Genetic Polymorphisms in Metabolizing Enzymes and Susceptibility of Chromosomal Damage Induced by Vinyl Chloride Monomer in a Chinese Worker Population

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Objective: To evaluate whether polymorphisms in metabolizing enzymes contributed to susceptibility of chromosomal damage induced by vinyl chloride monomer (VCM). **Methods:** Cytokinesis block micronucleus test was performed on 185 VCM-exposed workers and 41 control subjects to detect chromosomal damage in peripheral lymphocytes. The polymerase chain reaction and restriction fragment length polymorphism technique was applied to detect polymorphisms of GSTT1, GSTM1, GSTP1G/A, CYP2E1G/C, and CYP2D6G/C. Poisson regression analysis was performed. **Results:** Sex, age, VCM exposure, GSTP1, and CYP2E1 genotype can influence chromosomal damage. There was a 1.51-fold increased micronucleus frequency for GSTP1GG genotypes individuals compared with those GSTP1AA/GA genotype individuals ($P < 0.05$), the effect of polymorphism in CYP2E1 gene was more pronounced for allele C compared with allele G ($P < 0.05$). **Conclusions:** Polymorphisms of GSTP1G/A and CYP2E1G/C, which are potential susceptibility biomarkers of chromosomal damage in VCM-exposed worker.

Vinyl chloride monomer ($CH_2 = CHCl$, VCM) is a widely used industrial gas that produces polyvinyl chloride polymer. VCM exposure has been associated with liver angiosarcoma and hepatocellular cancer and was classified as a group 1 carcinogen by International Agency for Research on Cancer in 1987.[1–3] Although the relationship between VCM exposure and liver cancer has been established, the mechanism of VCM-related carcinogenesis remains unknown.

VCM is primarily metabolized in the liver by CYP2E1 into reactive chloroethylene oxide (CEO). Because CEO is unstable, it rearranges spontaneously to chloroacetaldehyde (CAA).[4] Both metabolites interact with DNA to form DNA adducts and induce errors during DNA and RNA synthesis.[5] Epidemiological studies have shown that VCM exposure was associated with increased genotoxicity in human studies. Chromosomal aberrations, micronuclei (MN), sister chromatid exchanges (SCEs), and DNA strand breaks have been observed in lymphocytes of individuals occupationally exposed to VCM.[6–8]

Among the biomarkers mentioned above, the frequency of MN in human cells has become one of the standard cytogenetic indexes for genetic toxicology testing. The cytokinesis-block micronucleus (CBMN) assay is the preferred method for measuring MN in cultured human cells because scoring is specifically restricted to once-divided cells. These cells are recognized by their bi-nucleated (BN) appearance after inhibition of cytokinesis by cytochalasin-B. Restricting scoring of CBMN to BN cells prevents confounding effects caused by suboptimal cell division kinetics, which is a major variable in this assay.[9] Because CBMN formation requires nuclear division, the scoring of those cells that have completed nuclear division is a prerequisite for the correct interpretation of the observed CBMN frequencies. This is achieved by scoring CBMN in BN cells using the CBMN technique.[10,11]

As we know, susceptibility to genotoxicity is modulated, at least in part, by polymorphisms in genes encoding metabolic enzymes, DNA repair proteins, and cell-cycle control proteins. Previously, many studies have been conducted to investigate the genotoxicity of VCM and the effect of genetic polymorphisms of genes involved in metabolism (*CYP2E1*, *GSTs*, *ADH2*, *ALDH2*) and DNA repair (*XRCC1*, *XPD*).[8,12–18]

To the best of our knowledge, however, there have been few investigations of the effect of genetic polymorphisms of genes involved in metabolism on CBMN frequencies in VCM workers. So CBMN was used in this study to monitor a group of subjects occupationally exposed to VCM, and the subjects were genotyped for several metabolizing genes: *GSTT1* (gene deletion), *GSTM1* (gene deletion), *CYP2E1G/C* (rs3813867), *CYP2D6G/C* (rs1135840), and *GSTP1G/A* (rs1695).

