International Journal of Occupational Medicine and Environmental Health, 2003; 16(1): 55—59

POLYMORPHISMS FOR VINYL CHLORIDE METABOLISM IN FRENCH VINYL CHLORIDE WORKERS

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Abstract. Genetic polymorphisms of aldehyde dehydrogenase 2 (ALDH2) and cytochrome P450 2E1 (CYP2E1) have been shown to influence the degree of genetic damage in Taiwanese workers exposed to the carcinogen - vinyl chloride (VC). Certain French VC workers have been found to express biomarkers of mutant forms of cancer-related proteins (*ras*-p21 and p53) that have been related to their exposure. ALDH2 and CYP2E1 polymorphisms were investigated in 211 of these workers in an attempt to correlate differences in VC metabolic capacity with differences in the presence of these biomarkers. All of the workers were found to have the normal, wild-type ALDH2 gene, and none of them were found to be homozygous for the variant CYP2E1 allele. Sixteen workers were found to be heterozygous for the variant CYP2E1 allele. After adjusting for age, smoking, drinking and cumulative VC exposure, the odds ratio for the presence of either the mutant *ras*-p21 or the mutant p53 biomarker in these heterozygous workers was found to be statistically significantly increased in comparison to their homozygous, wild-type counterparts (OR = 5.05; 95% CI = 1.10-23.25). However, as opposed to the case in Taiwanese workers, these polymorphisms are relatively uncommon, and thus differences in ALDH2 and CYP2E1 can account for only a small proportion of the variability in mutagenic response to VC exposure in a Caucasian population.

Key words:
Vinyl chloride, Mutations, Response biomarkers, Susceptibility biomarkers

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) [1]. However, the majority of workers exposed to VC do not develop neoplasms, suggesting that susceptibility factors involving inherited metabolic traits may explain the elevated risk in selected individuals.

In the liver, VC is metabolized by CYP2E1 to the reactive metabolites chloroethylene oxide (CEO) and chloro-

This work was supported in part by grants from the US Environmental Protection Agency (R-825361), the National Cancer Institute (R01-CA69243, T32-CA09529), the National Institute of Environmental Health Sciences (P30-ES09089) and the National Institute of Occupational Safety and Health (R01-OH04192).
Received: December 2, 2002. Accepted: February 6, 2003.
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acetaldehyde (CAA) which can bind to cellular macromolecules causing damage to DNA, including specific mutations in cancer-related genes such as *ras* and p53 [1]. CEO is further metabolized by glutathione-S-transferases, and CAA is further metabolized by aldehyde dehydrogenase 2 (ALDH2) to yield non-genotoxic metabolites for excretion [1]. Thus, it is possible that individuals who have polymorphisms of 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 could have elevated CEO/CAA and increased DNA damage. In fact, it has recently been shown in VC workers in Taiwan that individuals with the CYP2E1 c1c2/c2c2 genotypes in combination with the ALDH2 1-2/2-2 genotypes had a significantly increased frequency of sister chromatid exchange (SCE) in the DNA of their peripheral lymphocytes [2].

In studies of French VC workers, we have previously identified a dose-dependent increase in circulating mutant *ras*-p21 and p53 proteins presumably indicative of VC-induced genetic alterations [3,4]. However, among these workers, individuals with estimated similar exposures could have different patterns of expression of these mutant proteins. In this study, we have examined a subset of these French workers for CYP2E1 and ALDH2 polymorphisms to determine if this genetic susceptibility could account for the difference in VC-induced genetic injury, as it did in the Taiwanese workers.

MATERIALS AND METHODS

Subjects for study were selected from a previously described population of VC-exposed workers in France [3,4]. A cohort of 225 of these workers had been previously analyzed for circulating mutant *ras*-p21 and mutant p53 proteins in their serum [3,4]. A group of 211 of these workers with available lymphocytes for DNA extraction were selected for the study. The study protocol was reviewed and approved by the Columbia University Institutional Review Board for conformance with human subjects protections. All of the workers in the study were white males with the following characteristics: average age = 56 years (range = 35–74); average cumulative exposure = 5871 ppm-years (range = 6–46,702); 39.3% of current or former smokers; 19.9% of current drinkers; 62% of positive of at least one mutant oncoprotein biomarker (44.5% positive for mutant p53, 36.5% positive for mutant *ras*-p21, 19% positive for both).

