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Genetic polymorphisms of *XRCC1*, *HOGG1* and *MGMT* and micronucleus occurrence in Chinese vinyl chloride-exposed workers

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In this study, a group of 313 workers occupationally exposed to vinyl chloride monomer (VCM) and 141 normal unexposed referents were examined for chromosomal damage using the cytokinesis-blocked micronucleus (CBMN) assay in peripheral lymphocytes. We explored the relationship between genetic polymorphisms of *XRCC1* (*Arg194Trp*, *Arg280His* and *Arg399Gln*), *MGMT*(*Leu84Phe*) and *hOGG1* (*Ser326Cys*) and susceptibility of chromosomal damage induced by VCM. Polymerase chain reaction–restriction fragment length polymorphism techniques were used to detect polymorphisms in *XRCC1*, *hOGG1* and *MGMT*. It was found that the micronuclei (MN) frequency of exposed workers (4.86 ± 2.80)‰ was higher than that of the control group (1.22 ± 1.24)‰ ($P < 0.01$). Increased susceptibility to chromosomal damage as evidenced by higher MN frequency was found in workers with *hOGG1 326 Ser/Cys* genotype [frequency ratio (FR) = 1.21, 95% confidence interval (CI): 1.02–1.46; $P < 0.05$], *XRCC1 194 Arg/Trp* (FR = 1.12, 95% CI: 1.00–1.25; $P < 0.05$) and *XRCC1 280 Arg/His* and *His/His* genotypes (FR = 1.12, 95% CI 1.00–1.26, $P < 0.05$). Moreover, among susceptibility diplotypes, *CGA/CAG* carriers had more risk of MN frequency compared with individuals with wild-type *CGG/CGG* (FR = 1.67, 95% CI: 1.19–2.23; $P < 0.05$). MN frequency also increased significantly with age in the exposed group (FR = 1.13, 95% CI: 1.00–1.28; $P < 0.05$). Thus, CB-MN was a sensitive index of early damage among VCM-exposed workers. Genotype *XRCC1 Arg194Trp*, *Arg280His*, *hOGG1 Ser326Cys*, diplotype *CGA/CAG* and higher age may have an impact on the chromosome damage induced by VCM

Introduction

Vinyl chloride monomer (CH_2=CHCl, VCM), the main material used in the polymerization process of polyvinyl chloride, is a human carcinogen, according to the classification of International Agency for Research on Cancer (1987) (1). Previous studies have reported modest associations between many metabolism and/or DNA repair gene polymorphisms and genotoxicity or carcinogenicity in VCM workers (2).

In terms of DNA repair, the focus has been primarily on the base excision repair (BER) pathway in which human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) and X-ray repair cross-complementing group 1 (*XRCC1*) are key components. The involvement of *hOGG1* and *XRCC1* gene products in the repair of oxidized bases and single-strand DNA breaks, respectively, is well documented (3). Moreover, despite some controversial results (4–6), genetic variants in *hOGG1* and *XRCC1* genes have been associated with cancer risk. HOGG1 is responsible for the removal of the highly mutagenic 7, 8-dihydro-8-oxoguanine DNA lesion via its DNA glycosylase/apurinic lyase activities. The repair of single-strand DNA breaks, arising directly from damage to the deoxyribose moieties or indirectly as intermediates of the BER pathway (7), is facilitated by the scaffold protein XRCC1 via its ability to interact with DNA ligase IIIα, DNA polymerase β, apurinic/apyrimidinic endonucleases, polynucleotide kinase/phosphatase, poly (adenosine diphosphate-ribose) polymerases 1 and 2, hOGG1, hNEIL1 and DNA-dependent protein kinase (8–11).

A major defense against alkylating mutations is provided by O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein that transfers potentially carcinogenic O^6 alkylation adducts from the DNA to a cysteine residue of MGMT (12,13). For each adduct removed, an *MGMT* molecule is inactivated. Single-nucleotide polymorphisms (SNPs) of the *MGMT* gene have also been associated with increased risks of cancer, especially among those exposed to alkylating mutagens.

