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Effect of the *CYP2E1* genotype on vinyl chloride monomer-induced liver fibrosis among polyvinyl chloride workers

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Abstract

Although a relationship between vinyl chloride monomer (VCM) and liver cirrhosis has been reported, the underlying mechanisms are not clear. Cytochrome P450 2E1 (CYP2E1), aldehyde dehydrogenase 2 (ALDH2) and glutathione *S*-transferase theta 1 (GSTT1) enzymes are involved in activation and detoxification of VCM, and thus may be important determinants of interindividual susceptibility to VCM-induced liver damage, including liver cirrhosis. The objective of this study was to evaluate if metabolizing genetic polymorphisms could modify individual susceptibility to liver fibrosis of the VCM exposure. *CYP2E1*, *ALDH2*, and *GSTT1* polymorphisms were determined by the PCR-RFLP method among 320 workers who were employed in five polyvinyl chloride manufacturing plants. Cumulative VCM exposure levels for study subjects were calculated using a job exposure matrix model.

Thirteen workers were diagnosed as having liver fibrosis by using ultrasonography. We observed a dose–response trend between VCM exposure and liver fibrosis. Regarding the results on genetic polymorphisms, *CYP2E1 c2c2* genotype showed a significant increase in the risk of liver fibrosis as compared to those with *CYP2E1 c1c1* or *c1c2* genotypes. No differences were observed between *GSTT1* and *ALDH2* genotypes and liver fibrosis. In summary, our result suggests that genetic polymorphism in *CYP2E1* may be responsible for individual differences in susceptibility to liver fibrosis with regard to chronic VCM exposure. Thus, polymorphism analysis of metabolizing enzymes might be useful in the risk assessment of liver damage in workers with VCM exposure.

Keywords: Occupational exposure; Vinyl chloride monomer; Liver fibrosis; Metabolizing genetic polymorphisms

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1. Introduction

Vinyl chloride monomer (VCM, CAS No. 75-01-4), the main material used in the polymerization process of polyvinyl chloride (PVC), is a human carcinogen, according to the classification of International Agency for Research on Cancer (IARC, 1987). Occupational exposure to VCM occurs mainly via inhalation during the manufacturing process, especially when workers are involved in unloading PVC or VCM, adding catalyst, transportation and cleaning tanks (Du et al., 1996). Previous studies confirmed a causal relationship between occupational exposure to VCM and the development of liver angiosarcoma. In cases of liver angiosarcoma, there was extensive cirrhosis of a non-alcoholic type in addition to angiosarcoma (CDC, 1997). PVC manufacturing workers were found to be at greater risk of liver cirrhosis as compared with control subjects (Du and Wang, 1998; Mastrangelo et al., 2004). Advanced liver fibrosis results in cirrhosis, and our previous study also observed the association between VCM exposure and liver fibrosis after controlling the effects of potential confounders (Hsiao et al., 2004). Although the association between VCM exposure and liver fibrosis has been established, the underlying mechanisms remain unclear.

Metabolic polymorphisms which were involved in activation and detoxification of chemicals have been implicated in the interindividual susceptibility of health outcome. An earlier animal study noted that VCM is primarily metabolized in the liver by cytochrome P450 2E1 (CYP2E1) into chloroethylene oxide (CEO) and chloroacetaldehyde (CAA) (el Ghissassi et al., 1998), both of which may be reactive with DNA to form DNA adducts (Guengerich, 1992). Research has also found that reactive intermediates bind in the liver to proteins, DNA, RNA and lipids, thus eventually impairing liver cell function, inducing cytotoxicity and causing cell necrosis (ATSDR, 2006; Guengerich and Watanabe, 1979). Reactive intermediates were proposed to activate hepatic Kupffer cells and stellate cells, thus eventually leading to liver fibrogenesis (Mastrangelo et al., 2004; Prodan et al., 1975). Recently, liver fibrogenesis was also proposed to be a secondary effect of the initiation of immune responses to cytotoxicity and cell necrosis when protein adducts expressed on the liver cell membrane as neo-antigens (ATSDR, 2006; Robin et al., 1997). Further, those metabolizing enzymes for reactive metabolites of VCM such as aldehyde dehydrogenase 2 (ALDH2) and glutathione *S*-transferase theta 1 (GSTT1) may modulate the formation of these DNA adducts or affect the function of liver cells (Whysner et al., 1996).

