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• *BASIC RESEARCH* •

Evaluation in vinyl chloride monomer (VCM)-exposed workers and the relationship between liver lesions and gene polymorphisms of metabolic enzymes

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Abstract

AIM: The permissible exposure limit (PEL) of vinyl chloride monomer (VCM) in developed country was 1 p/m (2.79 mg/m^3); and threshold limit value-short term exposure limit (TLV-STEL) in China was 11 times higher [11 p/m (30 mg/m^3)] than it, till 2002. The mechanism of vinyl chloride monomer (VCM)-related carcinogenesis remains unclear. We aimed to analyze occupational health hazards exposure to doses lower than the Chinese occupational health standard in a selected VC polymerization plant in China, and also to elucidate the relationship between genetic polymorphisms and genetic susceptibility on liver lesions of workers exposed to VCM.

METHODS: In order to explore the mechanism of VCM-related health effects, we used a case-control design to investigate the association between the genetic polymorphisms of metabolic enzymes and liver lesions in workers occupationally exposed to VCM. Genotypes of *CYP2E1*, *GSTT1, GSTM1, ALDH2* and *ADH2* were identified using PCR and PCR-RFLP.

RESULTS: Even when the concentration of VCM was lower than the current Chinese occupational health standard, neurasthenia, pharyngeal irritation, liver ultrasonography abnormalities and hemoglobin disorders were significantly higher in exposure subjects compared to non-exposure subjects, and the relative risks (*RR* and 95% *CI*) were 1.74 (1.06-2.85), 1.97 (1.56-2.48), 10.69 (4.38-26.12), and 2.07 (1.20-3.57). *CYP2E1 c1c2/c2c2* genotype was significantly associated with liver damages (*OR* 3.29, 95% *CI* 1.51-7.20, *P*<0.01).

CONCLUSION: The incidences of neurasthenia and liver ultrasonography abnormalities significantly increase when the cumulative exposure dose increases. The genotypes of metabolic enzymes (*CYP2E1 c1c2/c2c2*, null *GSTT1* and *ADH2 1-1*) play important roles in VCM metabolism. Polymorphisms of *CYP 2E1*, *GSTT1* and *ADH2* may be a major reason of genetic susceptibility in VCM-induced hepatic damage.

Key words: Vinyl chloride monomer; Hepatic lesions; Cytochrome p450 2E1; Glutathione S-transferase; Aldehyde dehydrogenase-2

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INTRODUCTION

Vinyl chloride monomer ($CH_2=CHCl$, VCM) is a colorless gas and a synthetic chemical that does not naturally occur. It is also known as monochloroethylene and chloroethene. At normal temperature it has a mild, sweet odor. Because VCM is easily polymerized to polyvinyl chloride, it is widely used in industry primarily. In fact, in the seventies of last century, 95% of the vinyl chloride produced was polymerized, and the rest is used in the formation of other chemicals. VCM was found to be easily absorbed through the respiratory tract, where it passed into the blood. Most studies concluded that the target organs for the VCM action are the liver, brain, lung, lymphohematopoietic system and skin, and hepatic lesion is its characteristic manifestation[1-3]. In 1974, the US National Institute of Occupational Safety and Health and US Centers for Disease Control and Prevention (CDC), reported that several deaths at one of their plants in a chemical company could be related to occupational exposures of vinyl chloride[4,5]. VCM has been classified by the International Agency for Research on Cancer (IARC) as a Group I carcinogen[6]. Since vinyl chloride is a known carcinogen, it has caused great concern to people who may be exposed to it.

PVC manufacture is a major industry in China, with total annual production of two or three million tons till now. In 1970's, China began to produce PVC, the output increased from 780 000 tons in 1990 to nearly 2 400 000 tons in 2000, and the figure will increase to 3 million tons

in the near future. Thousands of workers could be exposed to VCM. The permissible exposure limit (PEL) of VCM in developed country was 2.79 mg/m^3, and in China which was 11 times higher (30 mg/m^3) than it till 2002. Some studies of health hazards of workers exposure to VCM in China had been done. The results of these studies showed a high prevalence of abnormal liver function and neurasthenia as well as other symptoms due to exposure to VCM[7,8], and more tumors in VCM-exposed workers can be expected in future. However, the workers studied in the aforementioned studies were exposed to VCM at levels higher than the Chinese PEL. There is a general lack of information on estimates of health effects of workers exposed to lower levels of VCM exposure.

