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ORIGINAL ARTICLE

The effect of genetic polymorphisms in the vinyl chloride metabolic pathway on mutagenic risk

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Abstract Vinyl chloride (VC) is a human carcinogen known to undergo metabolism by cytochrome P450 2E1 (CYP2E1) to reactive intermediates that can cause oncogene and tumor suppressor gene mutations and that are further metabolized by acetaldehyde dehydrogenase (ALDH2) and glutathione-S-transferases (GSTs) to non-mutagenic end products. These metabolic enzymes have known polymorphisms that could lead to increased levels of the VC reactive intermediates and thus an increased risk for mutations and cancer following exposure. Using restriction fragment length polymorphism (RFLP) analysis, we have examined a cohort of 597 French VC workers for polymorphisms in *CYP2E1*, *ALDH2*, *GSTM1* and *GSTT1* in relation to the occurrence of mutant oncogene and tumor suppressor gene biomarkers that are attributable to VC exposure. The presence of the biomarkers for mutant *ras*-p21 and mutant p53 was found to be highly significantly associated with cumulative VC exposure (P for trend <0.0001). The presence of the *CYP2E1* variant c2 allele was found to be significantly associated with the presence of either or both mutant biomarkers even after controlling for potential confounders including cumulative VC exposure (OR = 2.3, 95% CI = 1.2–4.1), and the effects of the c2 allele and VC exposure were approximately additive. *GSTT1* null status was found to have an increased, but not significant association with the presence of either or both biomarkers after controlling for confounders (OR = 1.3, 95% CI = 0.8–2.0). These results suggest the existence of a possible gene–environment interaction between polymorphisms in the VC metabolic pathway and VC exposure that could contribute to the variable susceptibility to the mutagenic effects of VC in exposed populations.

Keywords Gene–environment interaction · Polymorphisms · Mutations · Biomarkers · Cancer

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Introduction

Vinyl chloride (VC) is a known animal and human carcinogen capable of damaging DNA and producing a rare sentinel neoplasm, angiosarcoma of the liver (ASL) (ATSDR 2006). However, the majority of workers exposed to VC do not develop neoplasms, suggesting that susceptibility factors, including inherited metabolic traits, may explain the elevated risk in selected individuals.

In the liver, VC is metabolized by cytochrome P450 2E1 (CYP2E1) to the reactive intermediates chloroethylene oxide (CEO) and chloroacetaldehyde (CAA), which can bind to cellular macromolecules causing damage to DNA, including specific mutations in cancer-related genes such as *ras* and *TP53* (ATSDR 2006); as a result, we have been able to demonstrate that VC-exposed workers have an increasing prevalence of circulating biomarkers for the protein products of these VC-induced mutant genes (i.e., mutant *ras*-p21 and mutant p53) with increasing cumulative exposure to VC (Smith et al. 1998; Li et al. 1998a). CAA is further metabolized by aldehyde dehydrogenase 2 (ALDH2), and CEO is furthered metabolized by glutathione-S-transferases (GSTs) to yield non-genotoxic products

for excretion (ATSDR 2006). Thus, it is possible that individuals who have polymorphisms in the genes for the various enzymes involved in VC metabolism could have different levels of electrophilic, genotoxic intermediates despite similar levels of exposure. For example, individuals with high-activity variants of CYP2E1 and/or low-activity variants of ALDH2 and GSTs could have elevated CEO/CAA, increased DNA damage and increased biomarkers of mutant *ras*-p21 and mutant p53. In fact, we have recently found evidence in a cohort of 211 French VC workers that individuals who have the high-activity CYP2E1 c2 allele or who are GSTM1 or GSTT1 null may be more likely to have one or both of these mutant biomarkers than individuals who have only the wild-type CYP2E1 c1 allele or who are wild-type for GSTM1 or GSTT1, respectively (Li et al. 2003, 2005a).

In order to confirm and extend these studies, we have now expanded this cohort to include a total of 597 workers in the French VC industry. For all these workers, serum samples have been analyzed for the biomarkers of mutant *ras*-p21 and mutant p53, DNA samples have been genotyped for polymorphisms in *CYP2E1*, *ALDH2*, *GSTM1* and *GSTT1*, and the relationship between these polymorphisms and the VC-induced biomarkers examined.