The frequency of micronuclei (MN) in peripheral blood lymphocytes is extensively used as a biomarker of chromosomal damage and genome stability in human populations. The CB-MN assay is based on cytokinesis inhibition by cytochalasin B (Cyt-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 (14). CB-MN is easier to detect and more useful for predicting long-term risk associated with human exposure to mutagenic and carcinogenic agents in workplaces, in the environment and in lifestyles. (15)

As noted, previous investigations have reported modest associations between DNA repair gene polymorphisms and genotoxicity as well as carcinogenicity in cancer patients, and a small number of studies had investigated associations between these DNA repair gene polymorphisms and risks of chromosomal damage in VCM workers. The aim of the present study was to determine the influence of common polymorphisms in *hOGG1*, *XRCC1* and *MGMT* repair genes and VCM exposure levels on MN levels in peripheral blood lymphocytes of VCM exposure workers.

Material and methods

Study subjects

On the basis of employment records, information was collected from workers at a polyvinyl chloride polymerization plant in Shanghai, China, by use of personal interview questionnaires with appropriate informed consent during routine medical surveillance. A total of 313 workers who had been occupationally exposed to VCM for at least 1 year and for whom blood samples and completed questionnaires were available were included for analysis as study subjects. A total of 141 service workers and managers from the same factory with a similar age distribution but without VCM exposure who also agreed to provide a blood sample and completed questionnaires were selected as a reference group.

A 10 ml anticoagulated peripheral blood sample was collected from each subject. Blood samples were stored at room temperature in an insulated container and were delivered to the laboratory within 12 h of collection. Each control and exposed worker completed a detailed questionnaire, and cytokinesis-blocked micronucleus assays (CBMN) and polymerase chain reaction (PCR)–restriction fragment length polymorphism analyses were performed on their blood samples.

Assessment of vinyl chloride exposure

The level of VCM was measured at different worksites in the company overtime. The levels ranged from 0.18 p.p.m. (0.50 mg/m^3) to 108.30 p.p.m. (302.16 mg/m^3) in the air with a geometric mean concentration of 2.56 p.p.m. (7.15 mg/m^3). Because the VCM plant had kept VCM air concentration

Abbreviations: BER, base excision repair; CI, confidence interval; CBMN, cytokinesis-blocked micronucleus; FR, frequency ratio; *hOGG1*, human 8-oxoguanine glycosylase 1; MN, micronuclei; *MGMT*, O^6-methylguanine-DNA methyltransferase; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; VCM, vinyl chloride monomer; *XRCC1*, X-ray repair cross-complementing group 1.

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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:

$$\text{cumulative exposure dose (mg)} = \sum \left(C*M*T*A \times 70\% / 10^6 \right),$$

where *C*, the geometric mean of VCM exposure concentration (in milligrams per cubic meter) for each month in a given workplace (calculated for all the different worksites); M, the number of exposure months of each year for a VCM worker; T, the 2 h exposure time in each working day, 20 days in each month, giving 2400 min exposure time per month and A is alveolar ventilation (male average = 6500 ml/min and female average = 4300 ml/min, assuming 30% dead space). By this method, personal cumulative exposure doses in the VCM exposure group ranged from 10.3 to 301 992.0 mg. Based on this, the VCM-exposed subjects then were divided into high-exposure, middle-exposure and low-exposure groups with cumulative doses of >10 000, 1000–10 000 and <1000 mg, respectively.

CBMN assay

The CBMN assay was performed according to standard methods as described by Fenech (16). In brief, 0.5 ml heparin anticoagulated whole blood was added to 4.5 ml of medium (RMPI 1640) and incubated at 37°C, 5% CO_2 level. Cytochalasin-B (Sigma-Aldrich, St. Louis, MO) was added to each cell culture after 44 h at a final concentration of 6 μg/ml to prevent cytokinesis. Twenty-eight hours after the addition of cytochalasin B, cells were harvested by cytocentrifugation and fixed with methanol and acetic acid at a ratio of 3:1. Slides were air-dried and stained with Giemsa and scored for CBMN.