Earlier studies were more concerned with exposure levels and effects of vinyl chloride. More recently, there have been studies done on how vinyl chloride reacts in the body leading to cancer, both how it is bioactivated and how it interacts with DNA and forms DNA adducts. An earlier animal study has reported that VCM is primarily metabolized in the liver by CYP2E1 into active chloroethylene oxide (CEO), some of the CEO reacts to form adducts, 7-(2-oxoethyl) guanine. This adduct has been found in the most abundance, but it has not shown mispairing during DNA replication. Since CEO is unstable, it rearranges to chloroacetaldehyde (CAA)[9]. Once CAA is formed, it reacts with adenine, cytosine and guanine to form adducts. Reactions with guanine are less frequent than adenine and cytosine. There are four known etheno-adducts: 1, N^6-ethenoadenine (εA); 3, N^4-ethenocytosine (εC); N^2, 3-ethenoguanine (N^2, 3-εG); and 1, N^2 ethenoguanine (1, N^2 εG)[10]. These adducts contain an additional five-membered ring system. Although these adducts are known to be formed, it is still unclear which one induces errors during DNA and RNA synthesis, for both of which may be reactive with DNA to form DNA adducts[11], and are mutagenic in bacterial systems and mammalian cells[12].

Most of population-attributable cancer heritability is related not to the rare deleterious gene defects but to polymorphic variations in the DNA sequence. CYP2E1 has already been reported to be associated with lung cancer in smokers[13]. In a recent study performed in the Caucasian population, CYP2E1 can account for only a small proportion of the variability in mutagenic response to VCM exposure[14]. Furthermore, CEO may then be metabolized by GSTs, and simultaneously CAA may be metabolized by ALDH2 and ADH2. Thus, those with appropriate types of ALDH2, ADH2, and GSTs may also have elevated reactive intermediates, which can lead to increased liver damages. The metabolism of VCM is mostly concerned with the enzymes CYP2E1,GSTT1, GSTM1, ALDH2 and ADH2. Several studies have shown that all the enzymes exhibit their genetic polymorphisms[15-17], thereby causing different biological effects of metabolizing enzymes.

We aimed to analyze occupational health hazards exposure to doses lower than the Chinese occupational health standard in a selected VC polymerization plant in China, and provide information whether occupational health standard of VCM exposure needs to be revised in China, and also to elucidate the relationship between genetic polymorphisms and genetic susceptibility on liver lesions of workers exposed to VCM.

MATERIALS AND METHODS

Study subjects and epidemiological data

Information was collected from workers at the VC polymerization plant by using interviewer-administered questionnaires, subsequent to informed consent having been obtained during the medical surveillance process. The structured questionnaire contained questions that covered demographic characteristics, lifestyles, including cigarette-smoking habits and alcohol consumption, as well as compiling a detailed occupational history. Individuals who smoke once a day for over 6 mo were defined as smokers, and individuals who consumed once or more alcohol drinks a week for over 6 mo were considered drinkers.

Study subjects exposed to VCM for a period of >1 year in the plant were selected if the following criteria were met: detailed questionnaires had been completed; the hepatitis B, C viruses' status were known; and a blood sample could be provided. At final tally, a total of 163 male and 75 female workers in the industry, with an average age of 33 years (range, 19-54 years) and an average exposure time of 7 years, and who had been occupationally exposed to VCM were included for analysis. One hundred and twenty four male and 88 female employees, with an average age of 38 years (range, 23-60 years), were selected as the non-exposure group, who worked in the same industry as the exposure subjects but not occupationally or environmentally exposed to VCM previously. The workers infected with hepatitis B, C viruses were excluded from the study because hepatitis B, C also leads to liver lesions.

Based on the results of medical examination, we divided the workers in the VCM exposure group into two groups. Fifty eight workers were selected as "liver lesion group" for their liver ultrasonography abnormality and/or alanine aminotransferase (ALT) >40 for at least 2 years and did not get acute hepatitis, chronic hepatitis and cholelithiasis. The "control subjects" were employees who worked at the same worksites as the liver damage group and also had exposure to VCM. Each control was matched for gender, duration of work, worksite, and cumulative exposure dose.