Materials and methods

Subjects for study were selected from a previously described population of VC workers in France (Smith et al. 1998). A cohort of 597 of these workers with available serum samples and lymphocytes for DNA extraction were selected for this study. All the workers in the study were white males with the following characteristics: average age = 59.6 years (range = 33–89); average cumulative exposure = 2,912 ppm-years (range = 0–46,702), where cumulative VC exposure was calculated from estimated average exposures in parts per million of VC in given job categories during certain time periods times the number of years worked in those job categories for each worker, as described previously (Smith et al. 1998); 45.6% current or former smokers; and 23.6% current drinkers. From blood samples collected by venipuncture from each worker, serum and lymphocytes were separated by standard procedures, and lymphocytes were cultured and DNA extracted by routine techniques. This study was approved by the Columbia University Institutional Review Board for human subject research.

Serum samples were assayed for the mutant *ras*-p21 and mutant p53 biomarkers as previously described (Smith et al. 1998; Li et al. 1998a). Briefly, the mutant *ras*-p21 was detected by immunoblotting the serum samples using the primary mouse monoclonal antibody, D146, which is specific for the p21 protein with aspartic acid at amino acid residue 13 and does not cross-react with wild-type p21 or other mutant forms of p21, and the 21 kD bands were confirmed as c-Ki-*ras*-p21 by immunoblotting with the primary mouse monoclonal antibody, 147-67C6, which is specific for the Ki protein and does not cross-react with other *ras*-p21 proteins; this specific biomarker is thus consistent with the codon 13 c-Ki-*ras* gene mutations detected in a high proportion of resultant ASLs in VC-exposed workers. For all immunoblotting, band visualization was achieved via the enhanced chemiluminescence method (ECL, RPN 2106; Amersham, Buckinghamshire, UK). Positive and negative controls of cell lysate from appropriate cell lines were run on every gel with coded serum samples that were recorded as positive or negative in comparison to the controls blinded to exposure status. Immunoblotting specificity was confirmed by failure of reactions with a non-specific mouse myeloma antibody, MOPC-141, and competitive inhibition of positive reactions with cell lysate from positive control cell lines. The reliability of the assay has been demonstrated by the reproducibility of results on triplicate repeats of the same serum sample run on separate gels as well as consistency over time on repeat serum samples taken from the same individuals 1–2 years apart.

The mutant p53 biomarker was determined by two different approaches, direct detection of the mutant protein or detection of antibodies to the mutant protein in the serum samples, as previously described (Smith et al. 1998; Li et al. 1999). The former was performed with a sandwich enzyme-linked immunosorbent assay (ELISA) based on the mouse monoclonal antibody, PAb240, which is specific for the mutant conformation of the proteins produced by the VC-induced mutations; i.e., a high proportion of the resultant ASLs in VC-exposed workers have been found to contain a mutation in the *TP53* gene at codons 179, 249 or 255, and we have previously shown that each of these mutations produced a common conformational change in the resultant mutant p53 proteins that exposed the epitope that is detected by PAb240, which does not cross-react with wild-type p53. For the ELISA, color is developed by incubation of each microtiter well with a secondary polyclonal rabbit reporter antibody, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G, and the chromogenic substrate 2,2′-azino-di-[3-ethylbenthiazoline sulfate]. The absorbance of each well was read on a spectrophotometric plate reader at 405 nm, and the concentration of mutant p53 was determined by comparison with the absorbance of standard solutions of purified, recombinant, mutant human p53. The cut-off for positivity was determined on the basis of previous results obtained with serum from cancer cases compared to normal, healthy controls, which gave the best discrimination between the

two groups. In addition, since some individuals who express a mutant p53 protein develop a specific antibody response to the protein, presumably because the immune system recognizes the conformationally altered protein as ''foreign,'' and since in some cases the presence of such antibodies can hinder the detection of the mutant protein in serum, all samples were also analyzed for the presence of such antibodies to mutant p53 using an ELISA. This ELISA is based on matching microtiter plates coated either with GST-conjugated p53 protein or GST protein alone with split serum samples from each subject incubated on each plate. Then a solution of horseradish peroxidase-conjugated goat anti-human IgG was added to both plates and color developed using a 3,3′,5,5′-tetramethylbenzidine substrate solution with the absorbance read on a spectrophotometric plate reader at 450 nm. For each sample, the ratio of the optical density on the GST-p53 plate to the GST alone plate was calculated. The cut-off for positivity was determined on the basis of previous results from the ratio of optical densities of serum samples from cancer cases and normal, healthy controls, which gave the best discrimination between the two groups. Known antibody-positive and antibody-negative serum samples were included on each plate. This assay has been shown to be accurate and reproducible, yielding results in good agreement with those obtained by immunoblotting and on repeated assays on the same serum samples. Anyone who had a serum sample that was positive for the mutant p53 protein or the p53 auto-antibody or both was considered to be mutant p53 biomarker-positive.

