogenase 2 (ALDH2), and glutathione *S*-transferase T1 (GSTT1). A DNA-repair gene, X-ray repair cross-complementing group 1 (*XRCC1*, exon 10), may also be implicated in the process of VCM-related carcinogenesis. Thus, VCM-exposed workers with inherited susceptible metabolic and DNA-repair genotypes may experience an increased risk of genotoxiciy. This study was designed to investigate whether metabolic and DNA-repair genotypes affected sister chromatid exchange (SCE) frequency in occupationally VCM-exposed workers from polyvinyl chloride (PVC) manufacturing plants. Study subjects comprised 61 male workers having experienced VCM exposure, and 29 male controls. Questionnaires were administered to obtain detailed histories of cigarette-smoking habits, alcohol consumption behavior, and occupation. The frequency of SCE in peripheral lymphocytes was determined using a standardized method, and genotypes of *CYP2E1*, *ALDH2*, *GSTT1* and *XRCC1* were identified by the polymerase chain reaction (PCR) procedure. Our results demonstrated that smoking, age and VCM exposure and *XRCC1* ($P=0.03$), *CYP2E1* ($P=0.04$), and *ALDH2* ($P=0.08$) were significantly associated with an increased SCE frequency. Further analysis of gene combinations, including *CYP2E1*, *ALDH2* and *XRCC1*, revealed an increased trend for these genotypes to influence SCE frequencies for the low VCM-exposure group ($P<0.01$), but not so for the high VCM-exposure group ($P=0.29$) or for controls ($P=0.49$). These results suggest that workers with susceptible metabolic and DNA-repair genotypes, may experience an increased risk of DNA damage elicited by VCM exposure.

**Keywords** Vinyl chloride monomer · *CYP2E1* · *ALDH2* · *XRCC1* · Sister chromatid exchange

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## Introduction

Vinyl chloride monomer (VCM; CAS No. 75-01-4) has been associated with angiosarcoma of liver in previous epidemiological studies (Theriault and Allard 1981; Jones et al. 1988; Wong et al. 1991). Our previous retrospective cohort study revealed that Taiwanese polyvinyl chloride (PVC) factory workers have a greater standardized mortality ratio (SMR) of liver cancer than the general male population (Wong et al. 2002a). Importantly, only 25 of these VCM-exposed workers developed liver cancer between 1985 and 1997. Those individuals experiencing a greater risk of developing cancers may possess certain susceptibility factors including inherited metabolic and DNA-repair traits.

Subsequent to metabolic activation in the liver by cytochrome P450 2E1 (CYP2E1; el Ghissassi et al.1998), VCM is transformed into the alkylating intermediates chloroethylene oxide (CEO) and chloroacetaldehyde (CAA), both of which may bind covalently with DNA to form DNA adducts (Guengerich 1992). CEO may then be metabolized by glutathione *S*-transferases (GSTs; Whysner et al. 1996), and CAA by aldehyde dehydrogenase 2 (ALDH2; Whysner et al. 1996). Our previous study has also revealed that CYP2E1 and ALDH2 are

associated with an increased frequency of SCE amongst VCM-exposed workers, although the small number of subjects in that study has precluded us from drawing any firm conclusion in this regard (Wong et al. 1998).

In addition to metabolic traits, DNA repair capacity also plays an important role in VCM-related carcinogenesis. Evidence has been presented to indicate that VCM derivative etheno–DNA adducts may be repaired though the base excision repair (BER) pathway (Dosanjh et al. 1994). X-ray cross-complementing group 1 (XRCC1), a DNA-repair protein involved in single-strand breaks and the BER pathway, has been reported to be responsible for the efficient repair of DNA damage caused by ionization and alkylating agents (Thompson et al. 1990). Three polymorphisms of DNA-repair genes XRCC1 have been identified at codon 194 (Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln) (Shen et al. 1998). In particular, XRCC1 399Gln polymorphism resulting in single base substitution, which may affect binding with PARP [poly (ADP–ribose) polymerase], may lead to deficiency of DNA repair (Marintchev et al. 2000). Our recent study showed that VCM-exposed workers with higher cumulative dose had an increased plasma mutant p53 protein and anti-p53 antibody. In the same study, workers with susceptible metabolic- and DNA-repair traits experienced higher levels of plasma mutant *p53* protein and anti-p53 antibody (Wong et al. 2002b).

Previous studies have shown that workers exposed to VCM have a higher SCE frequency than controls (Sinues et al. 1991; Fucic et al. 1996). SCE frequency reflects recent VCM exposure in contrast to p53 gene mutation, which reflects cumulative VCM exposure. In this study, we used SCE as an indicator of DNA damage to investigate the effects of *XRCC1*, *CYP2E1*, and *ALDH2* genotypes on DNA damage amongst VCM-exposed workers.