Assessment of vinyl chloride exposure

Exposure levels of VCM in the plant were based on existing environmental monitoring data. The plant kept VCM air concentration data, and the data were generated at different worksites since the beginning of its establishment. The level of VCM was calculated at different worksites of the company, which ranged from 0.3 p/m (0.85 mg/m^3) to 17.8 p/m (48.41 mg/m^3) in air, with a geometric average concentration was 2.6 p/m (7.11 mg/m^3). And then the cumulative exposure dose of each worker was estimated relatively exact. The following equation was used to calculate cumulative exposure dose: Cumulative exposure dose (mg) = Σ (C * M * T) * A* 70%/10^6, where C (mg/m^3): Geometric mean of VCM exposure concentration each year in a special workplace, calculated for all different worksites; M: number of exposure months of each year for a VCM worker; T: 2 h exposure time at each working day, 20 d in each month (exposure time per month was 2 400 min); and A: alveolar ventilation (male average: 6 500 mL/min, female average: 4 300 mL/min, 30% dead space).

Cumulative exposure dose in VCM exposure group ranged from 1 047.41 mg to 33 357.11 mg. Then the VCM exposure subjects were divided into high-exposure and low-exposure groups. The individuals placed into the high-exposure group had been exposed to a cumulative dose of >15 000 mg and those in the low-exposure group with their cumulative dose of ≤15 000 mg.

Genotyping of polymorphic metabolic enzymes

GSTT1 and *GSTM1* genotypes were determined by co-amplification of two genes as previously described[18,19]. Amplification of human *β-globin* (350 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'-GCC CTC TGC TAA CAA GTC CTA C-3' and 5'-GCC CTA AAA AGA AAA TCG CCA ATC-3'. Individuals with one or more *GSTT1* alleles had a 459-bp fragment and individuals with one or more *GSTM1* alleles had a 350-bp fragment (Figure 1A).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was applied to detect polymorphisms of the *CYP2E1*[20]. The primers used for *CYP2E1* were 5'-TTC ATT CTG TCT TCT AAC TG-3' and 5'-CAG TCG AGT CTA CAT TGT C -3'. The PCR products were digested with restricted endonuclease *P*st 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 the three fragments (Figure 1B).

The *ALDH2-Mbo*II polymorphism was determined by a modification of the methods developed by Harada and Zhang[21]. 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'. 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 of the fragments (Figure 1C).

According to Chen *et al*.[22], the sequences of *ADH2* primers were 5'- AAT CTT TTC TGA ATC TGA ACA G-3' and 5'- GAA GGG GGG TCA CCA GGT TGC-3', and the PCR products were digested with *Mae* III. Homozygous *1-1* individuals demonstrated a single product fragment of 95 bp, whereas homozygous *2-2* individuals revealed both 60- and 35-bp fragments, and heterozygous *1-2* individuals exhibited all three of the fragments (Figure 1D).

Statistical analysis

SPSS 10.0 version and Epi-info 6.0 software packages were used for statistical analysis. Maentel-Haenszel χ^2-test was used to compare the differences between basic characteristics of the non-exposure and exposure group. Stratified analyses were used to explore the correlation between liver damages and genotypes of *CYP2E1, GSTT1, GSTM1, ALDH2* and *ADH2* among the workers exposed to different levels of VCM. Logistic regression model was used to select the best model and to estimate the *OR* of related risk factors. A *P* value less than 0.05 was considered statistically significant.