Genotyping of DNA repair genes

From 2 ml heparin anticoagulated whole blood samples collected from each worker, DNA was collected from peripheral lymphocytes using commercial DNA extraction kits and stored frozen at −80°C. Approximately 50 ng of genomic DNA was amplified in GeneAmp 9600 (Perkin Elmer Corporation, Waltham, MA) in a total volume of 15 μl consisting of 0.4 μl for each primer, 7.5 μl 2× PCR mix, 5.7 μl ddH_2O and 1μl DNA.

PCR was performed under the following conditions: 95°C for 5min, followed by 30 cycles of 94°C for 40 s, annealing temperature for 20 s (the annealing temperatures were 58°C for *Arg194Trp*, *Arg399Gln* and *MGMT Leu84Phe*; 65°C for *hOGG1 Ser326Cys* and 69.5°C for *Arg280His*) and 72°C for 25 s and a final elongation step at 72°C for 10 min.

The PCR products were digested at 37°C for 12 h with restriction endonucleases (Fermentas, Burlington, Ontario, Canada) as follows: MspI for *Arg194Trp*, *Arg399Gln* and *HOGG*; HinfI for *MGMT Leu84Phe* and RsaI for *Arg280His*.

XRCC1 polymorphisms (*Arg194Trp*, *Arg280His* and *Arg399Gln*) were detected using the following primers: F, 5′-GCCCCGTCCCAGGTA-3′ and R, 5′-AGCCCCAAGACCCTTTCACT-3′ for *Arg194Trp*; F, 5′-TGGGGCCTGGATTGCTGGGTCTG-3′ and R, 5′-CAGCACCACTACCACACCCTGAAGG-3′ for *Arg280His* and F, 5′-TTGTGCTTTCTCTGTGTCCA-3′ and R, 5′-TCCTCCAGCCTTTTCTGATA-3′ for *Arg399Gln.* The *XRCC1 194 Arg/Arg* genotype resulted in 346 and 37 bp bands; *Arg/Trp* in 383, 346 and 37 bp bands and *Trp/Trp* in a 383 bp band. The *XRCC1 280 Arg/Arg* genotype resulted in a 140 bp band and *His/His* in a 280 bp band. The *XRCC1 399 Arg/Arg* genotype resulted in 277 and 186 bp bands; *Arg/Gln* in 463, 277 and 186 bp bands and Gln/Gln in a 463 bp band.

HOGG1 polymorphism (*Ser326Cys*) was detected using the following primers: F, 5′-TTGCCTTCGGCCCTGTTCCCCAAGGA-3′ and R, 5′-TTGCTGGTGGCTCCTGAGCATGGCCG-3′. The *hOGG1 326 Ser/Ser* genotype resulted in 142 and 26 bp bands; *Ser/Cys* in 168, 142 and 26 bp bands and *Cys/Cys* in a 168 bp band.

MGMT polymorphism (*Leu84Phe*) was detected using the following primers: F, 5′-AAGAGTTCCCCGTGCCGAC-3′ and R, 5′-GCCAAACGCTGCCTCTGT-3′. The *MGMT 84 Leu/Leu* genotype resulted in 161 and 17 bp bands; Leu/Phe in 178, 161 and 17 bp bands and *Phe/Phe* in a 178 bp band.

Statistical methods

The risks of chromosomal damage associated with the genotypes were estimated by computing frequency ratios (FRs = eβ, e = 2.71828;β, regression coefficient) and 95% confidence intervals (CIs) from univariate and multivariate Poisson regression models with adjustments for age, sex, smoking status, alcohol drinking and cumulative VCM exposure. For categorical variables, the FR indicated a proportional increase/decrease of the MN frequency in a comparison group relative to the referent. All statistical analyses were done using the software SAS (SAS Institute Inc, Cary, NC). We assessed the statistical significance of tests for the Hardy–Weinberg equilibrium and linkage disequilibrium analysis using the method described by Shi and He (17).