DNA samples from each of the workers were analyzed for polymorphisms in *CYP2E1*, *ALDH2*, *GSTM1* and *GSTT1*, as previously described (Li et al. 2003, 2005a). For each DNA sample, the *CYP2E1* PstI polymorphism was determined by a modification of the method of Hayashi et al. (1991). The primers used were 5′-CCAGTCGAGTCTACATTGTCA-3′ and 5′-TTCATTCTGTCTTCTAACTGG-3′. For amplification, 20 ng of sample DNA was added to a PCR mixture containing 25 ng of primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 50 mM KCl, 10 mM Tris–HCl (pH 8.3) and 0.25 units of Taq polymerase in a final volume of 10 µl. Amplification was performed for 32 cycles, denaturing at 94°C for 30 s, annealing at 55°C for 30 s and extending at 72°C for 1 min. The PCR products were digested with PstI for 20 h at 37°C and analyzed with 2.2% agarose gel electrophoresis with appropriate controls. In this assay, homozygous c1c1 samples have a single product fragment of 413 bp, homozygous c2c2 samples have 295- and 118-bp fragments, and heterozygous c1c2 samples have all three fragments.

For each DNA sample, the *ALDH2* MboII polymorphism was determined by a modification of the methods of Harada and Zhang (1993). Primers were synthesized from the 5′ region of exon 12 (5′-CAAATTACAGGGTCAACTGCT-3′) and the 3′ region of exon 12 (5′-CCACACTCACAGTTTTCTCTT-3′). Using the same PCR mixture as above, samples were heated at 94°C for 5 min, and then 34 cycles of amplification were performed, denaturing at 94°C for 25 s, annealing at 59°C for 30 s and extending at 72°C for 45 s. The PCR products were digested with MboII for 4 h at 37°C and analyzed with 4% NuSieve 3:1 agarose gel electrophoresis with appropriate controls. In this assay, homozygous 2–2 samples have a single product fragment of 135 bp, homozygous 1–1 samples have 126- and 9-bp fragments, and heterozygous 1–2 samples have all three fragments.

For each DNA sample, the *GSTM1* or *GSTT1* null polymorphisms were determined as previously described (Ford et al. 2000; Wiencke et al. 1995). For *GSTM1*, 20 ng DNA was added to the PCR reaction mixture containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 units Taq polymerase, 20 ng *GSTM1* primer pairs (5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′) and 10 ng β-globin primer pairs (5′-CAACTTCATCCACGTTCACC-3′ and 5′-GAAGAGCCAAGGACAGGTAC-3′), used as an internal control. The reaction mixture was denatured at 94°C for 4 min and then subjected to 34 cycles of 94°C for 25 s, 55°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The PCR products were examined by 2% agarose gel electrophoresis with ethidium bromide staining. A 273-bp band (β-globin gene) and a 219-bp band (*GSTM1* gene) were identified in individuals who were *GSTM1* wild-type, while the latter was missing in those individuals who were *GSTM1* null.

For *GSTT1*, the PCR mixture as above contained 30 ng DNA, 0.4 units Taq polymerase, 30 ng β-globin primer pairs and 30 ng *GSTT1* primer pairs (5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAGCA-3′). The reaction mixture was denatured at 94°C for 4 min and then subjected to 34 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min followed by a final extension at 72°C for 5 min. The PCR products were examined by 1.8% agarose gel electrophoresis with ethidium bromide staining. A 273-bp band (β-globin gene) and a 480-bp gene (*GSTT1* gene) were identified in individuals who were *GSTT1* wild-type, while the latter was missing in those individuals who were *GSTT1* null.