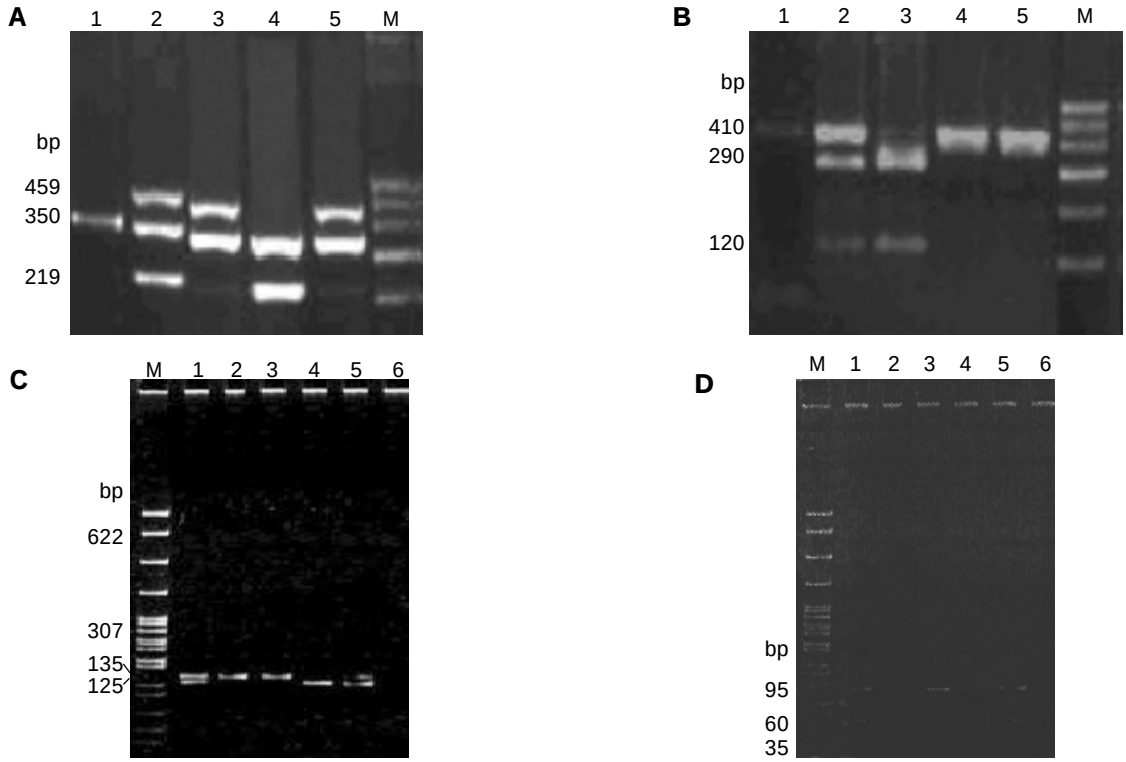

Figure 1 PCR products A: PCR products of GSTT1 and GSTM1. M: DNA marker; lanes 1-5 DNA products; lane 1: none; lane 2: GSTT1 and GSTM1; lane 4: GSTM1; lanes 3, 5: GSTT1. B: PCR-RLFP products of CYP2E1. M: DNA marker; lanes 1-5 DNA products. Homozygous c1c1 shows a single band with 410 bp; homozygous c2c2 shows two bands with 290 bp and 120 bp; and heterozygous c1c2 shows all three bands. C: PCR products of ALDH2. Lanes 1-6 DNA products; M: DNA marker. Homozygous 2-2 shows a single band with 135 bp; homozygous 1-1 shows two bands with 125 bp and 10 bp; and heterozygous 1-2 shows all the three bands. D: PCR products of ADH2. Lanes 1-6: DNA products; M: DNA marker. Homozygous 1-1 shows a single band with 95 bp; homozygous 2-2 shows two bands with 60 bp and 35 bp; and heterozygous 1-2 shows all the three bands.

RESULTS

Comparison of basic characteristics and clinical examinations between exposed and non-exposed groups

No significant difference was observed in age, duration of work, smoking, and drinking between the exposure and non-exposure subjects. All the standards of medical examination were selected as Chinese clinical diagnostic standards. There were significant differences in neurasthenia ($P<0.05$), pharyngeal irritation ($P<0.01$), liver ultrasonography abnormalities ($P<0.01$) and hemoglobin disorders ($P<0.05$) between the exposure and non-exposure groups, and relative risks were 1.74 (1.06-2.85), 1.97 (1.56-2.48), 10.69 (4.38-26.12), and 2.07 (1.20-3.57), respectively. Furthermore, abnormal ECG ($P<0.05$, *RR* 0.51, 95% *CI* 0.32-0.83) and fatty liver ($P<0.05$, *RR* 0.42, 95% *CI* 0.20-0.88) were found significantly higher in non-exposure subjects as compared to exposure subjects. Other symptoms and signs did not show any statistical difference (Table 1).

Stratified analysis of symptoms and signs caused by different cumulative exposure doses in exposure group

Trend chi-square test was used to compare symptoms and signs between the high-exposure and low-exposure groups. There were significant differences in neurasthenia, liver ultrasonography abnormality and pharyngitis between the high-exposure and low-exposure groups ($P<0.05$), with the chi-square values of 6.41, 5.33 and 4.56, respectively. Their incidences increased when the cumulative exposure dose increased (Table 2). However, the other symptoms and signs did not show any statistical difference.