PHASE software (version 2.0.2, University of Washington, Seattle) was used to obtain maximum-likelihood estimates of the *XRCC1* haplotype frequencies.

Results

Subject characteristics and risk estimates for demographic and lifestyle factors

Table I presents the study demographics and lifestyle characteristics of the workers, along with their associations with MN frequency. The average age of 313 ethnic Han Chinese workers was 36 years, range 23–57. There was a small increase in MN frequency among women but no statistically significant difference. No significant difference in MN frequency was detected in association with smoking and alcohol drinking status. However, the older age group exhibited more susceptibility to damage than the younger age group (FR = 1.13, 95% CI: 1.00–1.28; $P < 0.05$). The exposure group (4.86 ± 2.80)‰ showed higher MN frequency than the controls (1.22 ± 1.24)‰ ($P < 0.01$). Poisson regression showed that each VCM-exposed cumulative workers group had a significantly increased MN frequencycompared with the control group (FR = 3.27, 95% CI: 2.72–3.94; $P < 0.01$); (FR = 4.19, 95% CI: 3.69–4.92; $P < 0.01$) and (FR = 3.99, 95% CI: 3.38–4.73; $P < 0.01$), respectively.

Distribution of genotypes and risk assessment for genes polymorphisms

The frequencies of *hOGG1*, *MGMT*, *XRCC1* genotypes are found in Table II, and their association with chromosome damage (by CB-MN) in workers is presented in Table III. The allele frequencies for *hOGG1 326Cys*, *MGMT84Phe*, *XRCC1 194 Trp*, *XRCC1 280 His* and *XRCC1 399 Gln* were 0.65, 0.08, 0.30, 0.14 and 0.32, respectively. Genotype distributions at each locus were consistent with Hardy–Weinberg equilibrium. *MGMT 84* and *XRCC1 399* polymorphisms showed no significant associations with MN frequency. Nevertheless, individuals with *XRCC1 194 Arg/Trp* genotype had a significantly higher MN frequency compared with those carrying *Arg/Arg* genotype (FR = 1.12, 95% CI: 1.00–1.25; $P < 0.05$); *XRCC1 280 Arg/His* and *His/His* genotype had a significantly higher MN frequency compared with those carrying *Arg/Arg* genotype (FR = 1.12, 95% CI: 1.00–1.26; $P < 0.05$) and *hOGG1 326 Ser/Cys* genotype had a significantly higher MN frequency compared with those carrying *Ser/Ser* genotype (FR = 1.21, 95% CI: 1.02–1.46; $P < 0.05$).

Multiple Poisson analysis

Table IV shows the results of multivariate analysis using a backward stepwise selection of variables, including age, sex, smoking status, alcohol drinking, cumulative VCM exposure and polymorphisms of *XRCC1*, *hOGG1* and *MGMT*. The final model revealed subjects with *hOGG1 326 Ser/Cys*, *XRCC1 194 Arg/Trp* and *XRCC1 280 Arg/His* variant alleles (based on a dominant genetic inheritance model) each had a statistically significantly higher MN frequency, when compared with their respective wild-type homozygous counterparts. Higher age was also associated with higher MN frequency.

Diplotypes of XRCC1 and MN frequency

To further elucidate the relevance of *XRCC1* variants with MN frequency, linkage disequilibrium among the three *XRCC1* polymorphisms (*Arg194Trp*, *Arg280His* and *Arg399Gln*) were analyzed and haplotypes were reconstructed. The D′ value of the three loci of *XRCC1* were 0.883 (194 with 280), 0.651 (194 with 399) and 0.457 (280 with 399). For all subjects, 16 XRCC1 (194)-(280)-(399) diplotypes were identified in the analysis of the workers by the PHASE 2.0.2 software. Among these haplotype pairs, the rare diplotypes (<5% frequency) were analyzed as a group. The diplotype CGG/CGG that consists of the wild-type sequence in all loci was selected