MATERIALS AND METHODS

Study Population

Workers employed in a VC polymerization plant in China were studied. Blood had been collected for all the 272 workers in the VC polymerization plant from April to May, 2004. Before the study, a written informed consent form was obtained from each subject and a questionnaire was used to determine the lifestyle of each subject, namely, smoking and alcohol habits, medication, and occupational history. Study subjects exposed to VCM for a period >1 year were selected if the following criteria were met: detailed questionnaires had been completed; blood samples had been provided; and CBMN tests and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) for all the studied genes were completed successfully. A total of 185 (117 men and 68 women, mean age, 33.91 ± 0.484 years, range, 20 to 53 years) workers met these criteria, all of whom were ethnically Han Chinese.

As a control group, teachers and graduated students not exposed to VCM and other toxicants were selected, who came from the School of Public Health, Fudan University. Detailed questionnaires and the CBMN test were completed for each control, for a total of 41 Han Chinese (20 men and 21 women, mean age, 35.3 ± 11.1 year, range, 23 to 57 years) meeting these criteria.

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Environmental Monitoring and VCM Exposure Assessment

The suspension polymerization method was used to polymerize VCM in the VC polymerization plant in China. In the process, VCM and water are introduced into the polymerization reactor and a polymerization initiator, along with other chemical additives, are added to initiate the polymerization reaction. Once the reaction has run its course, the resulting polyvinyl chloride slurry is degassed and stripped to remove excess VCM (which is recycled into the next batch) then passed in a centrifuge to remove most of the excess water. The slurry is then dried further in a hot air bed and the resulting powder sieved before storage or pelletization.

Once a month, the level of VCM was measured for different worksites of the plant using gas chromatography. Because the VCM plant had kept VCM air concentration data for different worksites from the beginning of its establishment, we were able to estimate the cumulative exposure dose of each worker with a relatively high level of precision.

The following equation was used to calculate cumulative exposure dose[8,18]:

$$\text{Cumulative exposure dose (mg)} = \sum (C \times M \times T) \times A \times 70\%/10^6 C \ (\text{mg/m}^3),$$

where C denotes the geometric mean of VCM exposure concentration for each month in a specific workplace, calculated for all different worksites; M means the number of exposure months of each year for a VCM worker; T is a 2-hour exposure time in each working day for 20 days per month, or 2400 minutes of exposure time per month; and A is the alveolar ventilation (male average: 6500 mL/min, female average: 4300 mL/min, 30% dead space). By using the cumulative exposure dose equation above, we estimated personal cumulative exposure dose in the VCM exposure group and found the range to be 576 mg to 3,01,992 mg, with the median dose of 21,808,52 mg. The VCM exposed subjects then were divided into high-exposure ($\geq$21,809 mg) and low-exposure ($<$21,809 mg) groups by the median dose.

Cytokinesis-Block Micronucleus Assay

The CBMN assay was performed according to the standard method as described earlier.[9,19] Blood samples were collected by venipuncture in heparinized tubes and transferred to the laboratory within a few hours of collection. Aliquots of 0.5 mL of heparinized whole blood were cultured in 4.5 mL medium. Forty-four hours after phytohaemagglutinin stimulation, cytokinesis was blocked with 6 μg/mL cytochalasin-B (Sigma-Aldrich, St. Louis, MO); and after a total of 72 of hours culture, lymphocytes were harvested and fixed with methanol and acetic acid at 4:1 before being transferred to slides. For each subject, 1000 BN lymphocytes with well-preserved cytoplasm were scored blindly by the same reader.

DNA Extraction

DNA was isolated from the whole blood using a standard method.[20] Blood samples of all subjects were collected into 10-mL heparinized tubes and stored at -80°C until use. Genomic DNA was obtained from 3 mL of blood and each DNA sample was stored at -80°C until analysis.