Our previous study revealed that *CYP2E1* c2c2 and *GSTT1* positive genotypes were associated with VCM-induced abnormal liver function (Huang et al., 1997). One of our studies found that genotypes of *CYP2E1 c1c2/c2c2* and *ALDH2 2 × 1/2 × 2* were associated with an increased frequency of sister chromatid exchange amongst VCM-exposed workers (Wong et al., 1998). We also found that the *CYP2E1 c2c2* genotype may modify the effect of VCM-related mutant p53 protein and anti-p53 antibody (Wong et al., 2002). These findings suggest that genetic polymorphisms may affect the levels of reactive intermediates which can lead to hepatotoxicity or genotoxicity of VCM.

The role of metabolizing genetic polymorphisms on VCM-induced abnormal liver function had been investigated. However, the role of genetic polymorphisms in VCM-related liver damage, including cirrhosis, is less clear. In this study, we investigated whether genetic polymorphism of metabolizing enzymes modifies the risk of VCM exposure with regard to liver fibrosis being diagnosed by using liver ultrasonography among Taiwanese PVC workers.

2. Materials and methods

2.1. Study subjects and epidemiological information

The study subjects have been reported in a previous study (Hsiao et al., 2004; Wong et al., 2002). Subsequent to informed consent being obtained from each subject, medical surveillance, including physical examination and liver ultrasonography, was performed on each worker who was employed in one of five PVC manufacturing plants from 1994 to 1999.

Each worker completed an interviewer-administered questionnaire which pertained to demographic characteristics, history of alcohol consumption and cigarette-smoking, medication, and a detailed occupational history. The total amount of ethanol consumed for each worker was calculated, and habitual alcohol drinking was defined as drinking greater than 146,000 g of ethanol (40 g/day for 10 years). Individuals who smoke at least once a day for over 6 months were defined as active smokers. Cumulative smoking dose was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. Habitual cigarette smoking was defined as smoking more than 15 pack-years of cigarettes (Pessione et al., 2001). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Overweight was defined as BMI greater than or equal to 25.0 kg/m^2 (NHLBI, 1998). Hepatitis B virus surface antigen (HBsAg) and anti-hepatitis C virus antibody (anti-HCV) were determined by enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' recommendation (Abbott Laboratories, Chicago, IL, USA), respectively. Positive hepatitis viral infec-

tion was defined as either positive for HBsAg and/or anti-HCV. Considering the minimum induction period of chronic liver disease, we selected study subjects with a work history of polyvinyl chloride production of at least for 1 year if the following criteria were met: physical examination and liver ultrasonography were conducted, the genetic polymorphisms and status of hepatitis viral infection were known, and detailed questionnaires had been completed. Female workers were excluded because of their small number. Thus, a total of 320 male workers were enrolled for analysis. This study has been approved by the Institutional Review Board of the Cathay General Hospital before the analysis (CGHIRB No: CT9525, on 25 July 2006).

2.2. Assessment of vinyl chloride exposure

Cumulative VCM exposure levels for study subjects were calculated according to the job exposure matrix model. Because environmental monitoring data were not available before 1985, a mathematical model was developed for estimating the historical mean VCM exposure for 24 job categories for different calendar years in the PVC industry before 1985 (Du et al., 2001). Exposure levels of VCM after 1985 were based on existing environmental monitoring data.