## Materials and methods

### Study subjects and epidemiological data

Previously, we conducted a study to explore the association between SCE frequency and metabolic traits amongst 44 VCM-exposed workers at PVC manufacturing plants (Wong et al. 1998). In order to acquire sufficient statistical discriminatory power to detect differences in SCE frequency amongst various inherited polymorphisms in metabolic and DNA repair, we collected a greater number of study subjects than was the case for our previous study (Wong et al. 1998). In addition, we also included control subjects who had been exposed to neither VCM nor other mutagens at workplace.

A total of 61 male subjects from five PVC plants were recruited. Twenty-nine male controls were also selected from a resin-synthesis plant, where workers were exposed to dimethylformamide (DMF; CAS No. 68-12-2). Our previous study was unable to demonstrate any association between DMF exposure and SCE frequency alteration in peripheral lymphocytes from DMF-exposed workers (Cheng et al. 1999). Evidence from a comprehensive mutagenicity study, including an Ames test, indicates DMF is not a mutagen (Antoine et al. 1983).

In the current study, information was collected from study subjects by interviewer-administered questionnaires subsequent to informed consent having been provided by such subjects. The structured questionnaire contained questions that covered various demographic characteristics and life styles, including habits of cigarette-smoking and alcohol consumption, as well as a detailed occupational history. Each subject's smoking history included the number of cigarettes smoked daily and also the duration of the smoking habit. A parameter termed "pack-years" was coined as an indicator of cumulative smoking dose, and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking.

Our estimation of VCM exposure for this study's subjects was based upon our previously published work (Du et al. 1996) and also recently monitored air VCM levels (Cheng et al. 2001). A time-weighted average VCM-exposure level was assigned to each category of worker employment, those VCM-exposed workers in this study being categorized into one of two exposure groups, a high or a low VCM-exposure group. Individuals selected as belonging to the high VCM-exposure group had been exposed, occupationally, to a VCM concentration of greater than 1 ppm, whilst those from the low VCM-exposure group had been exposed to a VCM concentration of less than 1 ppm. An occupational exposure to VCM of 1 ppm is the current permissible exposure limit in most developed countries (IPCS 1999).

### Sister chromatid exchange assay

Sister chromatid exchange frequency assessment was performed according to a modified cytogenetic method (Wong et al. 1998). Briefly, 1 ml whole blood was mixed with 9 ml RPMI 1640 culture medium. Phytohemagglutinin was added at a concentration of 2 μg/ml to stimulate the division of lymphocytes. Cells were then incubated in 5% $CO_2$ in air at 37°C for 72 h. At 24 h, 50 μM 5-bromodeoxyuridine was added to the culture in order to achieve differential staining. Colcemid (0.1 μg/ml) was added 1.5 h before harvesting. The lymphocytes were then harvested with hypotonic solution for 10 min. Subsequently, cells were fixed in methanol:acetic acid (3:1, v/v). Slides were prepared and the chromosomes were stained by a modification of the fluorescence-plus Giemsa technique. For each subject, 50 cells at metaphase with 40 or more chromosomes present were scored to determine the individual mean SCE frequency per cell. All slides were scored by one reader who was blind to the determinants and exposure of each subject.

### Genotyping of polymorphic metabolic and DNA-repair traits

The determination of *CYP2E1*, *ALDH2*, and *GSTT1* genotypes was performed as we have indicated previously (Wong et al. 1998). Briefly, for the CYP450 2E1 gene analysis, any restriction fragment length polymorphism (RFLP) was detected by differences in *Pst*I sites in the 5′-flanking region following PCR amplification, using methods described by Hayashi et al. (1991). Primers used for the amplification of *CYP2E1* gene were 5′-CCA GTC GAG TCT ACA TTG TCA-3′ and 5′-TTC ATT CTG TCT TCT AAC TGG-3′. Amplification was carried out under conditions such that the denaturing step was conducted at 95°C, annealing at 55°C and extension at 72°C. The PCR products were digested with *Pst*I. Homozygous *c1c1* individuals exhibited a product fragment of 410 bp, whereas homozygous *c2c2* individuals revealed a 290 bp and a 120 bp fragment, and heterozygous *c1c2* individuals demonstrated all three fragments. The *ALDH2-Mbo*II polymorphism was determined by a modification of the methods as developed by Harada and Zhang (1993). The sequences of *ALDH2* primers were 5′-CAA ATT ACA GGG TCA ACT GCT ATG-3′ and 5′-CCA CAC TCA CAG TTT TCT CTT-3′. Amplification was carried out under conditions such that the denaturing step was conducted at 94°C, annealing at 52°C and extension at 65°C. The PCR products were digested with *Mbo*II and analyzed with 6% polyacrylamide gel electrophoresis

(PAGE). Homozygous *2-2* individuals demonstrated a single product fragment of 135 bp, whereas homozygous *1-1* individuals revealed both 125 and 10 bp fragments, and heterozygous *1-2* individuals exhibited all three fragments. The *GSTT1* genotype was determined as described by Pemble et al. (1994). 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′. The amplification of human $\beta$-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 $\beta$-globin were 5′-ACA CAA CTG TGT TCA CTA GC-3′ and 5′-CAA CTT CAT CCA CGT TCA CC-3′. The amplification procedure was carried out under conditions such that denaturing was conducted at 94°C, annealing at 52°C, and extension at 65°C. The reaction product then underwent electrophoresis in a 2% agarose gel. Individuals with *GSTT1* alleles demonstrated a 480-bp fragment.