PCR-RFLP Genotyping Assays

The *GSTT1* and *GSTM1* genotype were determined as described in previous investigation.[18] The primers used for *GSTT1* were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′. The primers used for *GSTM1* were 5′—GAA CTC CCT GAA AAG CTA AAG C- 3′ and 5′—GTT GGG CTC AAA TAT ACG GTG 3′. Amplification of human β-globin (350bp) was 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 459bp fragment and individuals with one or more *GSTM1* alleles had a 219bp fragment.

PCR-RFLP was applied to detect the polymorphisms of the *GSTP1*, *CYP2E1* and *CYP2D6*. The *GSTP1G/C* genotype was determined by endonuclease Alw26I, with the primers designed specifically for this study. The primers used for *GSTP1* exon 5 codon 105 Val/Ile (G/A, rs1695) polymorphism were 5′-CTT CCA CGC ACA TCC TCT TCC-3′ and 5′-AAG CCC CTT TCT TTG TTC AGC-3′. After the PCR products were digested with restricted Alw26I, homozygous Ile/Ile individuals exhibited a product fragment of 289bp, whereas homozygous Val/Val individuals revealed both 218 and 71bp fragments, and heterozygous Ile/Val individuals demonstrated all three of the fragments.

The *CYP2E1* genotype was determined as described by Zhu et al.[18] The primers used for *CYP2E1* promoter region-1293 site (G/C, rs3813867) polymorphism were 5′-TTC ATT CTG TCT TCT AAC TG-3′ and 5′-CAG TCG AGT CTA CAT TGT C-3′, respectively. The PCR products were digested with restricted endonuclease PstI. Homozygous c1c1 individuals reflected a single product fragment of 410 bp, whereas homozygous c2c2 individuals demonstrated both 290 and 120bp fragments, and heterozygous c1c2 individuals revealed all three of the fragments.

The CYP2D6 genotype was determined using a method reported previously.[21] The primers used for *CYP2D6* exon 9 codon 486 Thr/Ser (C/G, rs1135840) polymorphism were 5′-GAG ACA AAC CAG GAC CTG CCA-3′ and 5′-GCC TCA ACG TAC CCC TGT CTC-3′. The PCR products were digested with restricted endonuclease ECO91I. Homozygous Ser/Ser individuals exhibited a product fragment of 866bp, whereas homozygous Thr/Thr individuals showed 620bp and 246bp fragments, and heterozygous Ser/Thr individuals demonstrated all three of the fragments.

The PCR was done using 50 ng of genomic DNA, 0.2 μmol/L of each primer, 1 $\times$ PCR buffer, 0.2 μmol/L of each deoxynucleotide triphosphate, 2.0 mmol/L $MgCl_2$, and 0.625 units of Taq in a 25 μL reaction volume. PCR program was a 5-minute denaturation step at 94°C followed by 35 cycles of 94°C for30 seconds, 58°C (except 65°C for *CYP2D6*) for 30 seconds, 72°C for 30 seconds (except 60s for *CYP2D6*), respectively, and a final extension step at 72°C for 10 minutes. The PCR products were digested at 37°C for 16 hours by corresponding restriction enzyme (Fermentas, Inc., Burlington, Ontario, Canada), then the digested PCR products were separated by agarose gel electrophoresis and observed under UV image system (Gel Doc 2000, Bio-Rad, Hercules, CA). Ten percent of DNA samples were selected randomly for repeat and the concordance was 100%.