Our previous study found that the dose–response relationship between p53 over-expression and VCM exposure were less prominent in the high dose range (Wong et al., 2002). One possible explanation is that cumulative VCM dose of high exposure jobs may be underestimated in our reconstruction model, which could lead to the misclassification of VCM dose. Since tank cleaning was the most important exposure source, we recalculated the cumulative dose for tank cleaners. Based on the environmental surveillance conducted in 1992, the VCM concentration could reach 50,000 ppm when autoclaves were opened, but the vapor dispersed soon, in several minutes, before the inner temperature had dropped to allow workers to enter it. During cleaning of autoclaves, workers were estimated to be exposed to $\geq$3000 ppm of VCM vapor (Barnes, 1976). Thus we used 3000 ppm to recalculate the exposure of VCM concentration at that time for tank cleaners. Subsequently, an 8-h time-weighted (8-h TWA) job-exposure matrix for PVC workers was constructed. Cumulative VCM exposure level (ppm-years) for each worker was estimated using a summation of exposure concentration (8-h TWA, in ppm) of each job times the duration (in year) of that job (Wong et al., 2002).

2.3. Ultrasonographic examination of liver

Ultrasonographic examination of liver and spleen was performed with a Toshiba SAL-38B scanner with a 3.75-MHz convex-type transducer (Toshiba Co., Tochigi-Ken, Japan) on all workers by three well-experienced hepatologists at NTUH. They were blind to the exposure status of the workers and applied the same criteria to make the diagnoses of liver fibrosis (Yang et al., 1991; Saverymuttu et al., 1986). Liver fibrosis includes pre-cirrhosis and cirrhosis, both of which have heterogeneous echogenicity. Liver cirrhosis, a more advanced form of liver fibrosis, was diagnosed in the presence of coarse echogenicity, regeneration nodules and surface undulation of liver.

2.4. Genotyping of polymorphic metabolizing traits

The assays for genetic polymorphisms were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The methods of determination of *CYP2E1*, *ALDH2*, and *GSTT1* genotypes were performed as we have indicated previously (Wong et al., 1998, 2002).

Briefly, for the CYP450 2E1 gene analysis, any RFLP was detected by differences in PstI sites in the 5′-flanking region following PCR amplification, using methods described by Hayashi et al. (1991). The 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 that the denaturing step was conducted at 95 degree Celsius (°C), annealing at 55 °C and extension at 72 °C. The PCR products were digested with PstI. 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* MboII polymorphism was determined by a modification of the methods 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 that the denaturing step was conducted at 94 °C, annealing at 52 °C and extension at 65 °C. The PCR products were digested with MboII 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 2×1 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 β-globin (110 bp) was also performed as a positive internal 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 procedure was carried out under conditions 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* positive genotype (with at least one undeleted *GSTT1* allele; present GSTT1 activity) demonstrated a 480 bp fragment. Null genotype (with homozygous deleted alleles; no GSTT1 activity) did not present the amplified fragment.

2.5. Statistical analysis

Comparisons of the proportions of each determinant as well as genotypes between different VCM-exposed categories were made using multiple analysis of variance (MANOVA) tests for continuous variables and Mantel–Haenszel tests for discrete variables. The dose–response trend between VCM exposure and liver fibrosis was assessed by the Cochran–Armitage test. The distributions of *CYP2E1* and *ALDH2* polymorphisms among the subjects were tested for whether they fitted the Hardy–Weinberg equilibrium by χ^2 Goodness-of-Fit tests, but *GSTT1* was not tested because the PCR assays did not enable discrimination of heterozygous from homozygous *GSTT1* positive carriers. Because of a small number of cases, for the effects of genetic polymorphisms on liver fibrosis, odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated by exact logistic regressions models. All the statistical analyses were performed using the SAS program (Version 9.1.3, SAS Institute Inc., Cary, NC, USA), and all statistical tests were two-sided.

3. Results

The basic characteristics and results of ultrasonographic examination among different exposure groups of the 320 study subjects are shown in Table 1. The average age of these workers was 43.1 ± 10.4 (mean ± standard deviation, S.D.) years. The median cumulative dose of VCM exposure for all workers was 272.1 ppm-years. The average of BMI was 24.1 ± 2.8 kg/m^2. Ten (3.1%) workers had consumed alcohol regularly (at least 146,000 g of ethanol). Fifty-three (16.6%) workers had smoked regularly (at least 15 pack-years of cigarettes). Positive results for HBsAg and anti-HCV were found in 63 (19.7%) and 10 (3.1%) workers, respectively. Twelve subjects (3.8%) were diagnosed with pre-cirrhosis and one (0.3%) with cirrhosis of the liver. Statistically significant trends were found between increased exposure dose of VCM and different determinants, including age, working duration, proportion of habitual smoking, and proportion of liver fibrosis.