The *XRCC1* exon-10-*Msp*I polymorphism was determined using a PCR-RFLP technique (Duell et al. 2000). An *Arg* to *Gln* substitution in exon 10 (codon 399) was amplified to form an undigested fragment of 242 bp using the primer pair 5′-CCC CAA GTA CAG CCA GGT C-3′ and 5′-TGT CCC GCT CCT CTC AGT AG-3′. The amplification was carried out under conditions such that denaturing was conducted at 94°C, annealing at 59°C, and extension at 72°C. The PCR products were digested with *Msp*I and analyzed in a 2% agarose gel. Homozygous *Gln-Gln* individuals reflected a single product fragment of 242 bp, whereas homozygous *Arg-Arg* individuals demonstrated both 94 and 148 bp fragments, and heterozygous *Arg-Gln* individuals revealed all three fragments.

### Statistical analysis

Comparisons between low and high VCM-exposure groups with the controls for age, duration of employment, current smoking status, pack-years of smoking, and alcohol consumption were made using Student's *t*-test and analysis of variance (ANOVA) for continuous variables, and using a $\chi^2$-test for discrete variables. The $\chi^2$-test was used to test the prevalence of genotypes of *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* amongst low and high VCM-exposure groups and controls. Since workers possessing at least one *XRCC1 Arg* allele have previously been shown to possess a higher capacity for DNA repair than is the case for the *XRCC1 Gln* allele (Shen et al. 1998), those with at least one *XRCC1 Arg* allele were grouped as *XRCC1 Arg-Arg*/*Arg-Gln* genotypes. Similarly, individuals featuring the *CYP2E1* genotype with at least one *c1* allele demonstrate a lower enzyme activity level than is the case for those presenting a *CYP2E1 c2* allele (Hayashi et al. 1991), and hence those with at least one *CYP2E1 c1* allele were grouped as *CYP2E1 c1c1*/*c1c2*. Workers with at least one $ALDH2^2$ allele also reflect a lower enzyme activity than *ALDH2 1-1* (Crabb et al. 1989), and hence those with at least one $ALDH2^2$ allele were grouped as *ALDH2 1-2*/*2-2*. Subsequently, the crude SCE frequency was evaluated using an analysis stratified by VCM exposure and different factors. ANOVA was used to compare difference in SCE frequencies by different VCM-exposure status, and a Student's *t*-test was used to test the association between age, duration of employment, smoking status, alcohol consumption, DNA repair, and different metabolic traits and associated SCE frequency. The association of these variables with SCE frequency was further assessed using a general linear model (GLM). Finally, a least-squares mean was performed to predict the adjusted SCE frequency for individuals with different numbers of susceptible *XRCC1*, *CYP2E1*, and *ALDH2* genotypes. In addition, GLM was also conducted to test for any trends in SCE frequency.

## Results

Sixty-one male subjects with VCM exposure and 29 male controls were included in the analysis. The demographic characteristics of the study subjects are summarized in Table 1. The mean age of the study subjects was 41.0 years, and 55.6% of the subjects were current smokers. The mean age ($P = 0.29$; *t*-test), duration of employment at PVC plants ($P = 0.74$), proportion of current smokers ($P = 0.56$; $\chi^2$-test), the number of cigarette pack-years ($P = 0.37$) and alcohol consumption ($P = 0.31$) were not significantly different between the high and low VCM-exposure groups. In contrast, however, controls were significantly younger in age, had a shorter duration of employment, and had smoked less pack-years of cigarettes.

The genotypic prevalence of *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* amongst the study subjects is shown in Table 2. The frequencies of the 399*Arg* and 399*Gln* alleles of *XRCC1* were 67.8% and 32.2%, respectively, although a slightly higher prevalence of the 399*Gln* allele was observed for individuals who experienced a high VCM exposure ($P = 0.06$, $\chi^2$-test). The frequencies of the *c1* and *c2* alleles of *CYP2E1* were 77.8% and 22.2%, respectively, and the frequencies of the $ALDH2^1$ and $ALDH2^2$ alleles were 68.3% and 31.7%, respectively, whereas the prevalence of the *GSTT1* null-type was 54.4% and that for the non-null-type was 45.6%.

