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Polymorphisms in BER and NER pathway genes: Effects on micronucleus frequencies among vinyl chloride-exposed workers in northern China

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ABSTRACT

In this study, a group of 317 workers occupationally exposed to vinyl chloride monomer and 166 normal, unexposed referents in Shandong province (Northern China) were examined for chromosomal damage in peripheral lymphocytes using the cytokinesis-blocked micronucleus (CB-MN) assay. The exposure group (3.47 ± 2.65)‰ showed higher micronucleus frequency than the unexposed workers (2.51 ± 1.96)‰ ($P < 0.01$). We explored the relationship between genetic polymorphisms of *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln*), *APE1 Asp148Glu*, *XPA Ala23Gly*, *XPC.PAT*, *XPC Ala499Val*, *XPC Lys939Gln*, *XPF 5′-UTR T2063A*, *XPG Exon15 G-C*, *ERCC13′-UTR C8092A* and susceptibility of chromosomal damage in all the subjects. It was found that *XRCC1 −77*, *XRCC1 280*, *APE1148*, *XPC.PAT*, *XPG Exon15 G-C, and ERCC13′-UTR C8092A* polymorphisms showed no significant associations with micronucleus frequency in unexposed workers. However, among the exposed workers individuals with *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln*) polymorphisms had a significantly higher micronucleus frequency as seen in mean frequency ratios (FR) compared with their homozygous wild-type *genotypes* (FR = 1.21, 95% CI: 1.05–1.39; $P < 0.01$); (FR = 1.14, 95% CI: 1.00–1.38; $P < 0.05$) and (FR = 1.26, 95% CI: 1.11–1.44; $P < 0.01$); (FR = 1.23, 95% CI: 1.08–1.46; $P < 0.01$). Four SNP sites in the nucleotide excision repair (NER) pathway were associated with susceptibility for MN frequency in either unexposed or exposed workers. Further, we observed the gene–MN association changed with exposure for *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln*), *XPA Ala23Gly*, *XPC Ala499Val*, *XPC Lys939Gln*, *XPF 5′-UTR T2063A*. Moreover, Individuals carrying the *XPC* (*PAT*)-(*499*)-(*939*) diplotype, *PAT-CG/PAT-TG*, had a higher MN frequency, compared with individuals carrying the wild-type *PAT-CA/PAT-CA*.

1. Introduction

Vinyl chloride monomer (CH_2=CHCl, VCM), a main material used in the polymerization process of polyvinyl chloride (PVC), is a human carcinogen classified by the International Agency for Research on Cancer (IARC, 1987) [1].

Previous studies of VCM workers have reported modest associations between polymorphisms of many metabolism and/or DNA repair genes and genotoxicity as well as carcinogenicity [2,3]. The major repair pathway responsible for handling the most common forms of DNA damage is base excision repair (BER) [4]. It is also well documented that the X-ray repair cross complementing 1 (XRCC1) protein is required for viability and efficient repair of DNA single-strand breaks (SSBs) [5]. Moreover, despite some controversial results [6,7], functional variants in XRCC1 genes play a crucial role in the facilitation of human cancer development because of the scaffold protein XRCC1 via its ability to interact with DNA ligase IIIα, DNA polymerase b, apurinic/apyrimidinic (AP) endonuclease (APE1), polynucleotide kinase/phosphatase, poly (ADP-ribose) polymerases 1 and 2 (PARP-1 and 2), the DNA glycosylase hNEIL1 and DNA-dependent protein kinase (DNA-PK) to participate in the base excision repair pathway [8,9]. APE1, the rate-limiting enzyme in the BER pathway, assembles pol β onto AP sites and allows pol β and ligase III to engage in DNA repair. A previous study has reported the APE1 Asp148Glu polymorphism increased lung cancer risk among Japanese [10].

Nucleotide excision repair (NER) is one of the major repair pathways for removing DNA damage caused by tobacco carcinogens as well as other helix-distorting lesions that interfere with base pairing and obstruct replication and transcription [11]. Several critical

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genes participate in the NER process and have functions central to the ability of the cell to cope with different types of DNA damage and to maintain genomic integrity. Although a total of 40 nonsynonymous SNPs occur in these genes, only some of the common, potentially functional SNPs (*XPC.PAT*, *XPC Ala499Val*, *XPC Lys939Gln*, *XPF 5′-UTR T2063A*, *XPG Exon15 G-C*, *ERCC13′-UTR C8092A*) have been documented and suggested to correlate with risk of cancer in the general population [12].

The validation of frequency of micronuclei (MN) in peripheral blood lymphocytes (PBL) as a biomarker of chromosomal damage and phenotypic susceptibility to cancer has been supported from some studies [13]. MN originates mainly from chromosome breaks or whole chromosomes that fail to engage with the mitotic spindle during cell division. Micronuclei also formed as a byproduct of cellular defense to remove the extra chromosome or as separate linear fragment by a double-strand break in DNA. Since one the jobs of the liver to detoxify the blood, it picks up dangerous toxins which can act on the cells at the gene level to induce some sort of DNA damage, the increased formation of micronuclei could as an indication of increased DNA damage in liver cell which progress to angiosarcoma and spread to other parts o liver or the blood system. 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]. MN frequency is easier to detect and also an important index for predicting long-term risk associated with environmental exposure and identifying various related life-styles [15].

As well known, many polymorphisms of DNA repair genes influence MN frequencies in PBL [16]. In this current study, we evaluated the associations of the polymorphisms and their haplotypes with MN frequency. Also we investigate the possible gene–MN association across different exposure levels in the VCM induced DNA damage.

