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Prevalence and persistence of chromosomal damage and susceptible genotypes of metabolic and DNA repair genes in Chinese vinyl chloride-exposed workers

Fang Ji†, Wei Wang†, Zhao-Lin Xia*, Ying-Jia Zheng, Yu-Lan Qiu, Fen Wu, Wen-Bin Miao, Ru-Feng Jin, Ji Qian[1], Li Jin[1], Yi-Liang Zhu[2] and David C.Christiani[3]

Department of Occupational Health and Toxicology, School of Public Health, Fudan University, and Key laboratory of public health and safety of Ministry of Education of China, 138 Yixueyuan Road, Shanghai, 200032, China, [1]Institute of Genetics, School of Life Science, Fudan University, Shanghai, 200043, China, [2]Department of Epidemiology and Biostatistics, College of Public Health, University of South Florida, Tampa, FL 33612, USA and [3]Department of Environmental Health, Environmental and Occupational Medicine and Epidemiology Program, School of Public Health, Harvard University, Boston, MA 02115, USA

*To whom correspondence should be addressed. Department of Occupational Health and Toxicology, School of Public Health, Fudan University, 138 Yixueyuan Road, Shanghai, 200032, China. Tel/Fax: +86 21 54237050; Email: zlxia@shmu.edu.cn

Vinyl chloride (VC) was classified as a group 1 carcinogen by IARC in 1987. Although the relationship between VC exposure and liver cancer has been established, the mechanism of VC-related carcinogenesis remains largely unknown. Previous epidemiological studies have shown that VC exposure is associated with increased genotoxicity in humans. To explore chromosomal damage and its progression, and their association to genetic susceptibility, we investigated 402 workers exposed to VC, a 77 VC-exposed cohort and 141 unexposed subjects. We measured the frequencies of cytokinesis-block micronucleus (CBMN) to reflect chromosomal damage and conducted genotyping for six xenobiotic metabolisms and five DNA repair genes' polymorphism. Data indicate that 95% of the control workers had CBMN frequencies ≤3‰, whereas VC-exposed workers had the 3.73-fold increase compared with the controls. Among the cohort workers who were followed from 2004 to 2007, the mean CBMN frequency was higher in 2007 than in 2004 with ratio of 2.08. Multiple Poisson regression analysis showed that mean CBMN frequencies were significantly elevated for the intermediate and high exposure groups than the low. Exposed workers with *CYP2E1* or *XRCC1* variance showed a higher CBMN frequency than their wild-type homozygous counterparts, so did workers with *GSTP1* or *ALDH2* genotype. This study provides evidence that cumulative exposure dose of VC and common genetic variants in genes relevant to detoxification of carcinogens are the major factors that modulate CBMN induction in VC-exposed workers.

Introduction

Monomeric vinyl chloride (CH_2=CHCl, VC) is a widely used gas for manufacturing polyvinyl chloride. Although it has been established that VC exposure is associated with liver angiosarcoma and hepatocellular cancer and IARC classified VC as a group 1 carcinogen in 1987 (1,2), it carcinogenic mechanism remains inconclusive. At present, the most coherent view is that 2-chloroacetaldehyde and 2-chloroethylene oxide as the most important VC metabolic intermediates react with DNA bases to form adducts. These DNA adducts are promutagenic and genotoxic and, if not repaired, may eventually induce base pair substitution, chromosomal aberrations, micronuclei (MN), sister chromatid exchange and DNA strand breaks, as observed in lymphocytes of individuals occupationally exposed to VC (3,4).

Among the biomarkers of effects mentioned above, the frequency of MN in human cells has become one of the standard cytogenetic indices for genetic toxicology testing. In humans, MN assays are conducted using cultured peripheral lymphocytes, which lend themselves well to both genotoxicity testing and biomonitoring. The cytokinesis-block micronucleus (CBMN) assay has gained increasing popularity as one of the most promising methodologies presently available. It is based on cytokinesis inhibition by cytochalasin B and has facilitated MN analysis exclusively in binucleate cells that have completed their first *in vitro* division after treatment with the test agent or following culture initiation (5). The main reasons for this development are that CBMNs are easier to observe and count and are less time consuming than chromosomal aberrations, and thus would have immediate application since many studies have used MN frequency as the principal outcome parameter in humans exposed to environmental mutagens (6–8).