The overall mean SCE frequency per cell was $8.2 \pm 0.2$. Table 3 summarizes the crude association of SCE frequency with various factors amongst test individuals. In this study, individuals experiencing a high VCM exposure revealed the highest SCE frequencies of 8.9 SCE/cell, followed by those classified as low VCM exposure (8.5 SCE/cell), and controls (7.2 SCE/cell; $P < 0.01$, ANOVA). Similarly, workers older than 45 years of age or who were smokers also showed a

**Table 1** Demographic characteristics of vinyl chloride monomer (VCM)-exposed polyvinyl chloride workers and controls stratified by different intensity of exposure. Data represent numbers of individuals, or means ± SE for continuous variables

| Variable | Controls | VCM exposure | | All |
|---|---|---|---|---|
| | | Low | High | |
| Number of subjects | 29 | 29 | 32 | 90 |
| Age (years) | 34.0 ± 1.0** | 45.7 ± 1.6 | 43.2 ± 1.6 | 41.0 ± 1.0 |
| Duration of employment (years) | 7.9 ± 0.9** | 20.0 ± 1.8 | 19.2 ± 1.7 | 15.8 ± 1.0 |
| Smoking habit | | | | |
| Number of current smokers (%) | 14 (48.3%) | 16 (55.2%) | 20 (62.5%) | 50 (55.6%) |
| Pack-years | 4.4 ± 1.0* | 11.1 ± 2.3 | 8.7 ± 1.6 | 8.1 ± 1.0 |
| Alcohol consumption (g/week) | 20.8 ± 15.1 | 61.3 ± 30.1 | 27.0 ± 13.9 | 36.1 ± 11.9 |

*$P = 0.02$, **$P < 0.01$, ANOVA

**Table 2** Prevalence of genotypes of *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* amongst vinyl chloride monomer (VCM)-exposed workers and controls stratified by VCM exposure. Data represent the numbers of subjects (with percentage in parentheses, where shown)

| Genotype | | Controls | VCM exposure | | |
|---|---|---|---|---|---|
| Gene | Alleles | | Low | High | All |
| Number of subjects | | 29 | 29 | 32 | 90 (100%) |
| *XRCC1*-exon 10 | *Arg-Arg* | 15 | 15 | 9 | 39 (43.3%) |
| | *Arg-Gln* | 13 | 11 | 20 | 44 (48.9%) |
| | *Gln-Gln* | 1 | 3 | 3* | 7 (7.8%) |
| *CYP2E1* | *c1c1* | 16 | 18 | 19 | 53 (58.9%) |
| | *c1c2* | 13 | 10 | 11 | 34 (37.8%) |
| | *c2c2* | 0 | 1 | 2 | 3 (3.3%) |
| *ALDH2* | *1-1* | 13 | 15 | 15 | 43 (47.8%) |
| | *1-2* | 14 | 10 | 13 | 37 (41.1%) |
| | *2-2* | 2 | 4 | 4 | 10 (11.1%) |
| *GSTT1* | Null | 17 | 13 | 19 | 49 (54.4%) |
| | Non-null | 12 | 16 | 13 | 41 (45.6%) |