An exact logistic regression model was further used to analyze the association between VCM exposure and liver fibrosis, and the results are shown in Table 2. Pre-cirrhosis and cirrhosis were combined together as liver fibrosis because the number of cases was small. When workers with VCM exposure below 40 ppm-years (1 ppm × 40 years) were used as the reference group, workers with VCM exposure between 40 and 400, 400 and 800, or greater than 800 ppm-years had ORs of 1.2 (95% CIs, 0.1–71.5), 1.6 (95%, CIs 0.1–118.3) and 2.3 (95% CIs, 0.2–159.6) of liver fibrosis, and a significant dose–response trend was observed. Infection with hepatitis B and/or hepatitis C was also found to be an independent risk factor for liver fibrosis among PVC workers.

The genotype frequencies of the *CYP2E1*, *ALDH2*, and *GSTT1* among the study subjects are listed in Table 3. The genotype frequencies matched the prediction by the Hardy–Weinberg theorem based on the allele frequencies, even when the subjects were divided into two subgroups by 800 ppm-years (near-median dose of workers with liver fibrosis).

The possible modulation of liver fibrosis by different genotypes, controlled for the effect of the main confounding factor, hepatitis viral infection, is summarized in Table 4. Those possessing the *CYP2E1 c2c2* genotype had a higher OR of liver fibrosis as compared to those demonstrating the *c1c2*/*c1c1* genotypes (OR, 13.4; 95% CIs, 1.9–92.0). Since the transcriptional activities differ among *c2c2*, *c1c2*and *c1c1* genotypes, the ORs for the risk of liver fibrosis were analyzed. When workers with the *c1c1* genotype were used as the reference group, workers with *c1c2* and *c2c2* genotypes had ORs of 0.6 (95% CIs, 0.1–3.3) and 11.0 (95% CIs, 1.5–84.3). No differences were found in the risks for liver fibrosis among *GSTT1* and *ALDH2* genetic polymorphisms.

Subsequently, individuals were stratified to test the joint effect between VCM exposure and genetic polymorphisms on liver fibrosis. The *CYP2E1 c2c2* genotype was found to be a stronger risk indicator (OR 10.9, 95% CIs, 1.2–89.8) than hepatitis viral infection on liver fibrosis in the high-exposure group. In the low-exposure group, the risks did not have significant differences among the different genotypes. Subsequently, we tried to assess the interaction effects between different genotypes and other possible determinants of liver fibrosis, but the interpretations were limited because of a small number of cases.

4. Discussion

Previous studies have reported that prolonged exposure to VCM can increase the progress of liver fibrosis (Hsiao et al., 2004; Maroni et al., 2003). The aim of this study was to explore the association between metabolizing genetic polymorphism and VCM exposure on liver fibrosis. Our results revealed that PVC workers with the *CYP2E1 c2c2* genotype faced a significantly higher risk of liver fibrosis.

The frequency of the *CYP2E1 c2* allele (24.8%) found in this study was similar to that found in a previous study conducted amongst people of Taiwanese descent (20.3%) (Yu et al., 1995). The frequency of the *ALDH2* allele 1 (70.6%) was also comparable to the correspond-

Table 1
Basic characteristics of vinyl chloride-exposed workers stratified by exposure groups