Genotoxicity is a complex process involving interactions between multiple factors of endogenous and exogenous origin. Several inter-individual factors have been shown to be associated with variation of MN, such as demography (age and gender), occupational and personal exposures (alcohol drinking and smoking) (9–11). Host factors, including inherited metabolic and repaired traits, may also explain the elevated risk in selected individuals. VC is hepatotoxic as well as carcinogenic in humans who undergo metabolism by the enzyme CYP2E1 to reactive intermediates. These intermediates can cause oncogene and tumor suppressor gene mutations and are then further metabolized by ALDH2 and GSTs to non-mutagenic end products. Most of these xenobiotic-metabolizing enzymes are known to have polymorphic variants with altered activities (12–15) that could produce variable VC metabolism and change the risk of chromosomal damage caused by VC. In addition to metabolic traits, DNA repair capacity may result in inherited susceptibility to chromosomal damage, with individuals who are unable to repair DNA damage or who do so at a slower rate accumulating mutations that may modulate risk (16). Polymorphisms in gene coding for DNA repair enzymes (XRCC1, MGMT, TP53, OGG1 and TDG) have been studied in relation to cancer risk (17–19).

In this study, we investigated the difference in the MN frequency between VC-exposed workers and unexposed controls (402 and 141, respectively). To this aim, CBMN test was conducted in lymphocytes of the individuals. To assess progression of chromosomal damage caused by VC exposure, we followed 77 VC-exposed workers from 2004 to 2007. Moreover, we studied common variants in genes involved in metabolism pathways (phase I and phase II enzymes) and DNA repair pathways, to elucidate the relationship between genetic polymorphisms and chromosomal damage in workers exposed to VC.

Abbreviations: CBMN, cytokinesis-block micronucleus; FR, frequency ratio; MN, micronuclei; PCR, polymerase chain reaction; VC, Vinyl chloride.

†These authors contributed equally to this work.

Materials and methods

Study subjects and epidemiological data

After giving informed consent during medical surveillance processes, workers at two VC polymerization plants in Shanghai (China) were interviewed using interviewer-administered questionnaires. The study was approved by the Fudan University Ethical Review Board. The interview instruments covered demographics, lifestyle, including cigarette-smoking and alcohol consumption, as well as occupational history. Workers were selected if meeting the following criteria: completed detailed questionnaires; continuously exposed to VC for >1 year; provided a blood sample and completed CBMN tests and genotyped for all candidate genes. A total of 402 workers met these criteria, with 289 males and 113 females aged between 20 and 54 years (average 35.04 years). Exposure ranged between 1 and 33 years, with an average of 11.76 years (median 13 years). The controls were comprised of two groups:

group 1 consisted of 41 male and 56 female workers from the same two factories in Shanghai who were not exposed to VC or other known toxicants occupationally; group 2 included 23 male and 21 female healthy residents living in Shanghai. The controls age ranged between 24 and 60 years (mean 45.79 years) for group 1 and between 23 and 57 years (mean 34.70 years) for group 2. Seventy-seven VC-exposed workers were recruited for prospective follow-up, with 46 males and 31 females between the age of 21 and 50 years (average 33.34 years). The exposure duration of the follow-up ranged between 1 and 18 years (average 12.29 years) at the baseline. The follow-up took place between 2004 and 2007. CBMN tests and polymerase chain reaction (PCR)–restriction fragment length polymorphism were conducted again at the end of follow-up.

Environmental monitoring and VC exposure assessment

The concentration of VC was measured for different work sites of the plants using gas chromatography. Because the VC plants had kept VC air concentration data for different work sites from the beginning of its establishment, we were able to estimate the cumulative exposure of each worker with a relatively high level of precision. The cumulative exposure dose was calculated according to an equation as described previously (4,20,21). Personal cumulative exposure dose in the VC exposure group was found to range between 16.78 mg and 301 992 mg, with the median exposure of 8866.56 mg (mean 28 005.58 mg). Based on the estimated cumulative exposure dose, the VC-exposed subjects were further divided into high exposure (>40 000 mg), middle exposure (4000–40 000) and low exposure (<4000 mg).

CBMN assay

The CBMN assay was performed according to standard methods as described by Fenech *et al.* (5,22). For each subject, 1000 binucleated lymphocytes with well-preserved cytoplasm were scored blindly.

Genotyping of polymorphic metabolizing enzymes and DNA repair genes

Genomic DNA was extracted from blood samples by a routine phenol–chloroform method (23). All 402 VC-exposed workers were genotyped for a total of 10 single-nucleotide polymorphisms. Multiplex PCR was used to simultaneously amplify *GSTM1* and *GSTT1* (24), with human β-globin (350 bp) as a control gene. Other genotyping was performed using a PCR–restriction fragment length polymorphism technique.