Univariate analysis of genotypes of metabolic enzymes and variables for liver lesions in case-control design

Univariate analysis showed that *CYP2E1 c1c2/c2c2* genotype was significantly associated with liver damages (*OR* 3.29, 95% CI 1.51-7.20, $P<0.01$). Moreover, our results showed that the older or drinking subjects suffered from a higher frequency of liver damages but without statistical significance (OR 2.10, 95% *CI* 0.73-3.43, and *OR* 2.48, 95% *CI* 0.80-7.66). However, other factors did not show any association with liver damages (Table 3).

Correlation between cumulative exposure dose and genotypes of metabolic enzymes in the case-control design

ORs of liver lesions were calculated to investigate the joint effect of VCM exposure and genotypes. *CYP2E1* c1c2/c2c2 genotype was significantly associated with liver lesions (*OR* 4.6, 95% *CI* 1.4-15.1, $P<0.05$) in high-exposure group; and in low-exposure group, the distribution of non-null *GSTT1* was higher in the control group compared to liver lesion group (*OR* 0.3, 95% *CI* 0.1-0.9, $P<0.05$), and *ADH2 1-2/2-2* genotype was the same (*OR* 0.2, 95% *CI* 0.1 -1.0, $P<0.05$).

Table 1 Prevalence of symptoms and signs between exposure and control groups

Symptoms and signs	Exposure group	Control group	Relative risk (RR)
	n = 238(%)	*n* = 212(%)	
Neurasthenia[a]	40 (16.81)	21 (9.91)	1.74 (1.06-2.58)
Pharyngeal irritation[b]	138 (57.98)	63 (29.72)	1.97 (1.56-2.48)
Abnormal ECG[a]	24 (10.08)	40 (18.87)	0.51 (0.32-0.83)
Cholelithiasis	6 (2.52)	7 (3.30)	0.76 (0.26-2.24)
Renal cyst	2 (0.84)	3 (1.42)	0.59 (0.10-3.52)
Liver ultrasonography abnormality[b]	51 (21.41)	5 (2.36)	10.69 (4.38-26.12)
Fatty liver[a]	10 (4.20)	21 (9.91)	0.42 (0.20-0.88)
Hemoglobin disorders[a]	46 (19.33)	22 (10.38)	2.07 (1.20-3.57)
Hypertension	11 (4.62)	15 (7.07)	0.64 (0.29-1.42)
Hepatic hemangioma	3 (1.26)	3 (1.42)	0.89 (0.18-4.37)
Leucopenia	4 (1.68)	1 (0.47)	3.61 (0.40-32.53)

[a]$P<0.05$ *vs* control group; [b]$P<0.01$ *vs* control group.

Table 2 Prevalence of symptoms and signs among VCM-exposed workers stratified by VCM exposure

Symptoms and signs	VCM exposure					
	≤15 000 mg (*n* = 186)		>15 000 mg (*n* = 52)		Total (*n* = 238)	
Neurasthenia[a]	26	14.0%	14	26.92%	40	16.81%
Respiratory system	3	1.6%	1	1.92%	4	1.68%
Digestive system	4	2.15%	1	1.92%	5	2.10%
Pharyngitis[a]	104	55.9%	34	65.38%	138	57.98%
ECG	19	10.21%	5	9.61%	24	10.08%
Nephridium USG	1	0.53%	2	3.85%	3	1.26%
Gall bladder USG	4	2.15%	4	7.69%	8	3.36%
Liver USG[a]	34	18.3%	17	32.69%	51	21.43%
Fatty liver	7	3.76%	3	5.77%	10	4.20%
Hepatic hemangioma	1	0.53%	2	3.85%	3	1.26%
Splenomegaly	8	4.30%	2	3.85%	10	7.08%

[a]$P<0.05$ *vs* ≤ 15 000 mg group.