	Cumulative dose of VCM (ppm-years)				
	<40	40–400	400–800	≥800	Total
Numbers	71	115	46	88	320
Age (years)[a]	33.6 ± 6.4	40.7 ± 9.9	48.5 ± 7.0	50.9 ± 7.3	43.1 ± 10. 4**
Cumulative dose of VCM (ppm-years)					
Mean cumulative dose[a,b]	10.7 ± 10.2 (0.6–38.6)	184.8 ± 100.3 (40.9–395.7)	565.4 ± 113.8 (402.5–798.6)	1514.4 ± 628.9 (800.9–4302.2)	566.5 ± 694.6** (0.6–4302.2)
Median cumulative dose	7.1	156.5	535.0	1359.2	272.1
Working duration (years)[a]	6.7 ± 4.5	14.0 ± 9.2	21.4 ± 7.9	24.3 ± 5.9	16.3 ± 9.8**
Body mass index (kg/m^2)[a]	23.3 ± 2.5	24.2 ± 2.9	24.4 ± 2.6	24.4 ± 2.8	24.1 ± 2.8
Habitual alcohol drinking[c]	1 (1.4%)	3 (2.6%)	1 (2.2%)	5 (5.7%)	10 (3.1%)
Habitual cigarette smoking[c]	1 (1.4%)	15 (13.0%)	14 (30.4%)	23 (26.1%)	53 (16.6%)**
Hepatitis B or C infection[c]					
HBsAg (+)	12 (16.9%)	23 (20.0%)	14 (30.4%)	14 (15.9%)	63 (19.7%)
Anti-HCV (+)	2 (2.8%)	2 (1.7%)	2 (4.4%)	4 (4.6%)	10 (3.1%)
HBsAg (+) or anti-HCV(+)	13 (18.3%)	24 (20.9%)	15 (32.6%)	17 (19.3%)	69 (21.6%)
Severity of liver fibrosis[c]					
Normal	70 (98.6%)	112 (97.4%)	43 (93.5%)	82 (93.2%)	307 (95.9%)†
Pre-cirrhosis	0 (0.0%)	3 (2.6%)	3 (6.5%)	6 (6.8%)	12 (3.8%)
Liver cirrhosis	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)

*$p < 0.05$, **$p < 0.01$ by MANOVA and Mantel–Haenszel tests.
[a] Mean ± standard deviation (S.D.).
[b] Range.
[c] Number (%).
† Cochran–Armitage test revealed a significant linear trend in the prevalence of liver fibrosis for workers with VCM doses of 40–400, 400–800 and greater than 800 when compared to workers with doses less than 40 ppm-years; $p < 0.05$.

Table 2
Exact logistic regression model of liver fibrosis in vinyl chloride-exposed workers

Determinant	Liver fibrosis[a] ($n = 13$)		
	Yes	No	Adjusted OR (95% CI)
Age (years)			
≥40	11	180	2.0 (0.2–26.8)
<40	2	127	1.0
Viral hepatitis			
HBsAg (+) or anti-HCV (+)	9	60	9.3 (2.5–43.2)[**]
HBsAg (−) and anti-HCV (−)	4	247	1.0
Body mass index (kg/m^2)			
≥25.0	6	109	1.3 (0.3–5.2)
<25.0	7	198	1.0
Habitual alcohol drinking (g)			
>146,000	1	9	1.8 (0.0–20.8)
≤146,000	12	298	1.0
Habitual cigarette smoking (packs-years)			
>15	4	49	1.9 (0.4–8.5)
≤15	9	258	1.0
Cumulative dose of VCM (ppm-years)			
≥800	6	82	2.3 (0.2–159.6)[†]
400–800	3	43	1.6 (0.1–118.3)
40–400	3	112	1.2 (0.1–71.5)
<40	1	70	1.0

[a] Liver fibrosis includes both pre-cirrhosis and liver cirrhosis.
[**] $p < 0.01$ by exact logistic regression.
[†] Cochran–Armitage test revealed significant dose–response trend for workers with VCM doses of 40–400, 400–800 and greater than 800 those when compared to workers with doses less than 40 ppm-years, respectively; $p < 0.05$.

ing value for the control group of other alcoholic studies for the Taiwanese population (68–76%) (W.J. Chen et al., 1996; Thomasson et al., 1991). The frequency of the *GSTT1* null type (44.7%) was likewise consistent with the analogous figure as revealed by previous studies, 44–51.4% for Taiwanese populations (C.J. Chen et al., 1996; Chen et al., 2004). These findings, to some extent, validate the technique of our genotyping.