PCR amplification was accomplished in a volume of 25 μl containing 50 ng genomic DNA, 0.2 μM primer, 0.2 mM deoxynucleotide triphosphates, 2.0 mM $MgCl_2$ and 0.625 U of *Taq* polymerase in 1× reaction buffer. Cycling conditions were 94°C or 5 min, followed by 35 cycles at 94°C for 40 s, 55°C to 69°C for 40 s, 72°C for 30 s to 35 s and a final 10 min extension at 72°C. Details for each polymorphism analysis are summarized in Table I.

After digestion at 37°C (Alw26I, PstI, Bsh1236I, MspI, HinfI and RsaI are from MBI Fermentas, Hanover, MD) and 65°C (TspRI is from New England Biolabs, Beverly, MA) for 12 h and resolved on 2–3% Metaphor agarose gels (BBI, Toronto, Canada), the digested products were observed under UV image system (Gel Doc 2000, Segrate, Milan, Italy). For quality control, 10% masked random samples were tested twice by different researchers, and the results were 100% concordant.

Statistical analysis

SPSS (V15.0) and SAS (V9.13) were used for the statistical analysis. Pearson's χ^2 test was used to examine differences in characteristics among study subjects. The influences of genotype and individual characteristics on the frequencies of CBMN cells per 1000 binucleated cells were determined using univariate and multiple Poisson regression analyses. The significance level (alpha) was set at 5% for all analyses. Mean frequency ratio (FR) and its 95% confidence interval were estimated using $FR = e^{\beta}$ ($e \approx 2.71828$) where β is the regression coefficient for a categorical variable (i.e. binary) in the Poisson model fitted to the MN frequency data. Thus, an FR is the ratio of mean MN frequency in a study group to that of a reference group. Subjects were tested for deviation of genotype frequencies from Hardy–Weinberg equilibrium. Using the 95 percentile of the controls' MN frequency to define a threshold above which the level represents chromosomal damage, odds ratio was estimated to quantify relative risk of chromosomal damage caused by VC exposure.

Table I. Primers and restriction enzymes used for genotyping various single-nucleotide polymorphisms in metabolic and DNA repair genes and allele frequencies in VC-exposed workers

Polymorphisms	Primer sequence (5′–3′)	Annealing temperature (°C)	Restriction enzyme	Length	Fragment size (bp)
GSTT1	F: 5′-TTCCTTACTGGTCCTCACATCTC-3′ R: 5′-TCACCGGATCATGGCCAGCA-3′	58	—	459	—
GSTM1	F: 5′-GAACTCCCTGAAAAGCTAAAGC-3′ R: 5′-GTTGGGCTCAAATATACGGTGG-3′	58	—	219	—
GSTP1 *Ile105Val* (rs1695)	F: 5′-CTTCCACGCACATCCTCTTCC-3′ R: 5′-AAGCCCCTTTCTTTGTTCAGC-3′	58	Alw26I	289	*Val/Val*: 218,71 *Val/Ile*: 289,218,71 *Ile/Ile*: 289
CYP2E1 *c1/c2* (rs3813867)	F: 5′-CAGTCGAGTCTACATTGTC-3′ R: 5′-TTCATTCTGTCTTCTAACTG-3′	58	PstI	410	*c1/c1*: 410 *c1/c2*: 410,290,120 *c2/c2*: 290,120
ADH2 *Arg48His* (rs1229984)	F: 5′-GGGATTAGTAGCAAAACCCTCAAATACA-3′ R: 5′-CACTAACC$\underline{A}^{a}$CGAGGTCATCTG$\underline{C}^{a}$G-3′	60	Bsh1236I	165	*Arg/Arg*: 142,23 *Arg/His*: 165,142,23 *His/His*: 142,23
ALDH2 *Glu504Lys* (rs671)	F: 5′-AACCCATAACCCCCAAGAGT-3′ R: 5′-CAGGTCCCACACTCACAGTTT-3′	60	TspRI	180	*Glu/Glu*: 155,25 *Glu/Lys*: 180,155,25 *Lys/Lys*: 180
OGG1 *Ser326Cys* (rs1052133)	F: 5′-TTGCCTTCGGCCCTGTTCCCCAAGGA-3′ R: 5′-TTGCTGGTGGCTCCTGAGCATGGCCG-3′	65	MspI	168	*Ser/Ser*: 142,26 *Ser/Cys*: 168,142,26 *Cys/Cys*: 168
MGMT *Leu84Phe* (rs12917)	F: 5′-AAGAGTTCCCCGTGCCG$\underline{A}^{a}$C-3′ R: 5′-GCCAAACGCTGCCTCTGT-3′	58	HinfI	178	*Leu/Leu*: 161,17 *Leu/Phe*: 178,161,17 *Phe/Phe*: 178
XRCC1 *Arg280His* (rs25489)	F: 5′-TGGGGCCTGGATTGCTGGGTCTG-3′ R: 5′-CAGCACCACTACCACACCCTGAAGG-3′	69	RsaI	280	*Arg/Arg*: 140 *Arg/His*: 280,140 *His/His*: 280
P53 *Arg72Pro* (rs1042522)	F: 5′-GTCCCAAGCAATGGATGAT-3′ R: 5′-CAAAAGCCAAGGAATACACG-3′	55	Bsh1236I	551	*Pro/Pro*: 551 *Arg/Pro*: 551,443,108 *Arg/Arg*: 443,108
TDG *Gly199Ser* (rs4135113)	F: 5′-GGAAGTGTTTGTTTATGTCAGGGCTC$\underline{C}^{a}$GG$\underline{A}^{a}$GAG-3′ R: 5′-CACCAGCATACTCAAGGTTC-3′	55	MspI	187	*Gly/Gly*: 89,72,26 *Gly/Ser*: 161,89,72,26 *Ser/Ser*: 161,26