For the analysis of the data, first the exposed workers were stratified into quartiles by exposure level and the effect of cumulative VC exposure on the occurrence of the mutant *ras*-p21 and mutant p53 biomarkers determined by logistic regression analysis in comparison to the unexposed controls adjusting for potential confounders such as age, smoking and drinking. Then, among the exposed workers, the independent effect of the various polymorphisms on the

occurrence of the mutant *ras*-p21 and mutant p53 biomarkers was determined by logistic regression analysis adjusting for age, smoking, drinking, and cumulative VC exposure.

Results

Among the 597 workers, 51 were considered to have never been exposed to VC and could thus serve as comparable, unexposed controls. The remaining 546 workers had VC exposures ranging from 2 to 46,702 ppm-years and could be divided into tertiles of approximately equal size with the following exposure levels: 2–1,705 ppm-years ($N = 179$), 1,706–5,703 ppm-years ($N = 186$) and >5,703 ppm-years ($N = 181$). For the total cohort of 597 workers, 47.6% were positive for at least one mutant biomarker (18.3% positive for mutant *ras*-p21; 18.8% positive for mutant p53; 10.6% positive for both). The distribution of biomarker positivity by exposure status is shown in Table 1. Assigning an odds ratio of one to the unexposed controls and adjusting for potential confounders such as age, drinking and smoking, the odds ratio for the occurrence of either or both mutant biomarkers increased to 9.7 (95% CI = 3.3–28.7) in the low exposure tertile, to 14.5 (95% CI = 4.9–42.9) in the medium exposure tertile, and to 26.5 (95% CI = 8.7–80.2) in the high exposure tertile, with a trend that was highly significant ($P < 0.0001$), as we have seen previously in this cohort (Li et al. 1998b).

However, among the 546 exposed workers, within each tertile of exposure there were workers who were comparable in terms of age, smoking, drinking and cumulative exposure levels, but who could be positive for none, one or both mutant biomarkers. To determine whether this could be at least partially attributable to genetic variations, the effect of polymorphisms in *CYP2E1*, *ALDH2*, *GSTM1* and *GSTT1* was examined. None of the exposed workers were found to have the variant *ALDH2-2* allele, which is believed to produce lower enzymatic activity for phase II metabolism. For *CYP2E1*, 505 workers were homozygous wild-type (c1c1, 92.5%), 40 were heterozygous (c1c2, 7.3%) and 1 was homozygous variant (c2c2, 0.2%), consistent with a Hardy–Weinberg equilibrium ($\chi^2 = 0.05$) and previous results in this and comparable populations (Li et al. 2003; Nebert et al. 1996). For *GSTM1*, 243 workers were wild-type (44.5%) and 303 were null (55.5%), and for GSTT1, 465 workers were wild-type (85.2%) and 81 were null (14.8%), consistent with previous results in this and comparable populations (Li et al. 2005a; Habdous et al. 2004).

The effect of the *CYP2E1* polymorphism on the mutant biomarkers is shown in Table 2. Assigning an odds ratio of 1 to the c1c1 homozygotes and adjusting for age, smoking, drinking and cumulative VC exposure, the odds ratio for the occurrence of either or both mutant biomarkers increased to 2.3 (95% CI = 1.2–4.1) for individuals who were either c1c2 heterozygotes or c2c2 homozygotes; the variant genotypes were combined for this analysis since there was only one individual who was c2c2 (who was positive for the mutant *ras*-p21 biomarker). The effects of the *GST* polymorphisms on the mutant biomarkers are shown in Tables 3 and 4. *GSTM1* had no effect on the biomarkers; for the *GSTM1* null workers, the adjusted odds ratio for the occurrence of either or both mutant biomarkers was 1.0 (95% CI = 0.7–1.3) compared to the *GSTM1* wild-type workers. *GSTT1* had a small, non-significant effect on the mutant biomarkers; for the *GSTT1* null workers, the adjusted odds ratio for the occurrence of either or both mutant biomarkers was 1.3 (95% CI = 0.8–2.0) compared to the *GSTT1* wild-type workers.