In our study, workers with the *CYP2E1 c2c2* genotype were at higher risk of liver fibrosis than those lacking the genotype. The primary route of VCM metabolism is by the action of *CYP2E1*. Individuals with the *CYP2E1 c2c2* genotype demonstrate higher metabolizing activity than those expressing the *CYP2E1 c1c1*/*c1c2* genotypes (Hayashi et al., 1991), thus workers may experience higher CEO and CAA levels when VCM were absorbed

Table 3
Distribution of *CYP2E1*, *ALDH2*, and *GSTT1* genotypes among VCM-exposed workers

Genotypes	<800 ppm-years (%)	≥800 ppm-years (%)	Total
CYP2E1			
c1c1	129 (55.6%)	47 (53.4%)	176 (55.0%)
c1c2	95 (41.0%)	34 (38.6%)	129 (40.3%)
c2c2	8 (3.4%)	7 (8.0%)	15 (4.7%)
ALDH2			
1 × 1	122 (52.6%)	43 (48.9%)	165 (51.6%)
2 × 1	88 (37.9%)	34 (38.6%)	122 (38.1%)
2 × 2	22 (9.5%)	11 (12.5%)	33 (10.3%)
GSTT1			
Null	109 (47.0%)	34 (38.6%)	143 (44.7%)
Positive	123 (53.0%)	54 (61.4%)	177 (55.3%)

Table 4
Multiple logistic regression models for liver fibrosis among workers with different categories of exposure and genetic polymorphisms

Determinant	Liver fibrosis[a] ($n = 13$)		
	Yes (n%)	No (n%)	Adjusted OR (95% CIs)[b]
Total workers			
CYP2E1			
c2c2	4 (30.8%)	11 (3.6%)	13.4 (1.9–92.0)**
c1c1/c1c2	9 (69.2%)	296 (96.4%)	1.0
GSTT1			
Null	3 (23.1%)	140 (45.6%)	0.3 (0.0–1.3)
Positive	10 (76.9%)	167 (54.4%)	1.0
ALDH2			
2 × 1/2 × 2	7 (53.9%)	148 (48.2%)	1.9 (0.4–9.3)
1 × 1	6 (46.2%)	159 (51.8%)	1.0
Hepatitis viral infection			
Positive	9 (69.2%)	60 (19.5%)	9.7 (2.4–47.2)**
Negative	4 (30.8%)	247 (80.5%)	1.0
<800 (ppm-years)			
CYP2E1			
c2c2	1 (14.3%)	7 (3.1%)	9.0 (0.1–357.6)
c1c1/c1c2	6 (85.7%)	218 (96.9%)	1.0
GSTT1			
Null	1 (14.3%)	108 (48.0%)	0.1 (0.0–1.1)
Positive	6 (85.7%)	117 (52.0%)	1.0
ALDH2			
2 × 1/2 × 2	4 (57.1%)	106 (47.1%)	1.9 (0.2–23.2)
1 × 1	3 (42.9%)	119 (52.9%)	1.0
Hepatitis viral infection			
Positive	6 (85.7%)	46 (20.4%)	25.6 (3.0–>999.0)**
Negative	1 (14.3%)	179 (79.6%)	1.0
≥800 (ppm-years)			
CYP2E1			
c2c2	3 (50.0%)	4 (4.9%)	10.9 (1.2–89.8)*
c1c1/c1c2	3 (50.0%)	78 (95.1%)	1.0
GSTT1			
Null	2 (33.3%)	32 (39.0%)	0.9 (01–8.7)
Positive	4 (66.7%)	50 (61.0%)	1.0
ALDH2			
2 × 1/2 × 2	3 (50.0%)	42 (51.2%)	1.3 (0.1–14.1)
1 × 1	3 (50.0%)	40 (48.8%)	1.0
Hepatitis viral infection			
Positive	3 (50.0%)	14 (17.1%)	3.0 (0.3–27.8)
Negative	3 (50.0%)	68 (82.9%)	1.0