[a]Underlined base modifies primer sequence, introducing a restriction site in the presence of the nucleotide.

Results

Frequencies of CBMN in the study subjects and 3 year follow-up of chromosomal damage in the subcohort

The mean and median CBMN frequencies for the control group 1 were 1.24 ± 1.28 and 1.00 (‰), respectively, with a range of 0–5 (‰); the mean and median CBMN frequencies of control group 2 were 1.18 ± 1.16 and 1.00 (‰), respectively, with a range of 0–5 (‰). There was no difference between the two control groups (FR = 1.05; 95% CI: 0.76–1.46; $P = 0.78$). As a result, the two groups were pooled into one 'control group (1 + 2)', with a mean of 1.22 ± 1.24 (‰) and median 1.00 (‰), respectively, and a range of 0–5 (‰). By comparison, the mean and median CBMN frequencies of the 402 VC-exposed workers were higher, with mean 4.55 ± 2.72 (‰), median 4.00 (‰) and range of 0–15 (‰) (Table II). Unadjusted Poisson regression showed the highly significant difference in CBMN frequency between the exposed group and the pooled control group ($P < 0.0001$), with FR = 3.73 (95% CI: 3.20–4.38).

To explore the progression of chromosomal damage over time, we compared the CBMN frequencies between 2004 and 2007 of 77 VC-exposed workers in the follow-up subcohort. The mean of CBMN frequencies in 2004 and 2007 was 3.39 ± 2.20 (‰) and 7.07 ± 4.22 (‰), respectively. Unadjusted Poisson regression also showed that subjects in 2007 had higher mean CBMN frequencies than in 2004 ($P < 0.05$) with the FR = 2.08 and 95% CI: 1.81–2.41.

A normal reference values of CBMN frequencies

A normal reference value of CBMN frequency may be determined based on the CBMN frequencies of 141 controls that were presumably unexposed to VC and healthy. We adopted the 95 percentile of the controls' CBMN frequencies as a threshold, above which the CBMN frequency may indicate an aberration from being normal, hence indicating a chromosomal damage. This resulted an estimated threshold of 3‰ corresponding to the 94.3 percentile of the controls' CBMN frequencies (Table III). Thus, a CBMN frequency >3‰ in a VC-exposed worker is an indicator of chromosomal damage and <3‰ is normal.

Using this threshold (CBMN > 3‰), there were 8 and 246 cases of chromosomal damage in the controls and the exposure group, respectively. This was a 26.22-fold increase in risk of chromosomal damage in the VC-exposed workers from that in the controls (unadjusted OR thinsp;= 26.22; 95% CI: 12.49–55.01; $P < 0.0001$). Among the 156 VC-exposed workers without chromosomal damage (CBMN ≤ 3‰), the median cumulative dose was 70.46 mg/m^3-year or 25.25 p.p.m.-year (7573.10 mg), as compared with 84.14 mg/m^3-year or 30.16 p.p.m.-year(9708.08 mg) the 246 VC-exposed workers with chromosomal damage.