Statistical Analysis

SAS software package (version 9.13) was used for the statistical analysis. The influence of genotype, age, gender, cumulative exposure dose, alcohol consumption, and smoking status on the frequencies of CBMN cells per 1000 BN cells were determined using multiple Poisson regression analysis. The significance level (alpha) was set at 5% for all analysis. Frequency ratio (FR) and its 95% confidence interval (95% CI) were estimated using formulate, $FR = e^{\beta}$ ($e \approx 2.71828$). For categorical variables, the FR indicates the proportional increase of the micronucleus frequency in the study group; for example, an FR of 1.21 for women versus men means a 21% increase of micronucleus frequency in women. For continuous variables, the FR represents the proportional increase of micronucleus frequency due to the increase of one unit of the variable evaluated; for example, an FR for age of 1.02 means a 2% increase of micronucleus frequency per year of age.[22]

RESULTS

Ages of person in VCM group and controls group were 33.91 ± 0.484 and 35.3 ± 11.057, respectively. Chi square test showed that there is no difference in ages between two groups.

TABLE 1. Demographic Data of VCM Workers and Controls

Character	VCM Workers *n* (%)	Controls *n* (%)	χ^2	*P*
Gender				
Male	117 (63.2)	20 (48.8)	2.941	0.086
Female	68 (36.8)	21 (51.2)		
Smoking				
Never	109 (58.9)	38 (92.7)	16.828	<0.001
Current*	76 (41.1)	3 (7.3)		
Drinking				
Never	159 (85.9)	36 (87.8)	0.098	0.754
Current	26 (14.1)	5 (12.2)		

*$P < 0.05$ with regard to the corresponding group.

According to other characters of population, only smoking status appears significant in two groups, and demographic data of two groups showed in Table 1.

The mean and median CBMN frequencies of the 185 subjects exposed to VCM were 3.64 ± 2.360 (‰) and 4.00 (‰), respectively, with a range of 0 to 14 (‰). By comparison, the mean and median CBMN frequencies of the 41 control subjects were lower (mean, 1.22 ± 1.194 [‰]; median, 1.00 [‰]; range of 0 to 5 [‰]). Poisson regression showed this difference of CBMN frequencies between the two groups to be significant ($P < 0.001$), and the FR of exposure was found to be 2.99 (95% CI = 2.27 to 4.037). Number, ratio, and CBMN frequencies of subjects in different group categorized by demographic, personal factors, and genes of objects are reported in Table 2. The distributions of the *GSTP1G/A* and *CYP2E1G/C* alleles were in agreement with the Hardy–Weinberg equilibrium, but the same was not true for the *CYP2D6G/C* alleles.

The FR estimated by Poisson regression analysis included age, gender, smoking status, alcohol consumption, exposure, and polymorphisms as potential confounding factors in the two group peoples. The result showed that age, gstp1(gg), cyp2e1(c2), VCM exposure were risk factors for the increase MNs (Table 2).

TABLE 2. Risk Estimate for Demographic, Habitual Factors, and Genes in VCM Workers

Characteristic	*N*	Ratio (%)	MN Frequency (‰)	FR (95%CI)	χ^2	*P*
Sex						
Male	137	60.6	3.09 ± 2.117	1		
Female*	89	39.4	3.37 ± 2.748	1.089 (0.938–1.262)	1.28	0.258
Age						
<36	132	58.4	2.83 ± 2.124	1		
≥36	94	41.6	3.73 ± 2.628	1.321 (1.142–1.528)	14.05	<0.001
Smoker						
−	147	65.0	3.08 ± 2.568	1		
+	79	35.0	3.43 ± 1.992	1.113 (0.957–1.293)	1.95	0.163
Drinker						
−	195	86.3	3.16 ± 2.442	1		
+	31	13.7	3.45 ± 1.997	1.091 (0.884–1.333)	0.69	0.406
Dose						
0	41	18.1	1.22 ± 1.194	1		
<21809*	89	39.4	3.55 ± 2.195	2.912 (2.183–3.969)	49.3	<0.001
≥21809*	96	42.5	3.73 ± 2.511	3.058 (2.298–4.159)	54.81	<0.001
GSTM1						
−	62	27.4	2.98 ± 1.954	1		
+	164	72.6	3.29 ± 2.528	1.101 (0.934–1.305)	1.29	0.2568
GSTT1						
−	120	53.1	3.06 ± 2.360	1		
+	106	46.9	3.37 ± 2.412	1.101 (0.952–1.274)	1.68	0.195
GSTP1						
AA	152	67.3	3.21 ± 2.289	1		
GA	65	28.8	2.95 ± 2.294	0.920 (0.777–1.085)	0.96	0.328
GG*	9	3.98	4.89 ± 3.887	1.523 (1.103–2.047)	7.14	0.008
CYP2E1						
GG	144	64.9	3.00 ± 2.222	1		
GC*	67	30.2	3.58 ± 2.692	1.194 (1.018–1.397)	4.85	0.028
CC*	11	4.95	4.09 ± 2.427	1.364 (0.990–1.831)	3.92	0.048
CYP2D6						
GG	10	4.42	4.20 ± 2.486	1		
GC	109	48.2	3.28 ± 2.381	0.782 (0.575–1.092)	2.27	0.132
CC*	107	47.3	3.03 ± 2.373	0.721 (0.529–1.008)	3.98	0.046