2. Material and methods

2.1. Study subjects

Upon informed consent during routine medical surveillance, a total of 317 workers employed at a PVC polymerization plant in Shandong Province, China, 257 Han Chinese men and 60 women, aged 37.16 ± 8.07 years on average, were included in the study. The study participants had been occupationally exposed to VCM for at least one year, had their blood sample drawn, and completed questionnaires in a personal interview. A total of 136 service workers and managers from the same factory, 98 Han Chinese men and 38 women, with a similar age distribution (38.39 ± 5.89) to the exposures but without direct VCM exposure were enrolled as internal controls. All controls also agreed to provide a blood sample for micronucleus test and completed questionnaire**s**. But only 69 individuals from control group agreed to provide more blood for genotype test. Both age and gender were included as adjustment variables in all analyses.

Each study participant completed a detailed questionnaire, and also submitted a 5 mL anticoagulated peripheral blood sample. Blood samples were stored at room temperature in an insulated container and were delivered to the laboratory within 6 h of collection. Cytokinesis-blocked micronucleus assays (CBMN), DNA extraction and sequent PCR-restriction fragment length polymorphism analyses (PCR-RFLP) were performed on the blood samples.

2.2. Assessment of vinyl chloride exposure

The VCM plant had been monitoring ambient air VCM concentration at different worksites since the beginning of its establishment. We were able to estimate the cumulative exposure dose of each worker using the following equation:

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

where C = the geometric mean of VCM exposure concentration (in mg/m^3) for each month in a given workplace (calculated for all worksites); M = the number of exposure months in a year for the VCM worker; T = duration of exposure per month; for 2 h exposure time in each working day, 20 days per month, for example, T = 2400 min exposure time per month; A is alveolar ventilation (male average = 6500 ml/min, female average = 4300 ml/min, assuming 30% dead space), and 1 ml = 10^{-6} m^3. By this method, personal cumulative exposure doses (CU.DOSE) in the VCM exposure group ranged from 30.09 mg to 339278.01 mg and CU.DOSE was not assessed for the control group. We then grouped the VCM exposed subjects into high-exposure and low-exposure groups by the median cumulative doses of 14198.55 mg.

2.3. CBMN assay

The CBMN assay was performed according to standard methods as described by Fenech [17]. In brief, 0.5 ml heparin anticoagulated whole blood was added to 4.5 ml of medium (RMPI1640) and incubated at 37 °C, 5% CO_2 level. Cytochalasin-B (Cyt-B, Sigma) was added to each cell culture after 44 h at a final concentration of 6 ug/ml to prevent cytokinesis. Twenty-eight hours after the addition of Cyt-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. For each subject, CB-MN in 1000 binucleated lymphocytes with well-preserved cytoplasms was scored blindly by the same reader. MN frequencies are the number of micronuclei observed per 1000 lymphocytes, expressed as a count per thousand (‰).

2.4. Genotyping of DNA repair genes

From the 4.5 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 (PerkinElmer Corp.) 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 1 μl DNA.

PCR was performed according to previous published study [2,3,6,12] under the following conditions: 95 °C for 5 min, followed by 30 cycles of 94 °C for 40 s, annealing temperature for 20 s (the annealing temperatures were 55 °C for C-77/T, APE1 Asp148Glu, XPC.PAT, XPG Exon G15 C, 57 °C for XPA A23G, 58 °C for Arg194Trp, Arg399Gln, 60 °C for XPC Ala499Val, XPC Lys939Gln, 63 °C for ERCC1 Cys8092Ala, XPF T2063A, 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, Inc.). 10% of all samples were randomly chosen and were took independently genotype running for each polymorphism site as quality control to ensure the accuracy of genotype results.

3. Statistical methods

Adjusted MN frequency was reported in association with sample characteristics, VCM exposure, as well as the genotypes using mean frequency ratios and its 95% confidence intervals derived from univariate Poisson log-logistic regression models. The risks of chromosomal damage were estimated by computing frequency ratios (FR = e^{β}, e = 2.71828, β: regression coefficient) and 95% confidence intervals; the FR indicated a proportional increase/decrease of the micronucleus frequency in a comparison group relative to the referent. All statistical analyses were done using the software SAS (SAS Institute). We tested for the Hardy–Weinberg equilibrium and linkage disequilibrium using the method described by Shi et al. [18]. PHASE software (version 2.0.2) was used to obtain maximum-likelihood estimates of the *XRCC1* and *XPC* diplotype frequencies. Bonferroni approach was used for Type-I error adjustment.

4. Result

4.1. Subject characteristics and risk estimates for demographic and lifestyle factors

Table 1 presents gender, age, smoking and drinking status of the study population, along with their associations with micronucleus frequency. Most MN frequency significantly increased for the some factor in exposed workers compared with unexposed group. There was a small, but statistically not significant, increase in micronucleus frequency in female exposed workers than in male (FR = 1.12, 95% CI 0.97–1.30). No significant difference in micronucleus frequency was detected in association with smoking and alcohol drinking status in exposed workers. However, the older age group (>35 years) exhibited generally higher MN frequency than the younger age group (⩽35) (FR = 1.32, 95% CI: 1.17–1.50; P < 0.01) in exposed workers. The exposure group showed higher micronucleus frequency (3.47 ± 2.65)‰ than the unexposed controls (2.51 ± 1.96)‰ (P < 0.01) based on simple Poisson regression.

Table 1
Micronuclei (MN) frequency of VCM-exposed workers and unexposed controls by demographic characteristics.