Distribution of, and risk estimates for demographic, habitual factors and genotypes among the VC-exposed workers

Sample size, mean CBMN frequencies among the VC-exposed workers are reported in Table IV by demographic and lifestyle factors, as well as by genotype. It is shown that the mean CBMN frequency was higher in older workers (>35 years) than their younger counterparts (5.06 versus 4.08‰; $P < 0.0001$); mean CBMN frequency was higher for regular drinkers than non-regular or never drinkers (4.96 versus 4.40‰; $P < 0.019$). Mean CBMN frequencies were significantly elevated for the middle (4000–40 000 mg) and high (>40 000 mg) exposure groups compared with that of the low exposure group (4.74 and 4.78 versus 3.99‰, respectively; $P = 0.0030$, $P = 0.0137$, respectively). Difference in the mean CBMN frequencies was not significantly associated with gender and smoking status.

A significant increase in mean CBMN frequency was observed among workers with homozygous variant *CYP2E1 c1/c2* and *XRCC1 Arg280His* homozygous variant and/or heterozygous compared with their counterparts with wild-type homozygous ($P < 0.05$); individuals bearing the *Val/Val* genotype also demonstrated higher mean CBMN frequency than those with the *GSTP1 105Val/Ile*, *Ile/Ile* and *Val/Ile + Ile/Ile* genotypes ($P = 0.01$, 0.002 and 0.004, respectively); persons with the *ALDH2 504Glu/Glu* genotype had higher mean CBMN frequency than the *Lys/Lys* and *Glu/Lys + Lys/Lys* genotypes ($P < 0.001$ and $P = 0.011$, respectively). Differences in MN frequencies were statistically insignificant with respect to genetic polymorphisms in other genes in this study ($P > 0.05$).

Table II. Frequencies of MN in the study subjects

Groups	Number	MN frequencies (‰)	FR (95% CI)	χ^2	P
Control group 1	97	1.24 ± 1.281	1		
Control group 2	44	1.18 ± 1.161	1.047 (0.761–1.461)	0.08	0.78
Pooled group (1 + 2)	141	1.22 ± 1.243	1		
Exposure group	402	4.55 ± 2.751	3.734 (3.204–4.381)	272.89	<0.001

Table III. CBMN frequencies distribution in control group

Number of micronucleus (‰)	Controls			Exposed		
	Frequency	%	Cumulative %	Frequency	%	Cumulative %
0	49	34.8	34.8	12	3.0	3.0
1	47	33.3	68.1	31	7.7	10.7
2	20	14.2	82.3	51	12.7	23.4
3	**17**	**12.1**	**94.3**	**62**	**15.4**	**38.8**
4	6	4.3	98.6	63	15.7	54.5
5	2	1.4	100.0	63	15.7	70.1
6	0	0	100.0	37	9.2	79.4
7	0	0	100.0	31	7.7	87.1
8	0	0	100.0	21	5.2	92.3
9	0	0	100.0	10	2.5	94.8
10	0	0	100.0	8	2.0	96.8
11–15	0	0	100.0	13	3.2	100
Total	141	100.0	100.0	402	100.0	100.0

Multiple Poisson regression model for frequencies of total micronucleus

We performed a multiple Poisson regression analysis for CBMN frequencies, while adjusting for the potential impact of a host of genetic polymorphisms as well as demographic and lifestyle factors (e.g. cumulative exposure dose of VC, gender, smoking status and alcohol consumption). The results (Table V) revealed five factors that significantly altered CBMN frequencies: cumulative exposure dose of VC, *GSTP1*, *CYP2E1*, *ALDH2* and *XRCC1 Arg280His* genotypes. This analysis confirmed the increase in CBMN frequency with cumulative exposure dose of VC; mean CBMN frequencies were significantly elevated for the middle (4000–40 000 mg) and high (>40 000 mg) exposure groups compared with that of the low exposure group ($P = 0.003$, $P = 0.03$, respectively). Specifically, subjects with *CYP2E1* and *XRCC1 Arg280His* variance showed higher mean CBMN frequencies compared with their wild-type homozygous counterparts ($P = 0.02$); those with *GSTP1 Val/Val* genotype and *ALDH2 Glu/Glu* genotype showed higher mean CBMN frequencies than those with other genotypes ($P = 0.01$ and 0.003, respectively).

Discussion

We showed that workers exposed to VC have elevated levels of micronucleus (MN) induction when compared with the unexposed controls (25–27). Our data also showed that workers exposed to VC for an

Table IV. Demographics, lifestyle and genes as risk factors among VC-exposed workers