Table I. MN frequency of VCM-exposed workers and unexposed controls by various demographic characteristics

	Unexposed controls		Exposed workers		
	N	Mean MN ± SD (‰)	*N*	Mean MN ± SD (‰)	FR (95% CI)
Age (years)					
≤35	87	1.11 ± 1.24	151	4.48 ± 2.53	1
>35	54	1.83 ± 1.25	162	5.21 ± 2.99	1.13 (1.00–1.28)[a]
Gender					
Male	64	1.25 ± 1.36	232	4.74 ± 2.89	1
Female	77	1.32 ± 1.21	81	4.90 ± 2.77	1.04 (0.89–1.21)
Drinking					
No	113	1.28 ± 1.21	203	4.72 ± 2.84	1
Yes	28	1.04 ± 1.20	110	5.11 ± 2.72	1.05 (0.93–1.18)
Smoking					
No	106	1.20 ± 1.23	159	4.56 ± 2.81	1
Yes	35	1.26 ± 1.53	154	5.06 ± 2.79	1.04 (0.90–1.19)
Cumulative dose					
≤1000 mg	—	—	85	4.44 ± 2.14	3.27 (2.72–3.94)[b]
1000–10 000 mg	—	—	98	5.15 ± 3.12	4.19 (3.69–4.92)[b]
>10 000 mg	—	—	130	4.91 ± 2.91	3.99 (3.38–4.73)[b]
Total	141	1.22 ± 1.24	313	4.86 ± 2.80	3.76 (3.20–4.44)[b]

‰, per thousand lymphocytes; SD, standard deviation.
[a]Compared in same groups $P < 0.05$.
[b]Compared with control $P < 0.01$.

Table II. Distribution of genotypes and allele frequencies

Allele site	Genotype	Number	Rate (%)	Frequency
XRCC1 194	GG	156	49.8	G:0.703
	AG	131	41.9	A:0.297
	AA	26	8.3	
XRCC1 280	GG	230	73.5	G:0.860
	AG	80	1.0	A:0.140
	AA	3	1	
XRCC1 399	GG	156	49.8	G:0.679
	AG	116	37.1	A:0.321
	AA	41	13.1	
hOGG1	GG	35	11.2	G:0.353
	GC	151	48.2	C:0.647
	CC	127	40.6	
MGMT	CC	264	84.3	C:0.922
	CT	49	15.7	T:0.078
	TT	0	0	

Table III. MN frequency among VCM-exposed workers by DNA repair gene polymorphism

Genetype	*N*	Mean MN ± SD (‰)	FR (95% CI)	*P*-value
XRCC1 194				
Arg/Arg	156	4.72 ± 2.65	1	
Arg/Trp	131	5.18 ± 2.98	1.12 (1.00–1.25)*	0.05
Trp/Trp	26	4.00 ± 2.55	0.89 (0.72–1.12)	0.34
Arg/Trp and Trp/Trp	157	4.56 ± 2.75	0.94 (0.77–1.19)	0.14
XRCC1 280				
Arg/Arg	230	4.67 ± 2.76	1	
Arg/His and His/His	83	5.36 ± 2.86	1.12 (1.00–1.26)*	0.04
XRCC1 399				
Arg/Arg	156	4.81 ± 2.80	1	
Arg/Gln	116	4.44 ± 2.80	0.96 (0.80–1.13)	0.61
Gln/Gln	41	5.06 ± 2.81	1.04 (0.93–1.17)	0.46
Arg/Gln and Gln/Gln	157	4.65 ± 2.77	0.97 (0.86–1.21)	0.51
hOGG1 326				
Ser/Ser	35	4.11 ± 2.93	1	
Ser/Cys	151	5.13 ± 2.87	1.21 (1.02–1.46)*	0.03
Cys/Cys	127	4.73 ± 2.65	1.12 (0.94–1.35)	0.19
MGMT 84				
Leu/Leu	264	4.84 ± 2.73	1	
Leu/Phe and Phe/Phe	49	4.94 ± 3.19	1.02 (0.89–1.17)	0.77