		Unexposed workers		Exposed workers		FR 95% CI
		N	Mean MN ± SD (0/00)	*N*	Mean MN ± SD (‰)	
Gender	Male	98	2.46 ± 1.80	257	3.39 ± 2.67$^{\&\&}$	Reference
	Female	38	2.63 ± 2.34	60	3.82 ± 2.52$^{\&\&}$	1.12 (0.97–1.30)
Age	⩽35	17	1.47 ± 1.37	128	2.91 ± 2.44$^{\&}$	Reference
	>35	119	2.66 ± 1.99	189	3.85 ± 2.72$^{\&}$	1.32 (1.17–1.50)
Smoke	No	74	2.49 ± 2.02	158	3.44 ± 2.56$^{\&\&}$	Reference
	Yes	62	2.53 ± 1.90	159	3.50 ± 2.74	1.02 (0.90–1.15)
Drink	No	61	2.31 ± 2.11	202	3.46 ± 2.77$^{\&\&}$	Reference
	Yes	75	2.67 ± 1.83	115	3.48 ± 2.43$^{\&}$	1.01 (0.89–1.14)
Cu. dose	Low	–	–	157	2.91 ± 2.38	1.14 (0.75–1.44)
	High	–	–	160	4.03 ± 2.79**	2.68 (1.23–3.56)
Total	136	2.51 ± 1.96	317	3.47 ± 2.65**	2.31 (1.81–3.01)	

Compare with same factor in unexposed workers $^{\&}P < 0.05$; $^{\&\&}P < 0.01$.
Compare with total MN frequency in unexposed workers $^{*}P < 0.05$; $^{**}P < 0.01$.
MN – micronuclei; FR – frequency ratio, compare with reference MN frequency in exposed workers (total MN frequency as reference in unexposed workers for Cu.dose FR); VCM – vinyl chloride monomer; ‰ – per thousand lymphocytes; SD – standard deviation; Cu. dose – cumulative dose, VCM exposed subjects were divided into high-exposure, and low-exposure groups with median cumulative doses.

Poisson regression also showed an increasing MN frequency, compared with the unexposed controls, the lower VCM-exposure (FR = 1.94, 95% CI: 1.50–2.54; $P < 0.01$) to higher VCM-exposure group (FR = 2.68, 95% CI: 1.23–3.56; $P < 0.01$), respectively.

4.2. Distribution of genotypes and risk assessment for genes polymorphisms

The genotype association of NER pathway genes and BER pathway genes *APE1, XRCC1* with MN frequency in workers is presented in Table 2. Genotype distributions at each locus were consistent with Hardy–Weinberg equilibrium. *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln)*, *APE1 Asp148Glu* polymorphisms showed no significant associations with micronucleus frequency in unexposed workers. However, exposed individuals with *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln*) polymorphic genotypes had a significantly higher micronucleus frequency, with (FR = 1.21, 95% CI: 1.05–1.39; $P < 0.01$), (FR = 1.14, 95% CI: 1.00–1.30; $P < 0.05$), (FR = 1.26, 95% CI: 1.11–1.44; $P < 0.01$), (FR = 1.23, 95% CI: 1.08–1.40; $P < 0.01$) respectively, compared with those homozygous wild-type at these sites. Four SNP sites *XPA Ala23Gly*, *XPC Ala499Val*, *XPC Lys939Gln*, *XPF 5′-UTR T2063A* demonstrated a significant genotype-dependent MN frequency in both unexposed or exposed workers. At the site *XPA 23 Ala/Gly*, the exposure had FR = 1.42 (95% CI: 1.20–1.69; $P < 0.01$) relative to the *Ala/Ala* site. Both *XPC499* heterozygote and homozygote exhibited higher MN frequency with FR = 1.44 (95% CI: 1.02–2.05; $P < 0.05$) and FR = 1.25, 95% CI: 1.01–1.52; $P < 0.01$ at *Ala/Val* for the unexposed and exposed, respectively; and with FR = 1.85 (95% CI: 1.26–2.69; $P < 0.01$) and FR = 1.14 (95% CI: 1.00–1.30; $P < 0.05$) at *Val/Val* for the unexposed and exposed, respectively. At the site *XPC939 Gln/Gln* FR = 1.23 (95% CI: 1.08–1.40; $P < 0.05$) for the exposed workers, compared with wild-type genotype. In contrast, significant lower MN frequency was found at the site *XPF 5′-UTR Thr2063Ala*, relative to the homozygous wild-type genotype, with FR = 0.41 (95% CI: 0.21–0.70; $P < 0.05$) and 0.83 (95% CI: 0.70–0.99; $P < 0.01$) in the exposed group, respectively.

4.2.1. Genotype-MN association across different exposure level

Based on the results of genotype and risk assessment for gene polymorphism in Table 2, the further combined effect of the *XRCC1* (*−77C/T, Arg194Trp, Arg280His, Arg399Gln*), *XPA Ala23Gly*, *XPC Ala499Val*, *XPC Lys939Gln*, *XPF 5′-UTR T2063A* and cumulative exposure dose is shown in Table 3. Compared with the low CU.DOSE having the *−77TT* wild-tpye genotype, we observed an increased risk of DNA damage in low CU.DOSE *−77CT* carriers FR = 1.25 (95% CI: 1.00–1.54; $P < 0.05$) and higher risk in high CU.DOSE *−77TT* FR = 1.30 (95% CI: 1.03–1.61; $P < 0.05$) as well as *−77CT* workers FR = 1.74 (95% CI: 1.36–2.21; $P < 0.01$). This suggested a possible gene-exposure combined effect and some results also shown in *XRCC1* (*Arg194Trp*, *Arg280His*, *Arg399Gln*), *XPA Ala23Gly*, *XPC Ala499Val*, *XPC Lys939Gln.* While *XPF 5′-UTRT2063A* mutant homozygous and heterozygous in low exposure groups had lower MN frequency than their wild-type homozygous in low CU.DOSE FR = 0.75 (95% CI: 0.58–0.96; $P < 0.05$), but increased risk for wild-type genotype carriers FR = 1.10 (95% CI: 0.82–1.41; $P < 0.01$) and homozygous in high exposure groups FR = 1.37 (95% CI: 1.10–1.71; $P < 0.01$), suggesting a possible protect factor for *XPF 5′-UTRT2063A* mutant homozygous and heterozygous with different exposure level.