Table 3 Liver lesions status according to genotypes and variables of interest in VCM exposure group

	VCM exposure group		Adjusted *OR* (95%*CI*)
	Control (n = 58)	Liver lesions (n = 58)	
GSTT1			
Non-null	31 (53.4%)	37 (63.8%)	1.0 (reference)
Null	27 (46.6%)	21 (36.2%)	0.65 (0.31-1.37)
GSTM1			
Non-null	37 (63.8%)	36 (62.1%)	1.0 (reference)
Null	21 (36.2%)	22 (37.9%)	1.08 (0.51-2.29)
CYP2E1			
c1c1	43 (74.1%)	27 (46.6%)	1.0 (reference)
c1c2/ c2c2	13/2 (25.8%)	24/7 (53.4%)	3.29 (1.51-7.20) [b]
ALDH2			
1-1	37 (63.8%)	36 (62.1%)	1.0 (reference)
1-2/2-2	17/4 (36.2%)	17/5 (37.9%)	1.08 (0.51-2.29)
ADH2			
1-1	4 (6.9%)	9 (15.5%)	1.0 (reference)
1-2/2-2	24/30 (93.1%)	22/27 (84.5%)	0.40 (0.12-1.39)
Age (yr)			
<35	41 (70.7%)	35 (60.3%)	1.0 (reference)
⩾35	17 (29.3%)	23 (46.6)	2.10 (0.76-3.43)
Drinking			
No	53 (91.4%)	47 (81.0%)	1.0 (reference)
Yes	5 (9.6%)	11 (19.0%)	2.48 (0.80-7.66)
Smoking			
No	40 (69.0%)	41 (71.7%)	1.0 (reference)
Yes	18 (31.0%)	17 (29.3%)	0.92 (0.42-2.04)

[b]P<0.01 *vs* control.

The distribution of other genotypes did not have significant difference (Table 4).

Logistic regression analysis of liver lesions of workers exposed to VCM

Logistic regression analysis was employed to model the relationship between liver damages and genotypes of metabolic enzymes and related indicator among VCM workers. We observed a significant association between *CYP2E1 c1c2/ c2c2* and liver lesions (P<0.001, *OR* 3.173, 95%*CI* 1.329-7.572) and drinking (P = 0.10, *OR* 3.10, 95%*CI* 0.78-12.64), which might be associated with increased OR on liver lesions. But, we could not found any association between liver damages and age, duration of work, smoking and gender (Table 5).

Table 5 Logistic regression analysis of liver lesions

	Logistic regression analysis		
	Coefficient	P	*OR* (95% *CI*)
Age (yr)	0.47	0.33	1.60 (0.62-4.19)
Duration of work	0.24	0.60	1.27 (0.52-3.12)
Drinking	1.14	0.10	3.14 (0.78-12.64)
Smoking	-0.90	0.13	0.41 (0.13-1.29)
Gender	-0.06	0.91	0.94 (0.36-2.47)
GSTT1	-0.51	0.23	0.60 (0.26-1.39)
GSTM1	-0.15	0.75	0.86 (0.36-2.10)
CYP2E1	1.16	0.009[b]	3.17 (1.33-7.57)
ALDH2	0.09	0.84	1.09 (0.46-2.58)
ADH2	-0.72	0.31	0.49 (0.12-1.96)
Constant	0.24	0.84	

[b]P<0.01 *vs* control.

DISCUSSION

Several studies have proved VCM as a human carcinogen, and hence VCM exposure has strictly been regulated in many countries. In the USA, the Occupational Safety & Health Administration (OSHA) has adopted a permissible exposure limit (PEL) of 1 p/m for VCM as an 8 h time-weighed average (TWA). Taiwan has also adopted 1 p/m as the health standard. In many developing countries, for some reasons, they have not restricted the emissions of VCM to satisfy this standard. Therefore, their occupational health standard for VCM is still at a relatively higher level, for example, in China, the maximum allowable dose was 25 mg/m^3 which was 9-fold higher than that in the advanced countries (Since June 2002 the TWA has been revised to 10 mg/m^3 in China, and the

Table 4 ORs for analysis of correlation between exposure and genotypes

Genotypes	Cumulative VCM exposure					
	⩽15 000 mg			>15 000 mg		
	Control	Liver lesion	*OR* (95% *CI*)	Control	Liver lesion	*OR* (95%*CI*)
GSTT1						
Non-null	15	23	1.0	16	14	1.0
Null	17	9	0.3 (0.1-0.9)[a]	10	12	1.4 (0.5-4.1)
GSTM1						
Non-null	21	21	1.0	16	15	1.0
Null	11	11	1.0 (0.4-2.8)	10	11	1.2 (0.4-3.6)
CYP2E1						
c1c1	23	16	1.0	20	11	1.0
c1c2/ c2c2	9	16	2.6 (0.9-7.2)	6	15	4.6 (1.4-15.1)[a]
ALDH2						
1-1	20	22	1.0	17	14	1.0
1-2/2-2	12	10	0.8 (0.3-2.1)	9	12	1.6 (0.5-5.0)
ADH2						
1-1	2	8	1.0	2	1	1.0
1-2/2-2	30	24	0.2 (0.1-1.0)[a]	24	23	2.1 (0.2-24.5)

[a]P<0.05 *vs* ⩽ 15 000 mg group.

maximum allowable dose modified to 25 mg/m^3).