Compared with wild-type for each genotype case *$P < 0.05$. *N*, number in each category; ‰, per thousand lymphocytes; SD, standard deviation.

as reference. FRs associated with various diplotype in all study subjects are presented in Table V. Compared with individuals with wild-type *CGG/CGG*, multiple Poisson regression showed that FR adjusted by age, gender and cumulative exposure dose increased for the individuals with *CGA/CAG* (FR = 1.67, 95% CI: 1.19–2.23; $P < 0.05$), *TGA/TGA* (FR = 1.13, 95% CI: 1.07–1.36; $P < 0.05$), *TGG/CGG* (FR = 1.27, 95% CI: 1.09–1.44; $P < 0.05$), *CAG/CAG* (FR = 1.11, 95% CI: 1.07–1.46; $P < 0.05$) and *TAG/TAG* (FR = 1.18, 95% CI: 1.11–1.52; $P < 0.05$).

Discussion

The aim of this study was to explore the association between polymorphisms of DNA repair genes and chromosomal damage induced by VCM. Our results confirmed that VCM exposure was associated with the risk of chromosomal damage. Moreover, high-age workers had more evidence of chromosomal damage determined by the MN assay. We also observed that workers who possessed the *XRCC1 194 Arg/Trp*, *XRCC1 280 Arg/His* and *His/His* genotype and *hOGG1 326 Ser/Cys* genotype faced a significantly higher risk of chromosomal damage.

The detection of MN in binucleated cells by means of the *ex vivo/in vitro* CBMN assay (16) has been successfully employed as a reliable biomarker of exposure to chemical agents (18,19). More recently, a large international cohort study conducted within the Human MicroNucleus network provided evidence that the baseline MN frequency in cytokinesis-blocked lymphocytes is a predictive biomarker of cancer risk (20,21). Previous studies have also reported differences in MN frequency among different VCM-exposed workers (22,23). Based on these previous studies, we explored the MN frequency in VCM workers.

Although the difference of MN frequency between high-exposure, middle-exposure and low-exposure subgroups was not significant,

Table IV. Final multivariate Poisson regression analysis of the association between genetic polymorphism data (independent variable) and MN frequency (dependent variable)

Name	β	95% CI		*P*	FR (95% CI)
		Low	Upper		
Intercept	1.67	1.11	2.21	<0.0001	
Age	0.1405	0.0386	0.2424	0.0069	1.15 (1.03–1.27)
hOGG1	0.1916	0.0126	0.3706	0.0359	1.21 (1.01–1.44)
XRCC1 194	0.1222	0.0162	0.2282	0.0239	1.12 (1.01–1.25)
XRCC1 280	0.1165	0.0017	0.2317	0.0437	1.12 (1.00–1.26)

Because clinical characteristics (gender, smoking and alcohol status) and cumulative dose were each non-significantly associated with MN frequency, these variables were not included in the final regression model. Because the frequency of homozygous variants for several of the polymorphisms was low, a dominant genetic inheritance model is presented. Age is a continuous variable. β, beta regression term in Poisson regression model.

Table V. Associations between diplotypes of XRCC1 and MN frequency

Diplotype	*N* (%)	Mean MN ± SD (‰)	Adjusted FR (95% CI)
CGG/CGG	30 (9.61)	4.24 ± 2.52	1
TGG/TGG	54 (17.33)	4.03 ± 2.35	0.92 (0.65–1.18)
CGA/CGA	48 (15.37)	4.07 ± 2.31	0.91 (0.61–1.08)
TGA/TGA	41 (13.14)	4.74 ± 2.89	1.13 (1.07–1.36)*
CGG/CGA	25 (8.06)	4.44 ± 2.60	1.14 (0.94–1.42)
TGG/CGG	23 (7.37)	4.85 ± 3.18	1.27 (1.09–1.44)*
CAG/CAG	23 (7.37)	4.46 ± 3.89	1.11 (1.07–1.46)*
TAG/TAG	23 (7.37)	4.61 ± 2.77	1.18 (1.11–1.52)*
CAA/CAA	21 (6.79)	4.57 ± 2.65	1.13 (0.98–1.41)
TGG/TGA	7 (2.25)	4.21 ± 2.53	0.92 (0.72–1.24)
CAG/CAA	6 (1.94)	3.67 ± 1.80	0.81 (0.45–1.13)
TAA/TAA	5 (1.63)	3.27 ± 1.21	0.84 (0.61–1.36)
CGA/CAG	3 (0.96)	6.05 ± 2.79	1.67 (1.19–2.23)*
Others	4 (1.28)	5.04 ± 3.09	1.21 (0.97–1.51)