Characteristic	No. (%)[a]	MN frequency (‰)	FR (95% CI)	χ^2	*P*
Gender					
Male	289 (71.89)	4.51 ± 2.70	1		
Female	113 (28.11)	4.68 ± 2.89	1.04 (0.94–1.15)	0.55	0.46
Age					
Younger (≤35)	207 (51.49)	4.08 ± 2.51	1		
Elderly (>35)	195 (48.51)	5.06 ± 2.91	1.24 (1.13–1.36)	20.85	<0.001
Smoke habit					
Never smoke	213 (52.99)	4.43 ± 2.79	1		
Current + former smoke	189 (47.01)	4.69 ± 2.71	1.06 (0.97–1.16)	1.50	0.22
Drink habit					
No-habitual + never drink	289 (71.89)	4.40 ± 2.74	1		
Habitual drink	113 (28.11)	4.96 ± 2.74	1.13 (1.02–1.24)	5.54	0.02
Cumulative exposure Dose					
Low exposure (<4000 mg)	103 (25.62)	3.99 ± 2.07	1		
Middle exposure (4000–40 000 mg)	227 (56.47)	4.74 ± 2.99	1.19 (1.06–1.33)	8.82	0.003
High exposure (>40 000 mg)	72 (17.91)	4.78 ± 2.74	1.20 (1.04–1.38)	6.07	0.01
GSTM1					
−	158 (39.30)	4.45 ± 2.62			
+	244 (60.70)	4.62 ± 2.84	0.96 (0.88–1.06)	0.63	0.43
GSTT1					
−	209 (51.99)	4.44 ± 2.87	1		
+	193 (48.01)	4.67 ± 2.62	0.95 (0.87–1.04)	1.15	0.28
GSTP1 Ile105Val					
Val/Val	12 (2.98)	6.33 ± 3.14	1		
Val/Ile	162 (40.30)	4.67 ± 2.76	0.74 (0.59–0.94)	6.39	0.01
Ile/Ile	228 (56.72)	4.38 ± 2.70	0.69 (0.55–0.88)	9.64	0.002
Val/Ile + Ile/Ile	390 (97.02)	4.50 ± 2.72	0.71 (0.57–0.90)	8.51	0.004
CYP2E1 c1/c2					
c1c1	262 (65.34)	4.37 ± 2.67	1		
c1c2	121 (30.17)	5.02 ± 2.98	1.15 (1.04–1.27)	7.55	0.006
c2c2	18 (4.49)	4.33 ± 1.91	0.99 (0.78–1.24)	0.01	0.94
c1c2 + c2c2	139 (34.66)	4.93 ± 2.87	1.13 (1.03–1.24)	6.19	0.01
ADH2 Arg48His					
His/His	205 (51.12)	4.49 ± 2.70	1		
Arg/His	177 (44.14)	4.59 ± 2.73	1.02 (0.93–1.13)	0.23	0.63
Arg/Arg	19 (4.74)	5.00 ± 3.54	1.11 (0.90–1.37)	1.01	0.32
Arg/His + Arg/Arg	196 (48.88)	4.63 ± 2.81	1.03 (0.94–1.13)	0.46	0.50
ALDH2 Glu504Lys					
Glu/Glu	192 (48.12)	4.84 ± 2.73	1		
Glu/Lys	170 (42.61)	4.51 ± 2.81	0.93 (0.85–1.03)	2.13	0.14
Lys/Lys	37 (9.27)	3.38 ± 2.29	0.70 (0.58–0.84)	14.22	<0.001
Glu/Lys + Lys/Lys	207 (51.88)	4.30 ± 2.75	0.89 (0.81–0.98)	6.22	0.01
OGG1 Ser326Cys					
Ser/Ser	42 (10.45)	4.12 ± 2.76	1		
Ser/Cys	201 (50.00)	4.66 ± 2.83	1.13 (0.97–1.34)	2.24	0.14
Cys/Cys	159 (39.55)	4.53 ± 2.66	1.10 (0.94–1.30)	1.29	0.26
Ser/Cys + Cys/Cys	360 (89.55)	4.61 ± 2.75	1.12 (0.96–1.31)	1.95	0.16
MGMT Leu84Phe					
Leu/Leu	334 (83.08)	4.60 ± 2.70	1		
Leu/Phe	68 (16.92)	4.35 ± 3.01	0.95 (0.84–1.07)	0.73	0.39
XRCC1 Arg280His					
Arg/Arg	293 (73.80)	4.41 ± 2.73	1		
Arg/His	100 (25.19)	4.90 ± 2.79	1.11 (0.10–1.23)	3.89	0.05
His/His	4 (1.01)	6.25 ± 2.87	1.42 (0.93–2.06)	2.97	0.09
Arg/His + His/His	104 (26.20)	4.95 ± 2.79	1.12 (1.01–1.24)	4.89	0.03
P53 Arg72Pro					
Arg/Arg	116 (29.29)	4.77 ± 2.77	1		
Arg/Pro	193 (48.74)	4.64 ± 2.76	0.97 (0.82–1.18)	0.24	0.62
Pro/Pro	87 (21.97)	4.21 ± 2.72	0.88 (0.77–1.01)	3.44	0.06
Arg/Pro + Pro/Pro	280 (70.71)	4.51 ± 2.75	0.95 (0.86–1.05)	1.21	0.27
TDG Gly199Ser					
Gly/Gly	264 (65.84)	4.62 ± 2.82	1		
Gly/Ser	123 (30.67)	4.45 ± 2.54	0.96 (0.87–1.06)	0.58	0.45
Ser/Ser	14 (3.49)	4.14 ± 3.42	0.90 (0.68–1.15)	0.67	0.41
Gly/Ser + Ser/Ser	137 (34.16)	4.42 ± 2.63	0.96 (0.87–1.05)	0.86	0.35