*$P < 0.05$ with regard to the corresponding group.

In VCM exposure group, Poisson regression analysis showed a significant increase in MN with age and in women in VCM exposure groups. The older subjects had higher mean CBMN frequencies than the younger subjects ($P = 0.001$) and the FR (95% CI) was 1.298 (1.116 to 1.510) in VCM exposure. The gender effect was more pronounced in women compared with men (FR, 1.171; 95% CI = 1.004 to 1.366), but only a significant increase in MN with age in whole population.

A higher CBMN frequencies was found in *GSTP1GG* genotype individuals than in *GSTP1AA* genotype subjects ($P = 0.008$). When compared with *GSTP1GG* genotype subjects, mean CBMN frequencies of *GSTP1AG* and *GSTP1AA* genotypes subjects are significantly lower, and the frequency of variant *GSTP1AG* genotype subjects was closer to that of *GSTP1AA* genotype subjects than to that of *GSTP1GG* genotype subjects, so we combined the heterozygote *GSTP1AG* genotype and variant homozygote *GSTP1AA* genotype into one group. With this combination of data, we found that *GSTP1GG* genotype subjects had higher mean CBMN frequencies than the combine of GA and AA genotype of *GSTP1* subjects ($P = 0.004$) and the FR (95% CI) was 1.560 (1.134 to 2.089).

The mean CBMN frequencies rose with increases of allele C of the *CYP2E1* gene, so the CBMN frequencies of *CYP2E1* genotypes can be analyzed as ordinal data. Then the effect of polymorphism in *CYP2E1* gene was more pronounced for allele C compared with allele G (FR, 1.180; 95% CI = 1.046 to 1.328; *P*, 0.006). For the *CYP2D6* genotype, the allele G had no significant effect on micronucleus frequencies ($P = 0.3011$) when the mean CBMN frequencies of *CYP2D6* genotypes were processed as ordinal data in Poisson regression.

We performed multiple Poisson regression to explore the major factors which influence the CBMN frequencies of VCM workers, including sex, age, smoking and drinking status, exposure dose, and polymorphisms of the *GSTT1*, *GSTM1*, *GSTP1*, *CYP2E1* and *CYP2D6*. Subjects with *GSTP1AA* and *GSTP1AG* genotype were combined as one group and were compared with *GSTP1GG* genotype subjects; *CYP2E1* and *CYP2D6* were analyzed as ordinal data; other data were analyzed as binary data. Poisson regression model for all factors influence of MNs showed in Table 3. The results showed that the five factors including sex, age, VCM exposure, *GSTP1* and *CYP2E1* genotype were the major factors, which influence the CBMN frequencies of peoples. Step Poisson regression result also appears the same phenomenon, five factors and their risk estimate data were shown in Table 4.