*$P = 0.06$, $\chi^2$-test

**Table 3** Frequencies of sister chromatid exchanges per cell stratified by VCM-exposure status and various factors

| Variables | | Controls | | VCM exposure | | | | All | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | Class | | | Low | | High | | | |
| | | *n* | Mean ±SE | *n* | Mean ±SE | *n* | Mean ±SE | *n* | Mean ±SE |
| All | | 29 | 7.2 ± 0.1 | 29 | 8.5 ± 0.3 | 32 | 8.9 ± 0.3** | 90 | 8.2 ± 0.2 |
| Age | ≥45 years | 0 | | 18 | 8.8 ± 0.4 | 14 | 9.4 ± 0.5 | 32 | 9.0 ± 0.3$^{\#\#}$ |
| | < 45 years | 29 | 7.2 ± 0.1 | 11 | 8.0 ± 0.3 | 18 | 8.5 ± 0.2 | 58 | 7.7 ± 0.1 |
| Duration of employment | ≥15.8 years | 1 | 7.5 | 19 | 8.7 ± 0.4 | 22 | 8.8 ± 0.3 | 42 | 8.7 ± 0.2$^{\#\#}$ |
| | < 15.8 years | 28 | 7.2 ± 0.1 | 10 | 8.1 ± 0.3 | 10 | 9.0 ± 0.5 | 48 | 7.7 ± 0.2 |
| Smoking status | Yes | 14 | 7.6 ± 0.2$^{\#\#}$ | 16 | 9.1 ± 0.4$^{\#\#}$ | 20 | 8.9 ± 0.3 | 50 | 8.6 ± 0.2$^{\#\#}$ |
| | No | 15 | 6.8 ± 0.2 | 13 | 7.7 ± 0.2 | 12 | 8.9 ± 0.5 | 40 | 7.7 ± 0.2 |
| Alcohol drinking | Yes | 4 | 7.5 ± 0.5 | 7 | 8.8 ± 0.7 | 8 | 8.6 ± 0.4 | 19 | 8.5 ± 0.3 |
| | No | 25 | 7.1 ± 0.1 | 22 | 8.4 ± 0.3 | 24 | 9.0 ± 0.3 | 71 | 8.1 ± 0.2 |
| *XRCC1*-exon 10 | *Gln-Gln* | 1 | 6.3 | 3 | 11.4 ± 1.7$^{\#\#}$ | 3 | 8.7 ± 1.0 | 7 | 9.5 ± 0.9 |
| | *Arg-Arg/Arg-Gln* | 28 | 7.2 ± 0.1 | 26 | 8.1 ± 0.2 | 29 | 8.9 ± 0.3 | 83 | 8.1 ± 0.1 |
| *CYP2E1* | *c2c2* | 0 | | 1 | 11.6$^{\#}$ | 2 | 9.6 ± 0.3 | 3 | 10.3 ± 0.7$^{\#}$ |
| | *c1c1/c1c2* | 29 | 7.2 ± 0.1 | 28 | 8.4 ± 0.3 | 30 | 8.8 ± 0.3 | 87 | 8.1 ± 0.2 |
| *ALDH2* | *1-2/2-2* | 16 | 7.3 ± 0.2 | 14 | 8.8 ± 0.4 | 17 | 9.0 ± 0.3 | 47 | 8.4 ± 0.2 |
| | *1-1* | 13 | 7.0 ± 0.2 | 15 | 8.2 ± 0.4 | 15 | 8.7 ± 0.4 | 43 | 8.0 ± 0.2 |
| *GSTT1* | Null | 17 | 7.0 ± 0.2 | 13 | 8.3 ± 0.4 | 19 | 9.0 ± 0.3 | 49 | 8.2 ± 0.2 |
| | Non-null | 12 | 7.5 ± 0.1 | 16 | 8.6 ± 0.4 | 13 | 8.7 ± 0.4 | 41 | 8.2 ± 0.2 |

**$P < 0.01$, comparison between different VCM-exposure groups conducted with ANOVA
$^{\#}0.01 < P < 0.05$, $^{\#\#}P < 0.01$, comparison between different age, smoking status, duration of employment, or genotype groups conducted with *t*-test

significantly higher frequency of SCE. Significantly higher SCE frequency was also found in those with greater duration of employment. The frequencies of SCE were found to be higher for individuals featuring the *XRCC1 Gln-Gln*, the *CYP2E1 c2c2*, and *ALDH2 1-2/2-2* genotypes. Interestingly, workers from the low VCM-exposure group who featured *XRCC1 Gln-Gln* had significantly higher SCE than those who had *XRCC1 Gln-Arg/Arg-Arg*. Similarly, workers from the low VCM-exposure group who featured *CYP2E1c2c2* had significantly higher SCE than those who had *CYP2E1c1c2/c1c1*. The relationship is less prominent in high exposure groups. Those with *ALDH2 1-2/2-2* genotypes revealed the higher SCE frequencies than those with *ALDH2 1-1* genotypes in both high and low VCM-exposure groups, although the relationships were not significant. However, no significant association between SCE frequency and the *GSTT1* genotype was found.

A multiple regression model (GLM) for SCE frequency as a function of age, smoking habit, alcohol consumption, VCM exposure, and genotypes of *XRCC1*, *CYP2E1*, *ALDH2*, *GSTT1* is shown in Table 4. In order to ensure the variation in different periods on SCE frequency, we also included a variable for the time periods (batch) of SCE assays in the GLM model. Duration of employment was excluded in this model because the variables of duration of employment and age had high colinearity ($r = 0.88$, $P < 0.01$). The frequency of SCE was positively associated with an age greater than 45 ($P < 0.01$), and smoking status ($P = 0.03$). Relative to controls, a mean difference of 1.0 SCE/cell was noted for individuals experiencing a high VCM exposure ($P = 0.03$). Workers classified into the low VCM-exposure group experienced a mean difference of 0.6 SCE/cell compared to controls ($P = 0.16$). Interestingly, greater frequencies of SCE were observed