Discussion

These results suggest that polymorphisms in the genes for enzymes responsible for the metabolism of VC, particularly in phase I (i.e., CYP2E1) and possibly in phase II (i.e., GSTT1), can affect the risk for the occurrence of VC-induced mutations, independent of the level of VC exposure. These results extend our prior findings from a smaller sample of this cohort. For example, the prior studies did find an association between the *CYP2E1* c2 allele and the presence of the mutant biomarkers that just reached statistical significance and had very wide confidence intervals

Table 1 Association between cumulative VC exposure and mutant *ras*-p21 and mutant p53 biomarkers in VC workers

Exposure (ppm-years)	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
	Both –	Either +	Both +	
0 ($n = 51$)	47 (92%)	4 (8%)	0 (0%)	1.0
2–1,705 ($n = 179$)	99 (55%)	69 (39%)	11 (6%)	9.7 (3.3–28.7)
1,706–5,703 ($n = 186$)	96 (52%)	67 (36%)	23 (12%)	14.5 (4.9–42.9)
>5,703 ($n = 181$)	71 (39%)	81 (45%)	29 (16%)	26.5 (8.7–80.2)

* Adjusted for age, smoking and drinking; for trend, $P < 0.0001$

Table 2 Association between *CYP2E1* polymorphism and mutant *ras*-p21 and mutant p53 biomarkers in VC workers

CYP2E1 status	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
	Both –	Either +	Both +	
c1c1 ($n = 505$)	254 (50%)	195 (39%)	56 (11%)	1.0
c1c2/c2c2 ($n = 41$)	12 (29%)	22 (54%)	7 (17%)	2.3 (1.2–4.1)

* Adjusted for age, smoking, drinking and cumulative VC exposure

Table 3 Association between *GSTM1* polymorphism and mutant *ras*-p21 and mutant p53 biomarkers in VC workers

GSTM1 status	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
	Both –	Either +	Both +	
Wild-type ($n = 243$)	118 (49%)	96 (40%)	29 (12%)	1.0
Null ($n = 303$)	148 (49%)	121 (40%)	34 (11%)	1.0 (0.7–1.3)

* Adjusted for age, smoking, drinking and cumulative VC exposure

Table 4 Association between *GSTT1* polymorphism and mutant *ras*-p21 and mutant p53 biomarkers in VC workers

GSTT1 status	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
	Both –	Either +	Both +	
Wild-type ($n = 465$)	231 (50%)	181 (39%)	53 (11%)	1.0
Null ($n = 81$)	35 (43%)	36 (44%)	10 (12%)	1.3 (0.8–2.0)

* Adjusted for age, smoking, drinking and cumulative VC exposure

due to the small numbers of subjects examined (Li et al. 2003), whereas in the expanded cohort of the current analysis the statistical association is much more robust. These results also provide additional support for the existence of a potential gene–environment interaction in VC-exposed populations. For example, if we further examine the relationship among cumulative exposure levels, *CYP2E1* genotype and the occurrence of the mutant biomarkers (Table 5), we see an approximately additive effect of exposure and genotype when comparing the odds ratios in the high exposure tertile of the homozygous wild-type strata and the low exposure tertile in the heterozygous/homozygous variant strata with the odds ratio in the high exposure tertile of the heterozygous/homozygous variant strata (2.9 + 3.3 ~ 5.1), although the interaction term does not achieve statistical significance. Furthermore, there may be an additional gene–environment interaction with CYP2E1 and the personal habits of these workers. For example, CYP2E1 is involved in the metabolism of ethanol also and can be induced by ethanol exposure. Thus, one might expect that workers who consume alcohol on a regular daily basis and are exposed to VC would have higher levels of CYP2E1 and higher CYP2E1 activity and thus produce more reactive intermediates and generate more VC-induced mutations than workers who do not consume alcohol at any given level of VC exposure;

Table 5 Effect of interaction of *CYP2E1* polymorphism with VC exposure on mutant *ras*-p21 and mutant p53 biomarkers in VC workers

CYP2E1 status	Exposure (ppm-years)	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
		Both –	Either +	Both +	
c1c1 ($n = 167$)	2–1,705	95 (57%)	64 (38%)	8 (5%)	1.0
c1c1 ($n = 176$)	1,706–5,703	93 (53%)	61 (35%)	22 (13%)	1.6 (1.0–2.4)
c1c1 ($n = 162$)	>5,703	66 (41%)	70 (43%)	26 (16%)	2.9 (1.8–4.6)
c1c2/c2c2 ($n = 12$)	2–1,705	4 (33%)	5 (42%)	3 (25%)	3.3 (1.1–10.1)
c1c2/c2c2 ($n = 10$)	1,706–5,703	3 (30%)	6 (60%)	1 (10%)	3.1 (0.9–10.5)
c1c2/c2c2 ($n = 19$)	>5,703	5 (26%)	11 (58%)	3 (16%)	5.1 (2.0–13.1)