In this study, the level of VCM was lower than National Occupational Health Standard of China, but the incidences of neurasthenia, pharyngeal irritation and liver USG abnormality in the exposure group were still higher compared to non-exposure group, which indicated that even the concentration is around 9 p/m, we should also pay attention to the damage induced by VCM. By modeling the relationship between the symptoms and cumulative exposure dose, the results showed that when cumulative exposure dose increased, the incidence of the symptoms increased in accordance.

We found that, under the same working environment and exposure to the same level of VCM, not all but only some workers in VCM-exposure group had got liver damages, which indicated that there are genetic susceptible differences among the workers. Then we used case-control design to investigate the association between the gene polymorphisms of metabolic enzymes and liver lesions.

VCM is metabolized to CEO (chloroethylene oxide) and chloroacetaldehyde (CAA) by *CYP2E1*, which bind to macromolecular surface and form the electrophilic metabolites that can induce liver lesions, even worsen to hepatic angiosarcoma. A study showed the efficiency of conversion of c2 was higher than c1[23]. Studies by Huang *et al.*[24], and Li *et al.*[14], showed that the frequency of the *CYP2E1* c2c2 genotype was higher in the case group compared to the control group, but the degree of *CYP2E1* necessary to influence genetic susceptibility due to exposure to VCM was different between the Chinese and the Caucasian population. In our study, we found that the frequency of *CYP2E1 c1c2/c2c2* was higher in liver lesions group than that in controls. Stratified by cumulative exposure dose, in high dose group, the *CYP2E1 c1c2/ c2c2* genotype had higher frequency in liver lesions group than that in controls, indicating that *CYP2E1 c1c2/c2c2* genotype might be the main cause of genetic susceptibility of liver damage, which is consistent with previous reports.

All the metabolizing products are further metabolized and detoxified by GSTs; VCM metabolites can also directly bind to glutathione (GSH) after catalyzation by GSTs. This investigation showed that in liver lesions group, the frequency of the *GSTT1*-null genotype was 63.8%, which was higher than 47.4% as reported by Taiwan scholars[24]. And the frequency of the *GSTM1*-null genotype was 62.1%, which was consistent with 60.5% as reported by other scholars[23,24]. The study manifested that *GSTT1* non-null genotype protected the workers from exposure to low level VCM, but another study indicated that *GSTT1* non-null genotype could form reactive metabolites and produce adverse effects[25], which resulted in liver damages. However, we could not see any correlation between *GSTM1* and liver damage.

CEO may spontaneously transform into CAA, which may be subsequently metabolized by *ALDH2*. Although individuals with variant *ALDH2*2* allele have a lower enzyme activity, polymorphism of *ALDH2* has been reported to be associated with alcoholism[26]. Previous studies manifested that CEO produced DNA alkylating agent but CAA could not, suggesting that *ALDH2* had little effect on liver damage[27]. Our study also supports these results.

We found that *ADH2* genotypes *ADH2 1-2/2-2* and *1-1* were significantly different between liver lesions and controls in low-exposure group, which might be because the metabolized efficiency of *ADH2*2* was higher than *ADH2*1* [28]. Moreover, the incidence of liver damage in *ADH2 1-2/2-2* might be lower than *1-1*, if they were exposed to the same concentration of VCM.

The workers in our control group might have some potential VCM exposure based on environmental monitoring in the factory. However, our multivariate adjustment corroborates the possible mechanisms of VCM metabolism and carcinogenesis. In conclusion, VCM workers exposed to high concentration of VCM and with *CYP2E1 c1c2/c2c2* genotype may have a higher risk of liver lesions, and the incidence of liver damage in *ADH2 1-2/2-2* maybe lower than *1-1*, if they were exposed to the same concentration of VCM.

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