**Table 4** Multiple regression model for frequencies of sister chromatid exchange per cell

| Variables | Regression coefficient | SE | *P*-value |
|---|---|---|---|
| Intercept | 6.59 | 0.32 | < 0.01 |
| Age: ≥45 versus < 45 (years) | 0.83 | 0.31 | < 0.01 |
| Smoking status: yes versus none | 0.56 | 0.26 | 0.03 |
| Alcohol drinking: yes versus none | −0.21 | 0.33 | 0.52 |
| VCM exposure | | | |
| High versus controls | 1.00 | 0.46 | 0.03 |
| Low versus controls | 0.60 | 0.42 | 0.16 |
| Genotyping | | | |
| *XRCC1*-exon 10: *Gln-Gln* versus *Arg-Arg/Arg-Gln* | 1.09 | 0.49 | 0.03 |
| *CYP2E1*: *c2c2* versus *c1c1/c1c2* | 1.54 | 0.72 | 0.04 |
| *ALDH2*: *1-2/2-2* versus *1-1* | 0.44 | 0.25 | 0.08 |
| *GSTT1*: null versus non-null | 0.12 | 0.25 | 0.63 |
| Batch: before 1997 versus after 1997 | 0.14 | 0.36 | 0.70 |

amongst workers revealing the *XRCC1 Gln-Gln* genotype ($P = 0.03$), the *CYP2E1 c2c2* genotype ($P = 0.04$), or the *ALDH2 1-2/2-2* ($P = 0.08$) genotype. However, alcohol consumption ($P = 0.52$), the *GSTT1* genotype ($P = 0.63$) and the experimental batch ($P = 0.70$) appeared not to influence the frequencies of SCE for individuals when examining the data using GLM analysis. In this model, the partial $R^2$-value for age was 4.7%, for smoking was 5.1%, for alcohol consumption was 0.3%, for high VCM exposure was 11.9%, for low VCM exposure was 12.5%, for *XRCC1* was 3.7%, for *CYP2E1* was 3.7%, and for *ALDH2* was 1.5%.

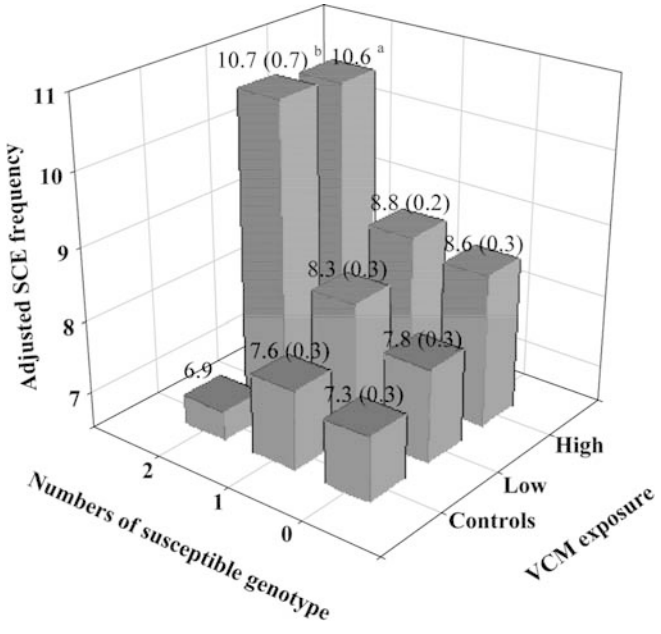


**Fig. 1** Adjusted mean sister chromatid exchange (SCE) frequencies (standard errors in parentheses) according to the numbers of susceptible *CYP2E1*, *ALDH2* and *XRCC1* genotypes displayed and the vinyl chloride monomer (VCM) exposure adjusted for age and smoking status. [a]Subjects with two or more susceptible genotypes showed a higher SCE frequency than those with one such genotype ($P = 0.12$) or no susceptible genotypes ($P = 0.09$) in the high VCM-exposure group. [b]Subjects with two or more susceptible genotypes also showed a higher SCE frequency than those with one such genotype ($P < 0.01$) or no susceptible genotypes ($P < 0.01$) in the low VCM-exposure group

Subsequently, a least-squares mean analysis was performed to assess the SCE frequency for study subjects expressing one or more susceptible *CYP2E1*, *ALDH2*, and *XRCC1* genotypes, and applying VCM exposure adjusted for age and smoking habits (Fig. 1). In the low and high VCM-exposure groups, individuals possessing no susceptible genotypes revealed the lowest mean SCE frequencies compared to all other test groups, namely 7.8 ($n = 14$) and 8.6 ($n = 11$), respectively, whereas those subjects reflecting one susceptible genotype featured the slightly greater SCE frequencies of 8.3 ($n = 12$) and 8.8 ($n = 20$), respectively, whilst those with two or more susceptible genotypes demonstrated the greatest mean SCE frequencies of 10.7 ($n = 3$) and 10.6 ($n = 1$), respectively. Amongst the control group, those presenting with no susceptible genotypes revealed a mean SCE frequency of 7.3 ($n = 13$), and those featuring one susceptible genotype, or those individuals demonstrating two or more susceptible genotypes reflected mean SCE frequencies of 7.6 ($n = 15$) and 6.9 ($n = 1$), respectively. In addition, the trend in SCE frequency with the numbers of susceptible genotypes was shown to be statistically significant at $P < 0.01$ (GLM) for the low VCM-exposure group, although no significant trend in SCE frequency with the numbers of susceptible genotypes was observed either for members of the high VCM-exposure group ($P = 0.29$) or for control subjects ($P = 0.49$).