* Adjusted for age, smoking and drinking; for trend, $P < 0.0001$

Table 6 Effect of interaction of *CYP2E1* polymorphism with drinking on mutant *ras*-p21 and mutant p53 biomarkers in VC workers

CYP2E1 status	Drinking	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
		Both –	Either +	Both +	
c1c1 (*n* = 374)	No	193 (52%)	143 (38%)	38 (10%)	1.0
c1c1 (*n* = 131)	Yes	61 (47%)	52 (40%)	18 (14%)	1.4 (0.9–2.0)
c1c2/c2c2 (*n* = 31)	No	9 (29%)	17 (55%)	5 (16%)	2.3 (1.1–4.6)
c1c2/c2c2 (*n* = 10)	Yes	3 (30%)	5 (50%)	2 (20%)	3.0 (0.9–9.9)

* Adjusted for age, smoking and cumulative VC exposure; for trend, $P = 0.03$

furthermore, one might expect this effect to be accentuated in workers who consume alcohol and have the high-activity *CYP2E1* c2 allele. As shown in Table 6, this seems to be the case. Assigning an odds ratio of 1 to workers who are *CYP2E1* c1c1 and not daily alcohol drinkers, the adjusted odds ratio for the occurrence of either or both mutant biomarker increases to 1.4 (95% CI = 0.9–2.0) in workers who are *CYP2E1* c1c1 and are daily alcohol drinkers, to 2.3 (95% CI = 1.1–4.6) in workers who are *CYP2E1* c1c2 or c2c2 and not daily alcohol drinkers, and to 3.0 (95% CI = 0.9–9.9) in workers who are *CYP2E1* c1c2 or c2c2 and daily alcohol drinkers, with a statistically significant trend ($P = 0.003$); once again, this interaction appears to be approximately additive (1.4 + 2.3 ~ 3.0). This is consistent with results of animal studies that show that rats exposed to both VC and ethanol have higher rates of tumor formation than animals exposed to VC alone (Radicke et al. 1981). Finally, although it is somewhat surprising that the effects of the *GST* polymorphisms were so minimal, there could be several explanations for this. The first would be that other GSTs may participate in the phase II metabolism when GSTM1 and GSTT1 are decreased. Although we have previously examined the effect of a *GSTP1* polymorphism in this pathway, and it also had no apparent effect (Li et al. 2005a), there are still other GSTs that have not yet been examined that may be able to compensate for decreased GSTM1, GSTP1 and/or GSTT1 activity. Alternatively, it is possible that if CEO converts readily to CAA, ALDH2 could provide an alternate compensatory pathway for phase II metabolism; in our study, no polymorphisms in *ALDH2* were found so that the pathway was intact in our cohort, whereas in other studies of VC-exposed cohorts with this polymorphism, its effect on VC metabolism was apparent (Wong et al. 2003; Zhu et al. 2005). However, this does not seem likely to be a major consideration since the rate constant for rearrangement of CEO to CAA is known to be small, suggesting that isomerization of CEO is a relatively minor reaction (Barbin et al 1990), and thus ALDH2 metabolism of CAA could provide only minimal compensation for loss of GST metabolism of CEO. Lastly, an important consideration may be the relatively short half-life of CEO itself (Barbin et al. 1975), which may suggest that its high reactivity outpaces the activity of GSTs even under normal circumstances, so the presence or absence of *GST* polymorphisms is of lesser relevance. However, the results of the present study may indicate possible gene–gene interactions between the polymorphisms in the enzymes of phase I and phase II metabolism when multiple defects are present simultaneously. As seen in Table 7, assigning an odds ratio of 1 to those workers who are wild-type for *CYP2E1*, *GSTM1* and *GSTT1*, the adjusted odds ratio for the occurrence of either or both mutant biomarkers increases to 1.2 (95% CI = 0.8–1.6) for those workers who have any one variant allele and to 1.5 (95% CI = 0.9–2.6) for workers who have two or more variant alleles, although these results still do not achieve statistical significance. Nevertheless, overall the findings of this study suggest the existence of potentially complex gene (*CYP2E1*)–gene (*GST*)–environment (alcohol)–environment (VC) interactions that could influence the carcinogenic risk in VC-exposed workers.