4.3. Diplotypes of XRCC1; XPC and MN frequency

To further elucidate the relevance of *XRCC1 and XPC* variants with MN frequency, linkage disequilibrium among the four *XRCC1* polymorphisms (*XRCC1 −77C/T, Arg194Trp, Arg280His, and Arg399Gln*) and three *XPC* Polymorphisms (*XPC PAT−/+, Ala499Val, and Lys939Gln*) was analyzed and diplotype was reconstructed in all subjects. The *D′* value of the four loci of XRCC1 were 0.803 (*XRCC1 −77* with 194), 0.876 (*XRCC1 −77* with 280), 0.852 (*XRCC1 −77* with 399), 0.833 (*XRCC1* 194 with 280), 0.859 (*XRCC1* 194 with 399) and 0.899 (*XRCC1* 280 with399). The *D′* value of the four loci of XPC were 0.871 (*XPC* 499 with PAT), 0.803 (*XPC* 939 with PAT), 0.849 (*XPC* 499 with 939). The diplotype *TCGG/TCGG and PAT-CA/PAT-CA* which consists of the wild type sequence for *XRCC1 and XPC* in all loci was selected as the reference. Among these diplotype pairs, the rare diplotypes (less than 5% frequency) were analyzed as a group. FRs associated with various diplotypes in all study subjects are presented in Table 4 and Table 5.

Compared with individuals with wild-type *TCGG/TCGG for XRCC1* (Table 4), the FR *CTAA/CTAA* diplotype was significantly higher (FR = 1.19, 95% CI: 1.02–1.32; $P < 0.05$) in the exposed workers. The *CCAA/CTAA* diplotype shares the same allele as *CTAA/CTAA* at two loci – *XRCC1 280* and *XRCC1 399* – and had higher MN frequency FR = 1.41, 95% CI: 1.02–1.87; $P < 0.05$) than the wild-type. However, only non-significant difference was found in unexposed workers.

For *XPC* (Table 5), *PAT-CG/PAT-TG* carriers in both exposed (FR = 2.45, 95% CI: 2.01–3.75; $P < 0.01$) or unexposed group (FR = 2.34, 95% CI: 1.21–3.31; $P < 0.01$) had higher MN frequency compared with *PAT-CA/PAT-CA.*

Table 2
Genotype association in micronuclei (MN) frequency with genes of DNA BER and NER repair pathway.