[a] Liver fibrosis includes both pre-cirrhosis and cirrhosis.
[b] Reference group (OR = 1.0) refers to workers with unsusceptible genotype or lack of hepatitis viral infection.
* $p < 0.05$.
** $p < 0.01$ by exact logistic regression model.

during the process. Reactive metabolites were also proposed to induce chronic liver damage and liver fibrogenesis. It has been observed that the metabolizing activity of toxins of *CYP2E1* increased with the number of variant c2 alleles, and that the *c2c2* genotype statistically differed from either the *c1c1* or the *c1c2* genotype (Marchand et al., 1999). Furthermore, because the frequencies of the *CYP2E1 c2c2* genotype are rare,

most studies stratified subjects into two groups by the *c2c2*/*c1c2* and *c1c1* genotypes. In this study, workers with the *c1c2* genotype have a similar risk of developing liver fibrosis when compared with workers with the *c1c1* genotype. Thus, we stratified workers into two subgroups by the *c2c2* genotype versus the *c1c2* and *c1c1* genotypes to compare the risk of liver fibrosis in the analysis.

Our result suggests that genetic polymorphism in *CYP2E1* may be responsible for individual differences in susceptibility to VCM-induced liver fibrosis. Though some epidemiologic studies suggest that genetic susceptibility may be more important at low levels of carcinogens but our study does not reveal similar finding (Vineis et al., 1994; Nakachi et al., 1993). The discrepancy may result from types of gene–environment interactions, affinity and capacity of the metabolic enzymes, and exposure through single or mixtures of chemicals (Vineis, 1997). One additional explanation for our study involves in the doses required for health outcomes, for example, types of liver damage and genotoxicity. To induce abnormal liver function and liver fibrosis, higher doses of VCM were required than those required for inducing genotoxicity and mutagenicity, which was supported by a chronic feeding study in rats (Til et al., 1991). They found that chronic VCM exposures resulted in non-cancerous types of liver damage, including cell necrosis, required a 100-fold dosage of VCM than the appearance of pre-neoplastic lesions. Our previous study also found that the modifying effect of the *CYP2E1 c2c2* genotype on the association between VCM exposure and abnormal liver function (ALT) was only observed in the high exposure group (Huang et al., 1997). Above findings suggest higher VCM exposure is required for inducing liver fibrosis. Thus our finding should be reasonable.

The susceptible *ALDH2* genotype did not modify the effects of VCM-related liver fibrosis in our study. Our previous studies also showed *ALDH2* might be associated with VCM-related genotoxicity (Wong et al., 2003). An animal study had previously demonstrated that VCM is metabolized by CYP2E1 to become CEO, and CEO may spontaneously transform into CAA, which may be subsequently metabolized by *ALDH2* (Whysner et al., 1996). CEO is more electrophilically capable of reacting with DNA bases to yield etheno-adducts than CAA (Guengerich, 1992; Gwinner et al., 1983). The metabolizing activity of toxins of ALDH2 decreased with the number of variant 2 alleles, and thus subsequently increased the risk of cytotoxicity. One of the probable reasons for this negative finding may lie in the small case number.

GSTs are phase II enzymes. Previous studies have also reported that GSTs may act as detoxifying enzymes by reacting with the epoxide product(s) of many different environmental chemicals (Ketterer, 1998). *GSTT1* null genotype can reduce detoxification capacity because foreign compounds cannot conjugate with glutathione. However, the role of GST enzymes in VCM metabolism, protective or harmful, has produced some controversial results. A recent study found that workers with the *GSTT1* positive genotype frequency showed higher prevalence of Reynaud's phenomenon at the lowest VCM exposure (Fontana et al., 2006); but another study showed that the *GSTT1* positive genotype protected the VCM workers from liver lesions (abnormal liver ultrasonography and/or alanine aminotransferase) in the low exposure group (Zhu et al., 2005). Our previous studies also showed the effect of the *GSTT1* positive genotype in inducing abnormal liver function and p53 over-expression differed with different levels of VCM exposure (Wong et al., 2002; Huang et al., 1997). The probable reasons for this discrepancy may lie in a difference in exposure assessment between the studies, the metabolism of VCM differing at different concentrations (Hefner et al., 1975), or the small sample sizes.