The diplotype is defined as the allele present at position 194(C/T), 280(G/A) and 399(G/A), respectively. ‰, per thousand lymphocytes; SD, standard deviation. Others, refers to the grouping of all diplotypes with <5% frequency.
*$P < 0.05$.

VCM-exposed workers had a significantly higher MN frequency compared with controls, indicating that VCM exposure was associated with increased genotoxicity in exposed workers. Such a finding is consistent with previous epidemiological studies showing that VCM exposure is associated with increased genotoxicity in humans (24,25). Chromosomal aberrations, MN, sister chromatid exchanges and DNA strand breaks have been observed in lymphocytes of VCM-exposed workers. The lack of significant difference among exposure workers might be due to the life span of lymphocytes. Because the lymphocytes had limited life span (from several weeks to several years) if individuals received VCM exposure for several years, the MN frequency in the lymphocytes of workers might be at its plateau phase (26).

Consistent with previous studies (27,28), our study found that there was no significant effect of smoking or alcohol drinking on MN frequency. The most plausible interpretations for the lack of such effects might be that VCM exposure was probably to mask the effect of smoking or alcohol drinking or that there was lower effective concentration of cigarette smoke genotoxins or alcohol genotoxins in the blood that could cause chromosomal damage in lymphocytes (29). Some previous epidemiological studies have investigated the effect of some lifestyle and biological factors on MN frequency in human lymphocytes. The most consistent demographic variable influencing the MN frequency was age, with MN frequency increasing significantly with age (30,31). In agreement with this, our results indicated that there was a significant increase in MN frequency among older workers (≥35 years of age) than among younger workers. The effects of aging on MN frequency might reflect accumulated chromosomal damage occurring during the life span.

In this study, the frequencies of the *XRCC1 194 Trp* (29.7%), *XRCC1 280 His* (14.0%) and *XRCC1 399 Gln* alleles (32.1%) (Table II) was similar to that noted in a previous study of normal Taiwanese subjects (27.0, 11.0 and 26.0%) (32). The figure of 64.7% for the prevalence of the *hOGG1 326 Cys* allele found in this study was also similar to that found in a previous study conducted among Chinese (64%) (33). The frequency of *MGMT 84 Phe* allele (7.8%) was likewise consistent with the analogous figure of 9.4% among Singapore Chinese (34). These findings, to some extent, support the validity of our genotyping techniques.

There are >60 validated SNPs in *XRCC1*, among which ~30 variants are located in exons or promoter regions. The most extensively studied SNPs are *Arg194Trp* on exon 6, *Arg280His* on exon 9 and *Arg399Gln* on exon 10 (5). In agreement with our study, the *Arg194Trp* variant has been shown to be associated with xenobiotic exposure (35). Our study showed the same result that the *XRCC1 194 Arg/Trp* genotype carriers have increased risk compared with *Arg/Arg* genotype workers (Table III). The *XRCC1 194 Arg/Trp* polymorphism is located in *XRCC1* nuclear localization signal domain, vicinal to other domains that mediate polymerase β and *apurinic/apyrimidinic endonucleases* interactions. Therefore, this polymorphism may disturb the *XRCC1* conformation, resulting in a decreased protein affinity or decreased DNA damage binding and ineffective DNA repair.