Polymorphism	Genotype	Unexposed workers			Exposed workers		
		N	Mean MN ± SD (‰)	Adjusted FR (95% CI)	*N*	Mean MN ± SD (‰)	Adjusted FR (95% CI)
XRCC1 −77	TT	50	2.70 ± 1.99$^{\Delta\Delta}$	Reference	226	3.20 ± 2.59	Reference
	CT	16	3.50 ± 2.13	1.35 (0.96–1.87)	79	3.97 ± 2.66**	1.92 (1.42–2.53)
	CC	3	4.33 ± 2.08	1.68 (0.89–2.90)	12	5.56 ± 3.13**	1.21 (1.05–1.39)
XRCC1194	Arg/Arg	35	2.42 ± 1.84$^{\Delta}$	Reference	158	3.20 ± 2.65	Reference
	Arg/Trp	27	3.26 ± 1.89$^{\Delta}$	1.23 (0.89–1.69)	126	3.69 ± 2.56*	1.25 (1.01–1.52)
	Trp/Trp	7	4.83 ± 2.93$^{\Delta\Delta}$	2.13 (1.34–3.27)	33	3.97 ± 2.92*	1.14 (1.00–1.30)
XRCC1280	Arg/Arg	44	2.71 ± 1.83$^{\Delta\Delta}$	Reference	209	3.19 ± 2.52	Reference
	Arg/His	24	3.21 ± 2.48	1.17 (0.86–1.59)	97	4.08 ± 2.72	1.11 (0.76–1.55)
	His/His	1	3.00 ± 0.00	1.09 (0.26–2.99)	11	3.90 ± 3.67**	1.26 (1.11–1.44)
XRCC1399	Arg/Arg	35	2.68 ± 1.71$^{\Delta}$	Reference	170	3.15 ± 2.54	Reference
	Arg/Gln	27	3.11 ± 2.34	1.16 (0.86–1.57)	121	3.84 ± 2.74	1.18 (0.93–1.47)
	Gln/Gln	7	4.75 ± 2.06	1.61 (0.95–2.60)	26	4.00 ± 2.66**	1.23 (1.08–1.40)
APE1148	Asp/Asp	27	3.07 ± 2.37	Reference	162	3.62 ± 2.67	Reference
	Asp/Glu	33	2.96 ± 1.90$^{\Delta}$	0.96 (0.71–1.30)	102	3.22 ± 2.67	0.91 (0.76–1.08)
	Glu/Glu	9	2.56 ± 1.67	0.77 (0.47–1.21)	53	3.38 ± 2.43	0.89 (0.77–1.02)
XPA23	Ala/Ala	16	2.41 ± 1.62$^{\Delta}$	Reference	85	3.01 ± 2.44	
	Ala/Gly	36	2.53 ± 1.87	0.93 (0.62–1.42)	154	3.32 ± 2.60**	1.42 (1.20–1.69)
	Gly/Gly	17	4.60 ± 2.20$^{\Delta}$	1.68 (1.10–2.61)	78	4.26 ± 2.82	1.11 (0.95–1.30)
XPC.PAT	PAT−/−	28	2.71 ± 1.78$^{\Delta}$	Reference	136	3.46 ± 2.69	Reference
	PAT+/−	33	3.18 ± 2.24$^{\Delta}$	1.08 (0.80–1.48)	148	3.61 ± 2.67	0.80 (0.64–1.01)
	PAT+/+	8	2.88 ± 2.23	0.98 (0.60–1.57)	33	2.94 ± 2.37	1.07 (0.94–1.22)
XPC499	Ala/Ala	33	2.09 ± 1.28$^{\Delta\Delta}$	Reference	158	3.20 ± 2.64	Reference
	Ala/Val	24	3.58 ± 1.64$^{*\Delta\Delta}$	1.44 (1.02–2.05)	126	3.69 ± 2.56*	1.25 (1.01–1.52)
	Val/Val	12	4.08 ± 2.94**	1.85 (1.26–2.69)	33	3.97 ± 2.92*	1.14 (1.00–1.30)
XPC939	Lys/Lys	36	2.50 ± 1.63$^{\Delta\Delta}$	Reference	170	3.15 ± 2.54	Reference
	Lys/Gln	25	3.40 ± 2.35	1.40 (0.99–1.91)	121	3.84 ± 2.74	1.18 (0.93–1.47)
	Gln/Gln	8	3.62 ± 2.50	1.34 (0.85–2.05)	26	4.00 ± 2.66**	1.23 (1.08–1.40)
XPF2063	Thr/Thr	44	3.34 ± 2.16	Reference	254	3.65 ± 2.70	Reference
	Thr/Ala	23	2.35 ± 1.70$^{\Delta}$	0.73 (0.53–1.00)	55	2.91 ± 2.43**	0.41 (0.21–0.70)
	Ala/Ala	2	1.50 ± 0.71	1.71 (0.14–1.59)	8	1.57 ± 0.79*	0.83 (0.70–0.99)
XPG15	Gly/Gly	16	3.12 ± 2.78	Reference	65	3.72 ± 2.93	Reference
	Gly/Cys	35	2.91 ± 1.79$^{\Delta}$	0.90 (0.64–1.29)	159	3.44 ± 2.73	0.88 (0.74–1.04)
	Cys/Cys	18	2.89 ± 1.88	0.90 (0.61–1.34)	93	3.37 ± 2.30	0.90 (0.77–1.05)
ERCC12063	Cys/Cys	40	2.90 ± 1.18$^{\Delta}$	Reference	159	3.35 ± 2.55	Reference
	Cys/Ala	25	3.24 ± 2.39	1.18 (0.86–1.61)	124	3.81 ± 2.86	0.82 (0.64–1.03)
	Ala/Ala	4	1.75 ± 2.06	0.58 (0.24–1.17)	34	2.83 ± 2.24	0.89 (0.99–1.28)

Compared with wild-type for each genotype case $^{*}P < 0.05$; $^{**}P < 0.01$.
Comparison of same genotype between different groups $^{\Delta}P < 0.05$; $^{\Delta\Delta}P < 0.01$.
Legend: MN – micronuclei; *N* – number in each category; adjusted FR – frequency ratio adjusted for age, sex, drink, smoke and cumulative exposure dose, CI – confidence interval; ‰ – personal thousand lymphocytes; SD – standard deviation.

Table 3
Micronucleus frequency by cumulative exposure dose (CU.DOSE) and DNA repair gene polymorphisms.

Genotype	Low CU.DOSE group			High CU.DOSE group		
	N	Mean MN ± SD (%)	Adjusted FR (95% CI)	*N*	Mean MN ± SD (‰)	Adjusted FR (95% CI)
XRCC1 −77 TT	117	2.77 ± 2.38	Reference	109	3.25 ± 2.33*	1.31(1.03–1.66)
CT/CC	40	3.24 ± 2.06*	1.27 (1.01–1.56)	51	4.78 ± 2.98**	1.75 (1.38–2.28)
XRCC1 194 Arg/Arg	83	2.81 ± 2.57	Reference	75	3.63 ± 2.68*	1.31 (1.01–1.71)
Arg/Trp and Trp/Trp	74	3.03 ± 2.15	1.12 (0.92–1.36)	85	4.38 ± 2.85**	1.59 (1.23–2.07)
XRCC1280 Arg/Arg	104	2.82 ± 2.24	Reference	105	3.55 ± 2.62*	1.24 (1.03–1.54)
Arg/His and His/His	53	3.19 ± 2.44	1.13 (0.90–1.34)	55	4.93 ± 2.89**	1.75 (1.44–2.17)
XRCC1399 Arg/Arg	89	2.86 ± 2.50	Reference	81	3.39 ± 2.63	1.24 (0.96–1.61)
Arg/Gln and Gln/Gln	68	3.03 ± 2.16	1.09 (0.87–1.31)	79	4.60 ± 2.95**	1.62 (1.31–2.05)
XPA23 Ala/Ala	43	2.06 ± 2.26	Reference	42	3.86 ± 2.37**	1.61 (1.16–2.24)
Ala/Gly and Gly/Gly	114	3.13 ± 2.45**	1.34 (1.02–1.63)	118	4.12 ± 2.86**	1.81 (1.35–2.47)
XPC499 Ala/Ala	83	2.81 ± 2.57	Reference	75	3.63 ± 2.68*	1.31 (1.01–1.71)
Ala/Val and Val/Val	74	3.03 ± 2.15	1.12 (0.92–1.36)	85	4.38 ± 2.85**	1.59 (1.23–2.07)
XPC939 Lys/Lys	89	2.87 ± 2.63	Reference	81	3.38 ± 2.23	1.24 (0.97–1.71)
Lys/Gln and Gln/Gln	68	3.03 ± 2.16	1.14 (0.92–1.23)	79	4.60 ± 2.95**	1.64 (1.30–2.05)
XPF2063 Thr/Thr	124	3.10 ± 2.37	Reference	130	4.17 ± 2.89**	1.37 (1.10–1.73)
Thr/Ala and Ala/Ala	33	2.21 ± 2.30*	0.75 (0.58–0.96)	30	3.38 ± 2.26	1.10 (0.82–1.47)