$P < 0.05$ with regard to the corresponding group.
[a]Some data were missing due to inability to amplify DNA.

Table V. Multiple (adjusted) Poisson regression model for CBMN frequencies in VC-exposed workers

Parameter	Estimate	Estimate 95% CI		χ^2	*P*	FR (95% CI)
		Lower	Upper			
Intercept	1.89	1.36	2.40	50.70	<0.001	—
Cumulative exposure dose (mg)						
<4000 (ref. group)	—	—	—	—	—	—
4000–40 000	0.18	0.06	0.30	8.96	0.003	1.19 (1.06–1.34)
>40 000	0.16	0.01	0.30	4.62	0.03	1.17 (1.01–1.35)
GSTP1 Ile105Val (*Ile*)	−0.31	−0.53	−0.07	6.69	0.01	0.74 (0.59–0.94)
CYP2E1 c1/c2 (*c2*)	0.11	0.02	0.21	5.47	0.02	1.12 (1.02–1.23)
ALDH2 Glu504Lys (*Lys*)	−0.14	−0.24	−0.05	8.74	0.003	0.87 (0.79–0.95)
XRCC1 Arg280His (*Arg*)	0.12	0.02	0.22	5.23	0.02	1.13 (1.02–1.25)

average of 11.72 years had elevated frequencies of CBMN than the unexposed controls. This implies that the induction of MN is a sensitive cytogenetic endpoint for detecting genotoxic activity caused by VC exposure. To explore the progression of chromosomal damage in Chinese workers exposed to VC, the CBMN test was performed prospectively in peripheral lymphocytes to detect chromosomal damage of 77 VC-exposed workers at both 2004 and 2007, the end of the 3 year followed. Ours is the first report of a prospective evaluation of CBMN in VC-exposed workers. Our findings show that CBMN frequencies observed in 2007 were significant higher than in 2004, suggesting that the increase in CBMN is correlated with the duration of VC exposure. Thus, CBMN frequency appears a biomarker suitable for estimating *in vivo* VC dose.

We have attempted to develop a normal reference value of CBMN frequencies in cultured peripheral lymphocytes. Using the observed 95 percentile of the controls, we were able to divide all subjects into two groups, normal ($\leq 3‰$) and chromosomal damaged ($>3‰$). Based on this working definition, the risk of chromosomal damage in the VC-exposed workers was significantly elevated (61.2%, 246 out of 402) over that of the controls. Of the 156 (38.8%) VC-exposed workers whose CBMN frequency was normal, the median cumulative exposure was 70.46 mg/m^3-year. Assuming a work life of 40 years, the annual exposure is 1.76 mg/m^3 (0.63 p.p.m.), lower than occupational exposure limits of VC in most developed counties.

Genotoxicity is a complex process involving interactions between multiple factors of endogenous and exogenous origin. As a result of this complexity, the contribution of a single risk factor to the intra- and inter-individual variation of MN is difficult to assess in many situations. In our study, we took into consideration several inter-individual factors such as demographic (age and gender), lifestyle (alcohol drinking and smoking) and occupational factors. The results of unadjusted Poisson regression analyses indicated that older workers (>35 years) had higher CBMN frequencies than younger workers ($\leq$35 years). Considering that age might be a confounding effect with exposure duration, we excluded age as a potential confounder of exposure in multiple (adjusted) Poisson regression analysis. Unadjusted Poisson regression analyses showed that the higher frequencies of CBMN were associated with alcohol drinking, which was not the case with multiple (adjusted) Poisson regression analysis. This inconsistency suggests that using CBMN as a biomarker for human VC genotoxicity must account for other risk factors and confounders. Consistent results showed that CBMN frequency trend to elevated with the increase of VC cumulative exposure dose ($P < 0.05$).