## Discussion

In this study, the metabolic *CYP2E1*, *ALDH2*, and DNA-repair *XRCC1* genotypes, age and smoking habits, as well as high level of VCM exposure were significantly associated with SCE frequency.

The frequency of the *XRCC1* 399*Gln* allele (32.2%) in this study was consistent with the results of a previous study conducted upon Taiwanese subjects (26.0%; Lunn et al. 1999). The figure for the prevalence of the *CYP2E1 c2* allele noted in our study (22.2%) appears to be quite similar to that reported in a previous study, again conducted upon people of Taiwanese descent (20.3%; Yu et al. 1995). The frequency of the presence of the

*ALDH2*$^{1}$ allele (68.3%) was also comparable to that for the control groups of some literature-cited studies dealing with the investigation of alcoholism in the Taiwanese population (68–76%; Thomasson et al. 1991; Chen et al. 1996b). The prevalence of the *GSTT1* null-type genotype in our study (54.4%) appeared to be similar to that reported in a previous study, both for people of Chinese descent (64.4%; Nelson et al. 1995) and of Taiwanese descent (51.4%; Chen et al. 1996a). These findings, to some extent, corroborate the practice and results of our genotyping technique.

Importantly, an earlier animal study has revealed that VCM is metabolized by CYP2E1 to become CEO (el Ghissassi et al. 1998), which has the potential to spontaneously transform into CAA and then be metabolized by ALDH2 (Whysner et al. 1996). Both CEO and CAA are electrophiles ready to react with DNA bases to yield etheno adducts (Guengerich 1992). Certain DNA adducts have previously been found to be associated with SCE frequency (Ross et al. 1990; Wiencke et al. 1990). In our study, VCM-exposed workers reflecting the *CYP2E1 c2c2* genotype demonstrated higher SCE frequencies than those without such a genotype. VCM-exposed workers expressing the *CYP2E1 c2c2* genotype reflect a higher metabolic activity than those featuring the *CYP2E1 c1c1*/*c1c2* genotypes (Hayashi et al. 1991), and thus the former may be likely to experience elevated levels of genetic damage. We also observed an association between the presence of *ALDH2 1-2*/*2-2* genotype and an increase in SCE frequency. From an earlier study, those individuals who revealed the presence of the *ALDH2*$^{2}$ allele reflected a lower metabolic activity level than those possessing the *ALDH2*$^{1}$ allele (Crabb et al. 1989), and hence the former may experience the accumulation of CAA in their bodies. Previous studies have also shown that GSTs may act as detoxification enzymes by reacting with the epoxide product of many different chemicals (Chen et al. 1996a; Nelson et al. 1995). However, there was no significant association between SCE frequency and GSTT1 genotype in the current study, although an association between GSTT1 and VCM-induced abnormal liver function in VCM-exposed workers has been reported (Huang et al. 1997). Thus, more studies are needed to determine the role of GSTT1 in VCM-related genotoxicity. Although GSTM1 has been reported to play a role in metabolizing epoxides of some compounds, our previous studies were unable to demonstrate any association between GSTM1 genotype and SCE frequency (Wong et al. 1998) or liver function (Huang et al. 1997) alteration in VCM-exposed workers. Thus, GSTM1 was not included in the analysis.

Several studies have already revealed the association between *XRCC1* and SCE frequency induced by alkylating agents (Zdzienicka et al. 1992) or by cigarette smoke (Duell et al. 2000; Lei et al. 2002). In this study, we also demonstrated that *XRCC1* plays an important role in the genotoxicity elicited by VCM. Similar to metabolic genes, the presence of the *XRCC1 Gln-Gln* genotype was observed to be associated with higher SCE frequencies than when the *XRCC1 Arg-Gln* or *Arg-Arg* genotype was present.

Our previous study also observed that an increased cumulative VCM exposure dose was associated with increased plasma p53 oncoprotein and anti-p53 antibody. In the same study, *CYP2E1* and *XRCC1* genes were shown to modulate this p53 manifestation among VCM-exposed workers, while the effects of *ALDH2* and *GSTT1* were not significant (Wong et al. 2002b). Both SCE and *p53* represent genetic damage. The consistent results indicate that metabolic and DNA-repair genes may modulate VCM-induced genotoxicity, particularly the *CYP2E1* and *XRCC1* genes.