Compared with wild-type in low cumulative groups case $^{*}P < 0.05$; $^{**}P < 0.01$.
Legend: MN – Micronuclei; *N* – Number in each category; Adjusted FR – Frequency ratio adjusted for age, sex, drink, smoke; CI – Confidence interval; % – Per thousand lymphocytes; SD – Standard deviation.

Table 4
Associations between diplotypes of XRCC1 And micronuclei frequency.

Groups	Diplotypes	*N*	Mean MN ± SD (‰)	χ^2	*P*	Adjusted FR (95% CI)
Exposed	*TCGG/TCGG*	141	3.00 ± 2.50			Reference
	CTAA/CTAA	51	3.86 ± 2.61*	6.55	0.0041	1.19 (1.02–1.32)
	TTGA/TTGA	24	3.29 ± 2.36	0.36	0.4723	1.04 (0.68–1.41)
	TTGG/TTGG	9	2.88 ± 1.83	0.02	0.8578	0.75 (0.46–1.37)
	TCGG/TTGA	7	3.28 ± 1.25	0.07	0.7321	1.03 (0.56–1.49)
	TTAA/TTAA	24	3.75 ± 2.84	1.34	0.0643	1.10 (0.86–1.46)
	CCAA/CTAA	7	4.71 ± 3.14*	4.31	0.0232	1.41 (1.02–1.87)
	TCGG/TTAA	6	2.00 ± 3.03	1.34	0.2636	0.55 (0.27–1.22)
	All OTHERS	48	4.52 ± 2.97**	17.18	<0.0001	1.32 ((1.17–1.64)
Unexposed	*TCGG/TCGG*	61	2.92 ± 1.84			Reference
	CTAA/CTAA	8	3.25 ± 3.41	0.15	0.6124	1.08 (0.63–1.58)

Compared with reference diplotypes: *$P < 0.05$; **$P < 0.01$.
The diplotype is defined as the allele present at positions *−77*(*C/T*), *194*(*C/T*), *280*(*G/A*) and *399*(*G/A*) respectively. Adjusted FR – frequency ratio adjusted for age, sex, drink, smoke and Cu.dose; CI – confidence interval; ‰ – per thousand lymphocytes; SD – standard deviation. OTHERS – refers to the grouping of all diplotypes with less than 5% frequency.

5. Discussion

This study found that workers who were cumulatively exposed to VCM at higher dose also faced a significantly higher risk of chromosome damage, when compared to less exposed workers and to unexposed controls. Among Chinese VCM-exposed workers, some genotypes in BER and NER pathway were associated with increased MN frequency. Moreover, genotype-MN changed across different VCM exposure level.

Previous epidemiologic studies have investigated the effect of various lifestyle and biological factors on MN frequency in human lymphocytes [19,20]. The dominating role of age and gender among variables affecting MN frequency in PBL is well document [21,22]. Our study found that the most consistent demographic variable influencing the MN frequency was age. There was significant increase in MN frequency among older workers compared with younger workers MN frequency was increased significantly with age (Table 1). However, no significant difference between female and male workers was found. A possible reason for these may be the limited number of female workers in this study. There was no significant effect of smoking or alcohol drinking on MN frequency. The most plausible interpretation for this lack of association is that the magnitude of association with VCM exposure was so strong that relationships with smoking or alcohol drinking were masked. Alternatively, blood concentrations of cigarette smoke or alcohol-related genotoxins may have been too low to cause chromosomal damage in lymphocytes [23].

We noticed that the micronucleus frequency in control group from this study was higher than our previous study from different study area [24]. As for the different Micronucleus frequency in these two separate studies, our explanation was that the leaky of VC air in workplace of Shanghai was well monitor and controlled, While this leaky situation was badly controlled in this study area due to lack of local government's funding support. This difference made inter control group in our study get more chance to passively exposure to VC and thus increased MN frequency.

BER and NER are the predominant pathways for averting the mutagenic and cytotoxic effects of damaged DNA generated by either endogenous or exogenous factors, including lesions that arise spontaneously due to the intrinsic instability of DNA and those that are induced by environmental chemicals. Among Chinese VCM-exposed workers, the following genotypes, *XRCC1 194 Trp/Trp*, *XRCC1 399 Gln/Gln*, *XPA 23 Gly/Gly*, *XPC499 Ala/Val and Val/Val*, *XPC939 Lys/Gln*, *XPF 5′-UTR 2063T* were each associated with increased MN frequency in this study.