TABLE 3. Poisson Regression Model for all Factors Influence of MNs

		Estimate 95% CI			
Parameter	**Estimate**	**Lower**	**Upper**	χ^2	***P***
Intercept	−0.70	−1.406	−0.07	4.61	0.032
Sex*	0.17	−0.022	0.37	2.97	0.085
Age*	0.22	0.0676	0.375	7.98	0.005
Smoke	−0.03	−0.239	0.185	0.07	0.797
Drink	0.12	−0.117	0.353	1.02	0.313
gstt1	0.09	−0.055	0.241	1.52	0.218
gstm1	0.11	−0.056	0.285	1.66	0.197
gstp1(GG)*	0.41	0.0875	0.71	6.76	0.009
cyp2e1*	0.13	0.0043	0.25	4.19	0.041
cyp2d6	−0.10	−0.218	0.034	2.1	0.148
VCM exposure	1.16	0.8588	1.487	52.6	<0.001

*$P < 0.10$ with regard to the corresponding group.

DISCUSSION

The application of cytogenetic biomarkers, such as chromosomal aberrations (CAs), SCEs, and MN (CBMN), in studies of individual susceptibility has been attempted in investigations of individuals exposed to potential or known genotoxic agents. However, the value for these biomarkers in occupationally exposed individuals has not been as high as expected. The MN assay is one of the most sensitive markers of DNA damage and has been used to investigate the genotoxicity of a variety of chemicals. As a cytogenetic marker, the MN assay using interphase cells is more suitable than studies using SCEs or CAs, because it is not limited to metaphase cells and has the advantage of allowing rapid screening of large numbers of cells.[23–26] MN are formed from acentric chromosome- or chromatid-type fragments and whole chromosomes that have lagged behind in cell division and accordingly have been left outside both daughter nuclei. Thus, MN analysis appears to be a good tool for investigating the effects of clastogens and aneuploidogens in occupational and environmental exposures. However, several factors have been shown to be associated with the intra- and inter-individual variation of MN. These factors include demographic (age and gender) and personal factors, such as drinking and cigarette smoking.[27–29]

This study found that subjects occupationally exposed to VCM were at higher risk for MN frequency than unexposed controls. Previous research[29] showed that a greater frequency of the mean standardized CBMN values was observed with increasing age through analysis of a population sample that included data from several bio-monitoring studies performed during the last few decades in 12 Italian laboratories. Studies[23] also indicated that subjects >40 years of age had higher MN frequencies than younger subjects (≤40 years). Our study also demonstrated that subjects >35 years of age had higher MN frequencies than their younger counterparts (≤35 years). Another important finding of this analy-

TABLE 4. Poisson Regression Model and Risk Estimate for Risk Factors of MNs

		Estimate 95% CI				
Parameter	**Estimate**	**Lower**	**Upper**	χ^2	***P***	**FR (95%CI)**
Intercept	−0.764	−1.2951	−0.2349	8.00	0.005	–
Sex	0.1408	−0.0096	0.2898	3.40	0.065	1.151 (0.990–1.336)
Age	0.2232	0.0723	0.3735	8.44	0.004	1.250 (1.075–1.453)
cyp2e1	0.1141	−0.0083	0.2339	3.41	0.065	1.121 (0.992–1.264)
gstp1(GG)	0.4102	0.0868	0.707	6.75	0.009	1.507 (1.091–2.028)
VCM exposure	1.1683	0.8716	1.4926	54.65	<0.001	3.217 (2.391–4.449)

$\alpha = 0.10$.

sis is the observation that women occupationally exposed to VCM were at higher risk for CBMN frequency than occupationally exposed men. This observation confirmed the work of earlier investigation.[22] We did not find the personal factors of drinking and smoking to be associated with a variation in MN frequencies in VCM exposed workers.