Further analysis of *XRCC1* and other VCM-related metabolic genes, including *CYP2E1* and *ALDH2*, together revealed an increasing trend for these genotypes to reflect as an increase in SCE frequency for individuals from the low VCM-exposure group. This trend was not significant in the high VCM-exposure group probably due to a small number of subjects with susceptible genotypes. This might also be explained, from a metabolic point of view, by the fact that at high dose levels of VCM the relevant VCM-metabolizing enzyme becomes saturated for both rapid and slow metabolizers. This concept is supported by a previous study of rats, from which the metabolism of VCM at low concentrations was found to follow first-order kinetics and is perfusion-limited, reaching a plateau with increasing concentrations of VCM (Filser and Bolt 1979). Further, *CYP2E1* is involved in the activation of VCM, *ALDH2* acting as a detoxifying enzyme for reactive metabolites of VCM, whilst *XRCC1* is involved in the subsequent DNA repair process. This also illustrates that VCM-exposed subjects who carry susceptible metabolic and/or DNA-repair genotypes are more likely to demonstrate an increased level of genotoxicity.

The limitation of this study is the small number of subjects with susceptible genotypes. We have tried to increase the number of susceptible genotypes in each cell by grouping *XRCC1 Gln-Gln* and *Gln-Arg*, and *CYP2E1 c2c2* and *c1c2*, respectively. However, SCE frequencies in subjects with at least one *XRCC1 Gln* or *CYP2E1 c2* were not higher than those without susceptible gene alleles. It is not surprising for *CYP2E1* because *CYP2E1 c1c2* has much lower enzyme activity than *CYP2E1c2c2* (Hayashi et al. 1991). The relative enzyme activity for homozygous *XRCC1 Gln-Gln* and heterozygous *XRCC1 Gln-Arg* is not clear. Our recent study conducted in smokers also observed that the *XRCC1 Gln-Gln* genotype significantly modified the smoking-related SCE frequency, while *XRCC1 Gln-Arg* did not (Lei et al. 2002). We also had similar findings in our previous study conducted in VCM-exposed workers. *CYP2E1 c2c2* and *XRCC1 Gln-Gln* modified the relationship between cumulative VCM doses and plasma p53 mutant protein (Wong et al. 2002b). Thus, results using either SCE or p53 as outcome indicators gave a similar finding on the role of *XRCC1 Gln-Gln* and *CYP2E1c2c2* on VCM carcinogenesis.

Our study has found that smoking and age are associated with an increased SCE frequency. Many studies have also shown that smoking can cause an increased SCE frequency (Hedner et al. 1982; Sarto et al. 1985; Cheng et al. 1999; Duell et al. 2000). Cigarette smoke contains a variety of known carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aldehyde derivates, and vinyl chloride (Hoffmann et al. 1976). Thus, smoking is generally believed to be a strong inducer of SCE. In addition, it has previously been reported that there is a tendency amongst older individuals to show a higher SCE frequency than is the case for younger individuals (Hedner et al. 1982; Sarto et al. 1985). The observed increase in level of SCE amongst older subjects could be either due to the presence of cumulative damage caused by long-term exposure to unidentified carcinogens and/or mutagens, or from other factors related to cellular aging such as the relative integrity of the DNA repair process. In addition, an increased SCE frequency has reported in chronic alcoholics (Butler et al. 1981), and alcohol consumption has been reported to generate reactive oxygen species presumably through the effect of cytochrome P450 2E1 (Bailey and Cunningham 1998; Mali et al. 2001). However, the effect of alcohol on SCE frequency was not observed in our study. This was probably due to a small range in the level of alcohol consumption.

Age and smoking have been reported to account for about 30% of the variance in SCE frequency (Sarto et al. 1985; Hirsch et al. 1992). In this study, age and smoking accounted for about 10% of the variation, while VCM exposure contributed 20%. Since VCM-exposed workers were older and tended to be smokers, the effects between age, smoking and VCM exposure cannot be separated. Interestingly, genotypes explained about 10% of the variation in SCE. A previous study also showed that genetic factors accounted for about 30% of the variation in SCE frequency (Hirsch et al. 1992). It will be intriguing to further investigate whether other genotypes contribute to the unexplained variation in SCE frequency.

The DMF-exposed workers and the VCM-exposed workers participating in this study were employed by the same plastics manufacturing company and they reflected a similar socioeconomic status. Thus, workers exposed to DMF are considered to be comparable to VCM-exposed workers, although DMF-exposed workers were younger than VCM-exposed workers in this study. In addition, we performed the cell cultures for SCE assay over two separate periods of time, and thus the potential for an influence on the results arising from a ''batch effect'' could not be totally excluded. However, the cultures were performed in the same laboratory and followed an identical protocol, and our model analysis indicated that the seasonal culture variation in SCE frequency was very small.

In summary, our results suggest that both metabolic and DNA-repair genes play a role in VCM-induced carcinogenesis. Further study to investigate the relationship of metabolic and DNA-repair genes with liver cancer is warranted.

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