XRCC1 gene is located on chromosome 19q13.2-13.3. Association between genetic variants in the XRCC1 gene and the risk of developing certain cancers and/or clinical prognostic factors has been reported [5]. Although there are more than 60 validated genetic polymorphisms in XRCC1, the most extensively studied

Table 5
Associations between diplotypes of XPC And Micronuclei (MN) frequency.

Groups	Diplotypes	*N*	Mean MN ± SD (‰)	χ^2	*P*	Adjusted FR (95% CI)
Exposed	*PAT-CA/PAT-CA*	114	2.93 ± 2.28			Reference
	PAT-CG/PAT-TG	5	8.80 ± 1.92**	33.15	<0.0001	2.45 (2.01–3.75)
	PAT + TG/PAT + TG	97	3.56 ± 2.55*	5.35	0.033	1.08 (1.02–1.32)
	PAT + TA/PAT + TA	13	3.07 ± 2.17	0.07	0.6345	1.01 (0.64–1.63)
	PAT + CA/PAT + CA	27	4.25 ± 3.33**	8.34	0.0043	1.35 (1.13–1.57)
	PAT-CA/PAT + TG	12	2.83 ± 2.20	0.02	0.7356	0.83 (0.57–1.27)
	PAT + TG/PAT + TA	5	4.60 ± ± 1.67*	3.23	0.0246	1.57 (1.02–1.96)
	PAT-TG/PAT + TG	5	3.60 ± 2.79	0.47	0.3778	1.19 (0.73–1.91)
	PAT-CA/PAT + CA	8	1.00 ± 1.41**	7.58	0.0084	0.25 (0.12–0.57)
	All OTHERS	31	4.45 ± 2.86**	13.48	<0.0001	1.43 (1.19–1.69)
Unexposed	*PAT-CA/PAT-CA*	12	2.25 ± 1.14			Reference
	PAT-CG/PAT-TG	5	5.80 ± 2.95**	5.74	0.0082	2.34 (1.21–3.31)
	PAT + TG/PAT + TG	8	3.25 ± 1.75	1.03	0.354	1.38 (0.44–2.91)
	PAT + TA/PAT + TA	6	3.33 ± 1.03	1.06	0.2345	1.31 (0.62–2.77)
	PAT + CA/PAT + CA	5	1.40 ± 1.52	0.57	0.5657	0.59 (0.17–1.53)
	PAT + CG/PAT + CG	5	2.20 ± 2.05	0.02	0.9441	0.98 (0.41–1.89)
	All OTHERS	28	3.00 ± 2.11	0.57	0.445	1.19 (0.45–1.44)

Compared with reference diplotypes: *$P < 0.05$; **$P < 0.01$.
The diplotype is defined as the allele present at positions *PAT−/+*, *499*(*C/T*) and *939*(*G/A*) respectively. Adjusted FR – frequency ratio adjusted for age, sex, drink, smoke and Cu.dose; CI – confidence interval; ‰ – per thousand lymphocytes; SD – standard deviation. OTHERS – refers to the grouping of all diplotypes with less than 5% frequency.

polymorphisms are *Arg194Trp* on exon 6, *Arg280His* on exon 9 and *Arg399Gln* on exon 10 [5]. In our study, the exposed workers carrying *XRCC1 194 Arg/Trp* and *Trp/Trp* genotypes, and the unexposed workers carrying *XRCC1 194 Trp/Trp* had statistically higher MN frequency, when compared to workers carrying the *XRCC1 194Arg/Arg* wild-type genotype. The *XRCC1 194* polymorphic site is located in the XRCC1 NLS domain, in proximity to other domains which mediate polymerase β and APE1 interactions [25]. Therefore, this polymorphism may disturb XRCC1 protein conformation, resulting in a decreased protein affinity or decreased DNA damage binding and ineffective DNA repair. Zhu et al. [26] Wang [27] and Zhang et al. [28] reported that this polymorphism significantly decreased DNA damage repair in 1,3-butadiene, vinyl chloride monomer and benzene exposed workers, respectively.

A dominant T > C variant located at nucleotide *−77* in the promoter region of XRCC1 has been identified, and this variant was reported by Hao et al. [29] association with risk of oesophageal squamous cell carcinoma in a Chinese population. In our study, the XRCC1 -T77 C polymorphism was also associated with chromosome damage risk in VCM exposure workers (Table 3) based on the C-allele-containing promoter greater Sp1 binding affinity and may have the potential to alter XRCC1 transcription [30]. This finding is consistent with previous studies, which have reported an association between XRCC1 -T77 C polymorphisms and lung cancer development in Northern and Southern Chinese populations [31].

Several studies [32] have found an association between the presence of the variant genotypes of the *XRCC1 Arg399Gln* polymorphism and reduced DNA repair capacity, including data showing increased MN frequency. Some studies [33,34] all reported a significant increase in CA frequency in the presence of a variant allele of the *XRCC1 Arg399Gln* polymorphism in benzene exposed workers. However, others have reported no detrimental effect of variant *XRCC1 399 Gln* allele and XRCC1-mediated repair [35,36]. In our study, either exposed or unexposed workers who carried the *XRCC1 399 Arg/Gln* or *Gln/Gln* genotypes had higher chromosome damage compared to the *Arg/Arg* genotype carriers.

Our previous study had reported polymorphisms in *XPD*; one core gene in NER pathway, to be associated with increased DNA damage cell frequency by single cell gel electrophoresis (SCGE) in VCM exposed workers [26]. This study further investigated association of other core genes polymorphism in NER pathway and MN frequency. Our results here show only four SNPs site that significantly altered MN frequency difference both in unexposed and exposed workers (*XPA 23 Gly/Gly*, *XPC499 Ala/Val and Val/Val*, *XPC939 Lys/Gln*, *XPF 5′-UTR 2063T*) compared with wild-type genotype for each gene.