In our study, VCM-exposed workers featuring the *XRCC1 280 Arg/His* and *His/His* genotype had a higher risk of chromosomal damage than those lacking that allele (Table III). This appears to be supported by a previous study that reported that only the *XRCC1-Arg280His* variant protein is defective in its efficient localization to a damaged site on the chromosome, thereby reducing the cellular BER efficiency (36). This is plausible because it is located close to sequences that mediate protein–protein interactions with poly (adenosine diphosphate-ribose) polymerase and DNA polymerase β (37,38), which is involved in the formation of unstable chromosomal aberrations (39). Previous studies showed that the 399 allele may be associated with higher mutagen sensitivity, higher levels of carcinogen adducts, mutations as well as sister chromatid exchanges and, inducing theoretically, higher incidence of cancer. On the contrary, some studies found no evidence of association between *XRCC1 399* genotypes and cancer (40,41). The relationship between MN frequency and polymorphism of *XRCC1 399* was not observed in this study, and the mechanistic basis requires further examination.

Linkage disequilibrium analysis of *XRCC1* found that the three polymorphisms of *XRCC1* (*Arg194Trp*, *Arg280His* and *Arg399Gln*) are in linkage disequilibrium, which agreed with another earlier study by Kim *et al.* (42). Several studies have shown that the haplotypes composed of variants of multiple SNPs of *XRCC1* may be more appropriate for assessing environment disease associations compared with individual SNPs. Leng *et al.* (43) suggested that the *XRCC1* haplotypes are associated with risk of chromosomal damage in Chinese coke oven workers. Consistent with this previous finding (44), we saw that *CGA/CAG* carriers had more risk of chromosome damage among these susceptibility diplotypes. Such a statistically significant association may be attributable to changes in XRCC1 function because the DNA repair capacity of mutant alleles was lower than that of wild-type alleles.

Our analysis also revealed an increased risk of chromosomal damage with the *hOGG1 326 Ser/Cys* genotypes (Table III). HOGG1 is an important component of the BER process and is responsible for the removal of the highly mutagenic 7, 8-dihydro-8-oxoguanine DNA lesion via its DNA glycosylase/apurinic lyase activities. Several polymorphisms have been described in the *hOGG1* gene. The most common polymorphism is the substitution of serine (Ser) for cysteine

(Cys) at codon 326 in exon 7. It has been shown in a bacterial complementation assay system that the DNA repair activity of the *hOGG1-Cys326* protein is lower than that of *hOGG1-Ser326* protein. Several investigators have demonstrated that the *Cys/Cys* homozygous variant corresponds to a phenotype that is deficient in 8-oxoG repair (45). In epidemiological studies, the *hOGG1-Ser326Cys* polymorphism has been found to be associated with risks of several cancer types (34). Our results verified that a functional impact of this *hOGG1 326* polymorphism is a decreased ability to repair chromosomal damage.

In conclusion, our results reported that exposed workers had higher frequencies of MN compared with controls. These results indicated a pronounced clastogenicity of VCM. The genotypes of *XRCC1 194 Arg/Trp*, *280 Arg/His*, diplotype *CGA/CAG* and *hOGG1 Ser326Cys* demonstrated an association with the levels of chromosomal damage in Chinese workers exposed to VCM in this study. Even though limited blood samples in the control group prevented further gene polymorphism analysis and a definitive conclusion on the possible influence of genetic polymorphisms in DNA repair genes, nevertheless, our experimental evidences highlighted the usefulness of the MN assay, as a biological marker for assessing genetic damage in populations exposed to VCM, and suggest that *XRCC1* and *hOGG1* polymorphisms might contribute to increase the genetic damage, possibly due to reduced DNA repair function. In addition, association between MN frequency and DNA repair genotypes of *XRCC1* and *hOGG1* in non-exposed group and the frequency of these genes in Asian populations from other reports also provide supportive evidence for this study (33,34,42,43). In the future, larger studies of other DNA repair polymorphisms could be targeted to healthy individuals under controlled lifestyle conditions, known to interfere with MN formation.

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