All these results are entirely consistent with the proposed carcinogenic pathway for VC (Li et al. 1998b). As noted, VC is thought to be initially metabolized in the liver

Table 7 Association between polymorphisms in *CYP2E1*, *GSTM1* and *GSTT1* and mutant *ras*-p21 and mutant p53 biomarkers in VC workers

CYP2E1/GSTM1/GSTT1 status	p21 and p53 biomarkers			Adjusted odds ratio* (95% CI)
	Both –	Either +	Both +	
All wild-type alleles (*n* = 191)	100 (52%)	69 (36%)	22 (11%)	1.0
1 polymorphic allele (*n* = 287)	138 (48%)	118 (41%)	31 (11%)	1.2 (0.8–1.6)
2 or 3 polymorphic alleles (*n* = 68)	28 (41%)	30 (44%)	10 (15%)	1.5 (0.9–2.6)

* Adjusted for age, smoking, drinking and cumulative VC exposure; for trend, $P = 0.13$

primarily by CYP2E1, which generates the reactive intermediates CEO and CAA, which can be further metabolized by GSTs to non-genotoxic products that are excreted. However, CEO and CAA are known to form etheno-DNA adducts (Guichard et al. 1996; Barbin 1998), including etheno-G and etheno-A, which are pro-mutagenic and could be responsible for the high proportion of VC-induced ASLs that are found to have G $\rightarrow$ A transitions in the Ki-*ras* oncogene and A $\rightarrow$ T transversions in the *TP53* tumor suppressor gene, respectively, and consequently could be responsible for the high proportion of VC-exposed workers who have biomarkers for mutant *ras*-p21 and mutant p53. Thus, at any given level of VC exposure, any factor that leads to increased CYP2E1 activity (induction by alcohol or the c2 polymorphism) or to decreased GST activity (null polymorphism) would be expected to lead to higher levels of CEO and CAA, an increase in etheno-DNA adducts and etheno-adduct generated mutations, and an increased occurrence of the biomarkers for these mutations, as the current results indicate. These results are also consistent with studies in other VC worker cohorts. For example, studies of VC-exposed workers in Taiwan have found the CYP2E1 polymorphism to be associated with a higher frequency of sister chromatid exchanges, as well as a higher prevalence of the same mutant p53 biomarkers examined in this study (Wong et al. 2002, 2003). Studies of VC-exposed workers in China have found the CYP2E1 polymorphism to be associated with elevated DNA damage as detected by the Comet assay or increased liver abnormalities detected by hepatic ultrasonography (Zhu et al. 2005).

Identification of workers at elevated risk from VC due to these effects could lead to more effective interventions to reduce their risk. For example, individuals with these polymorphisms could be placed in job categories with lower or no VC exposure and/or advised to minimize their alcohol intake. Alternatively or in addition, pharmacological interventions that reduce the accumulation of the reactive intermediates due to these polymorphisms may be helpful. For example, it is known that some ingredient in watercress effectively inhibits CYP2E1's metabolic activity (LeClercq et al. 1998), and thus high-risk workers given watercress or its active ingredient should generate lower levels of CEO and CAA at any given level of VC exposure. Finally, in workers who have already experienced VC-induced mutations, it may be possible to reverse the carcinogenic effect. For example, we have developed a synthetic peptide analogue for a region of p53 that causes mutant p53 to revert to normal function, reinstating its ability to cause apoptosis in cancerous cells and pre-cancerous cells that contain a p53 mutation both in cell culture and in animal models (Li et al. 2005b; Senatus et al. 2006); we have recently shown that this peptide can effectively kill HAEND cells in culture that are derived from an ASL in a VC-exposed worker (Hoover et al. 1993). Thus, such a treatment could potentially be used as chemotherapy for VC-exposed workers who have ASLs as well as chemo-prophylaxis for VC-exposed workers who do not yet have ASLs, but are at high risk of developing ASLs in the future due to their increased susceptibility from genetic polymorphisms or other factors as evidenced by their increased rate of mutant biomarkers.

In summary, the results of the current study suggest the existence of potential gene–environment and gene–gene interactions in individuals exposed to VC. In addition, it should be noted that this model could have much broader implications, since other potential carcinogenic exposures are also metabolized by these pathways and polymorphisms in these enzymes can be considerably more common in other ethnic groups.

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