Environmental carcinogens are converted into DNA-reactive metabolites by phase I and phase II enzymes that are involved in the activation and detoxification of xenobiotics. The electrophilic metabolites, CEO and CAA, considered to be the most important intermediates in the VCM carcinogenic process, react with DNA bases to form adducts. The DNA-adducts are mutagenic in bacterial systems, as well as in mammalian cells, and covalently bind to proteins impairing cell function. These two metabolites may be further processed by glutathione S-transferases.

The activities of these enzymes, GSTs and CYPs, are known to vary genetically among individuals. Thus, inter-individual differences in susceptibility to VCM exposure may be attributable to the differences in the activities of CYPs and GSTs. Susceptibility to diseases and polymorphisms of metabolizing genes, including *GSTs* and *CYPs*, have been reported to be linked in many earlier studies.[14,30–35] The results of this study suggest that polymorphisms in the genes for enzymes responsible for the metabolism of VC, in phase I (ie, *CYP2E1*) and in phase II (ie, *GSTP1*), can affect the risk of VC-induced MN occurrence.

Huang et al[35] showed that, with low VCM exposure, non-null GSTT1 genotype was significantly associated with an elevated odds ratio for abnormal ALT (OR = 3.8, 95% CI = 1.2 to 14.5), but *CYP2E1* genotype was not associated with abnormal ALT. At high VCM exposure, a c2c2 *CYP2E1* genotype was associated with an increased OR for abnormal ALT (OR 5.4, 95% CI = 0.7 to 35.1) and non-null *GSTT1* genotype was significantly associated with a decreased OR for abnormal ALT (OR 0.3, 95% CI = 0.1 to 0.9). Multiple linear and logistic regression analyses also showed strong interactions of the VCM exposure with *CYP2E1* and *GSTT1* genotypes. These results showed that the two genotypes, *CYP2E1* and *GSTT1*, may play important roles in the biotransformation of VCM, contributing to liver damage. A study[36] showed that, assigning an odds ratio of 1 to the c1c1 homozygotes and adjusting for age, smoking, drinking and cumulative VC exposure, the odds ratio for the occurrence of either or both VC-induced mutant biomarkers (p53 and p21) increased to 2.3 (95% CI = 1.2 to 4.1) for individuals who were either c1c2 heterozygotes or c2c2 homozygotes. Thus, increasing evidence suggests that the *CYP2E1* c2 allele can be a risk factor in VCM-exposed workers, as found in our study.

In rodents, overexpression of GSTP is a characteristic feature of foci of cellular alteration and neoplastic liver lesions induced by genotoxic chemicals.[37] GSTP1 has particular importance in the detoxification of inhaled toxicants because it is the most abundant GST isoform in the lungs.[38] The polymorphisms in exon 5 (Ile105Val) of this gene give rise to enzymes with different thermal stability and substrate affinity.[39] In this case, the results indicated that the presence of the *GSTP1GG* genotype in subjects caused a higher mean MN frequencies than the combined GA and AA genotypes ($P = 0.004$). However, because there are so few studies on the effect of *GSTP1* polymorphisms on chromosome damage, further study is needed to support the role of *GSTP1GG* genotype as a risk factor of chromosome damage in VCM workers.

Identification of workers at elevated risk from VC due to these effects could lead to more effective interventions to reduce their risk. For example, individuals with these polymorphisms could be placed in job categories with lower or no VC exposure and/or advised to minimize their alcohol intake. Alternatively or in addition, pharmacological interventions that reduce the accumulation of the reactive intermediates due to these polymorphisms may be helpful.

In summary, these results suggest that human studies of the genotoxic effects of VCM examining cytogenetic markers like CBMN need to take into account not only the individual demographic or exposure factors but also genetic factors, in particular *CYP2E1* and *GSTP1* polymorphisms. In addition, it should be noted that this model could have much broader implications, because other potential carcinogenic exposures are also metabolized by these pathways, and polymorphisms in these enzymes can be considerably more common in other ethnic groups.

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