A case-control study reported the common G allele of the *XPA A23G* polymorphism was associated with an increased risk in 886 basal cell carcinoma cases and 682 squamous cell carcinoma cases compared with 796 controls, and this polymorphism appeared to be the determining polymorphism in XPA that altered cancer susceptibility [37]. This phenomenon has also been demonstrated in an esophageal cancer study [38].

The XPC protein plays a key role in global NER by recognizing the distortion of damaged DNA. This pathway repairs bulky adducts induced by chemical carcinogens. Interestingly, emerging evidence suggests an additional role for XPC in the removal of oxidative damage [39]. All three nsSNPs in XPC have been well characterized in both functional and epidemiologic studies. Several case-control studies have been reported the associations between the *XPC Ala499Val* polymorphism and risk of cancers. In a study of 320 patients with lung cancer and 322 cancer-free controls in a Chinese population, the *499Val* variant allele was found to be associated with an increased risk of lung cancer [40]. Similar results were reported in an epidemiology study of bladder cancer [41]. Also, another study has suggested that the *XPC 939Gln* allele is associated with risk of colorectal adenoma [42].

The protective effect of the *XPF 5′-UTR T2063A* minor allele was observed in our study. This SNP previously was associated with decreased risk of pancreatic cancer in a study of 481 cases and 625 controls [43]. A larger, two-stage study of >1700 cases and 1900 controls found no increased risk for patients with *XPF 5′-UTR 2063T* minor alleles and possibly a protective effect from an intronic SNP [44].

In this study, no association of polymorphisms in *XRCC1 T-77C*, *XRCC1 Arg194Trp*, *XRCC1 Arg280His*, *XRCC1 Arg399Gln*, *XPA Ala23Gly*, *XPF Thr2063Ala, and ERCC13′-UTR C8092A* with the genetic damage in unexposed workers was found. However, the significant MN frequency difference in some genes showed in exposed workers. An explanation for this is possible VCM exposure-gene interactive effect resulted in the altered MN frequency. Further investigation for gene–MN association across different exposure level confirmed this assumption. With increase of cumulative exposure dose, wild-type genotype, mutant homozygous and heterozygous workers showed significant different joint effect on chromosomal damage. If the gene allele has potential susceptibility to DNA damage induced by VCM, then, the risk could rise with increase of number of allele and exposure level. On the contrast, if the gene allele has potential protection to chromosomal damage induced by VCM, then, the risk could decrease with increase of number of allele and exposure level. Moreover, according to interaction results in *XPF 5′-UTR T2063A*, we observed exposure had more impact on DNA damage than gene allele in this case (Table 3). This association between variant genotypes and exposure level provides further evidence for gene–environment interaction effect.

Haplotype is a set of single-nucleotide polymorphisms (SNPs) on a single chromosome of a chromosome pair that are statistically associated. It is thought that these associations, and the identification of a few alleles of a haplotype block, can unambiguously identify all other polymorphic sites in its region and be a more appropriate tool for assessing host-environment disease associations compared with individual single nucleotide polymorphisms. Such information is very valuable for investigating the genetics behind common diseases, and has been investigated in the human species by the International HapMap Project. Several studies have shown that the haplotypes composed of variants of multiple single nucleotide polymorphisms of XRCC1 may be useful for assessing cancer risk [45] and environment disease associations compared with individual single nucleotide polymorphisms. Leng et al. [46] suggested that the XRCC1 haplotypes are associated with risk of chromosomal damage in Chinese coke-oven workers. Although some diplotypes in exposed workers demonstrated higher MN frequency, similar results were not observed in unexposed workers possibly due to limited samples (Table 4). One diplotype for *XPC* was found to be indicative of susceptibility for genetic damage in all the subjects (Table 5). Such a statistically significant association may be attributable to changes in *XPC* function because the DNA repair capacity of mutant alleles was lower than that of wild-type alleles. However, no more studies date has confirmed that these diplotypes are more strongly associated with altered XRCC1 and XPC activity than the individual polymorphisms. If confirmed, diplotypes differences in cancer risk and occupational health surveillance might be expected.

In conclusion, our results reported that VCM exposed workers had higher frequencies of MN compared with controls and indicates a pronounced clastogenicity of VCM. *XRCC1 T-77C*, *XRCC1 194 Trp/Trp*, *XRCC1 399 Gln/Gln*, *XPA 23 Gly/Gly*, *XPC499 Ala/Val and Val/Val*, *XPC939 Lys/Gln*, *XPF 5′-UTR 2063T* were each associated with higher levels of chromosome damage among Chinese workers exposed to VCM. Moreover, the combined effect of the *XRCC1* (*−77C/T*, *Arg194Trp*, *Arg280His*, *Arg399Gln*), *XPA Ala23Gly*,

XPC Ala499Val, *XPC Lys939Gln*, *XPF 5′-UTR T2063A* and cumulative exposure dose were observed in our study. These results may have important implications for mechanisms of VCM-induced carcinogenesis. In order to understand better the mechanisms of VCM-induced genotoxicity and ultimately carcinogenicity, it is important to evaluate additional variants of genes that participate in the metabolic pathway of VCM. Particular attention should be paid to identify individual internal exposure levels as well as gene–gene and gene–exposure combined effect that may predict individual susceptibility to genetic damage and identify novel genetic markers.

Conflicts of interest

None.

Acknowledgments

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