Inter-individual variability in human responses to occupational toxins has been extensively studied. For instance, polymorphic loci have received increasing attention with respect to coding for phase I and II enzymes in the activation and detoxification of carcinogen. Previous studies have revealed that *CYP2E1 c1c2/c2c2* and *ALDH2 1-2/2-2* polymorphisms are associated with an increased frequency of sister chromatid exchange (28) and that *CYP2E1 c2c2* and *GSTT1* wild-type is associated with abnormal liver function among VC-exposed workers (29) and that the *CYP2E1* variant *c2* allele is significantly associated with an increased mutagenic risk even after controlling for potential confounders (30). Our present study confirms that the *CYP2E1 c2* allele may be a risk factor in VC-exposed workers, as described previously (21,30). GSTP1 is a major GST enzyme in the human lung; it is thought to be of particular importance in the detoxification of inhaled carcinogens. Enzyme activity is significantly lower in individuals with the *GSTP1 105Val* allele (31). The results of our study show that the presence of the *GSTP1 105Val/Val* genotype had a higher risk of chromosomal damage than the *105Val/Ile + Ile/Ile* genotypes ($P = 0.01$). This is perhaps because that the catalytic activity, substrate specificity and thermal stability of the enzyme are influenced by the *GSTP1 Ile105Val* substitution, leading to decreased enzymatic activity for some substrates (32,33) and increasing the risk of chromosomal damage caused by VC. *ALDH2* is 1 of 19 members of the human *ALDH* gene family of NAD(P)$^+$-dependent enzymes (34). In addition to principal enzyme involved in acetaldehyde oxidation, ALDH2 is the dehydrogenase that catalyzes 2-chloroacetaldehyde to yield non-genotoxic products for excretion. In this study, multiple (adjusted) Poisson regression analysis revealed a higher MN frequency with *ALDH2 504Glu/Glu* genotype than other genotypes. More genetic epidemiological investigations in China are required to disclose any possible reciprocal relationship between genetic damage and the *ALDH2* genotypes and identify the other risk factors that appear to be present.

In addition to metabolic traits, DNA repair system also plays an important role in VC-related carcinogenesis. The reactive intermediates of VC generate promutagenic etheno–DNA adducts that can cause the types of mutations in the exposed workers and an increased occurrence of the biomarkers for these mutations. Evidence has been presented to indicate that VC derivative etheno–DNA adducts may be removed by the base excision repair pathway (35). The X-ray repair cross-complementing gene 1 (*XRCC1*) is a key factor in the base excision repair pathway and is required for an efficient repair of DNA single-strand breaks (36). From our investigation, we found that subjects having *XRCC1 Arg280His* variant allele had significantly more chromosome damage as determined by the higher CBMN frequencies compared with those of subjects having the wild-types. This elevated risk is further evidence that the polymorphisms in *XRCC1* can be an important biomarker of susceptibility in populations exposed to VC.

As far as our study is concerned, we acknowledge some limitations. First, the cumulative VC exposure employed in this study may not completely reflect personal exposure, and the use of more accurate methods such as personal air sampling or VC metabolites may improve the assessment of the actual dose. Secondly, CBMN frequencies as biomarker of genotoxic effect produced by VC can indicate potential health impairment, although are not specific to particular endpoints. DNA adducts produced by the reaction of VC metabolites with DNA may serve as a specific indicator of VC's genotoxic potential. Thirdly, we investigated only 11 genes involved in metabolic and DNA repair pathways. Further understanding of the complexity of the relationships between VC exposure and multiple genes within pertinent pathways is needed. Technological advances in genotyping will help move the field forward.

In conclusion, we have demonstrated in this study that the persistence of residual genotoxic damage induced by VC can be characterized in CBMN frequencies, and progression in chromosomal damage was evident at the end of a 3 year follow-up. Chromosome damage as determined by exceedingly high levels of CBMN, defined as $>3‰$, appear suitable as a biodosimetry and biomarker for estimation of *in vivo* dose of VC exposure. Furthermore, our findings provide evidence that the combination of demographic variables, lifestyles and genetic polymorphisms related to VC metabolism and DNA repair play a role in VC-induced chromosome damage. Further study to investigate the relationship of individual characteristics and genetic susceptibility with VC-caused chromosome damage is warranted. These findings may have more general implications. Because other potential carcinogenic exposures are also metabolized and repaired by these same pathways considered here, what we have learned here contributes to our knowledge of human carcinogenesis. Finally, the elevated risk of chromosome damage among the VC-exposed workers invites re-evaluation of the health risk associated with existing occupational exposure limits employed in many industrial counties.

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