Hepatitis viral infection is a prominent risk factor for workers' liver fibrosis in our study, and the prevalence rates of subjects with hepatitis B and C were similar to those for the Taiwanese general population (Chen et al., 2007). Our analysis revealed there was no difference in the positive rates of HBsAg and Anti-HCV among workers with different metabolizing genotypes and VCM exposure categories. The progress to chronic liver disease from hepatitis B or C infection does not relate to the metabolizing pathway of xenobiotics, and previous studies also revealed that genetic polymorphisms of *CYP2E1*, *ALDH2* and *GSTT1* did not significantly correlate with the progression of liver cirrhosis and liver cancer among cases with hepatitis B or C (Mohammadzadeh Ghobadloo et al., 2006; Yu et al., 2002). However, we still kept hepatitis B or C infection as confounders in the analysis.

Some limitations of this study need to be outlined. The number of cases was small causing wide confidence intervals in the odds ratios and making the interpretation of the estimated effects of genetic polymorphisms on liver fibrosis less reliable. Lagakos suggests a stricter criterion should be applied to avoid false positive results when multiple subgroup analyses are conducted (Lagakos, 2006). When we used 0.0125 (0.05 divided by 4 determinants) as the threshold for significance, the *p*-value of the *CYP2E1 c2c2* genotype was still below this

figure. Furthermore our result was consistent with previous studies and with biologically plausible mechanisms. Thus the result still suggested the modifying effect of *CYP2E1* genetic polymorphisms on VCM-induced liver fibrosis.

There might still be some misclassification if the diagnosis of fibrosis is based solely on ultrasonography. A previous study showed that the sensitivity and specificity of detecting liver fibrosis was 57% and 88%, respectively (Saverymuttu et al., 1986). Another study also demonstrated that ultrasound evaluation of liver fibrosis stage based on the scoring system was a reliable and effective alternative to histological staging in chronic liver diseases (Nishiura et al., 2005). As the hepatologists in our study performing the ultrasonographic examination were blind to the VCM exposure status, the misclassification was assumed to be non-differential. Nonetheless, data analysis showed that the results were compatible with the prior knowledge that viral hepatitis infection, obesity and VCM exposure are associated with liver fibrosis. We concluded that the accuracy of the ultrasonographic diagnosis was acceptable. In this study, we focused on the asymptomatic workers with liver fibrotic change, and possible limitations on the extrapolation from liver fibrosis to liver cirrhosis need to be carefully considered.

The other issue is exposure assessment. Although we have improved the measurement through the job-matrix model, either under or overestimation of VCM exposure for tank cleaning cannot be excluded. However, we still observed that an increased cumulative VCM exposure dose was associated with an increased risk of liver fibrosis. Previous study found that ethanol causes a reduction in the rate of VCM metabolism in rats exposed to VCM (Hefner et al., 1975). Chronic ethanol consumption has also been reported to generate microsomal reactive oxygen species, presumably through the effect of cytochrome P450 2E1, and this has been implicated in the etiology of liver cell damage (Bailey and Cunningham, 1998). However, in this study, the effect of ethanol on liver fibrosis was not observed. This was probably because only 10 (3.1%) workers consumed alcohol regularly. Thus the contribution of ethanol consumption to cytochrome P450 2E1 activity can be ignored in this study.

In summary, our results have revealed that increased VCM exposure associated with liver fibrosis and the risk of liver fibrosis was modulated by the metabolizing cytochrome P450 2E1 in Taiwanese VCM-exposed workers. Those VCM-exposed workers, particularly individuals with the susceptible *CYP2E1* genotype, may need intensive medical screening, particularly for liver cirrhosis and cancer. Polymorphism analysis of metabolizing enzymes might be useful in the risk assessment of liver damage in workers with VCM exposure. Clearly, the role of metabolizing genes in VCM-related liver damage requires further study.

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