From blood samples from each of the workers, lymphocytes were cultured and DNA extracted by routine techniques. For each DNA sample, the ALDH2-MboII polymorphism was determined by a modification of the methods of Harada and Zhang [5]. Primers were synthesized from the 5' region of exon 12 (5'-CAAATTACAGGGTCAACTGCT-3') and the 3' region of exon 12 (5'-CCACACTCACAGTTTTCTCTT-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. Samples were heated at 94°C for 5 min, and then 34 cycles of amplification were performed, denaturing at 94°C for 25 sec, annealing at 59°C for 30 sec and extending at 72°C for 45 sec. 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 CYP2E1 PstI polymorphism was determined by a modification of the method of Hayashi et al. [6]. The primers used were 5'-CCAGTCGAGTCTACATTGTCA-3' (sense) and 5'-TTCATTCTGTCTTCTAACTGG-3' (antisense). Using the same PCR mixture as above, amplification was performed for 32 cycles, denaturing at 94°C for 30 sec, annealing at 55°C for 30 sec 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 bp and 118 bp fragments, and heterozygous c1c2 samples have all three fragments.

RESULTS AND DISCUSSION

In contrast to the Taiwanese cohort, all individuals in this study were found to have the normal, homozygous, wild-type ALDH2 status (1-1). Thus, variation in ALDH2 cannot explain the differences in sero-positivity for markers of genetic damage in this cohort of VC workers.

Among the 211 workers in this study, 195 were found to have the normal, homozygous, wild-type CYP2E1 status (c1/c1) and 16 were found to have the heterozygous CYP2E1 status (c1/c2). No one was homozygous for the variant CYP2E1 status (c2/c2). There was no significant difference in terms of the distribution of exposure levels between those workers who were c1/c1 and those who were c1/c2; e.g., the exposure breakdown for the c1/c1 workers was 21% at $\leq$500 ppm-years, 25% at 501–2500 ppm-years, 23% at 2501–5000 ppm-years, and 31% at >5000 ppm-years, whereas the exposure breakdown for the c1/c2 workers was 13% at $\leq$500 ppm-years, 31% at 501–2500 ppm-years, 31% at 2501–5000 ppm-years, and 25% at >5000 ppm-years ($\chi^2 = 1.39$, $p = 0.7$). Of the 195 who were c1/c1, 85 (44%) were positive for the mutant p53 biomarker, whereas, of the 16 who were c1/c2, 9 (56%) were positive for the mutant p53 biomarker. After adjusting for age, smoking, drinking and VC exposure, the odds ratio for mutant p53 positivity among the c1/c2 workers was found to be elevated in comparison to the c1/c1 workers, although this was not statistically significant (OR = 1.62, 95% CI = 0.54–4.88) (Table 1). Correspondingly, of the 195 who were c1/c1, 69 (35%) were positive for the mutant *ras*-p21 biomarker, whereas, of the 16 who were c1/c2, 8 (50%) were positive for the mutant *ras*-p21 biomarker. After adjusting for age, smoking, drinking and VC exposure, the odds ratio for mutant *ras*-p21 positivity among the c1/c2 workers was found to be elevated in comparison to the c1/c1 workers, although again this was not statistically significant (OR = 1.84, 95% CI = 0.65–5.19) (Table 2). However, when the biomarkers are considered in combination, a significant effect of CYP2E1 heterozygosity is seen. After adjusting for age, smoking, drinking and VC exposure, the odds ratio for either

Table 1. Association between CYP2E1 polymorphism and mutant p53 biomarker in VC workers

CYP2E1 status	p53 biomarker		Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
	–	+		
c1c1 (n = 195)	110 (56%)	85 (44%)	1.00	1.00
c1c2 (n = 16)	7 (44%)	9 (56%)	1.66 (0.60–4.62)	1.62 (0.54–4.88)
c2c2 (n = 0)	0	0	–	–

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

Table 2. Association between CYP2E1 polymorphism and mutant *ras*-p21 biomarker in VC workers

CYP2E1 status	p21 biomarker		Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
	–	+		
c1c1 (n = 195)	126 (65%)	69 (35%)	1.00	1.00
c1c2 (n = 16)	8 (50%)	8 (50%)	1.83 (0.66–5.03)	1.84 (0.65–5.19)
c2c2 (n = 0)	0	0	–	–

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

mutant p53 or mutant *ras*-p21 positivity among the c1/c2 workers was found to be statistically significantly elevated in comparison to the c1/c1 workers (OR = 5.05; 95% CI = 1.10–23.25) (Table 3).

Since ethanol is also metabolized by CYP2E1, and ethanol and VC have been shown to have a combined effect on carcinogenesis in animal models [7], the effect of drinking on the relationship between CYP2E1 status and biomarker positivity was further examined. Assigning an odds ratio of 1 to those workers who were c1/c1 and non-drinkers, the odds ratios for the presence of either biomarker were 1.11 (95% CI = 0.53–2.29) in workers who were c1/c1 and drinkers, 2.59 (95% CI = 0.68–13.73) in workers who were c1/c2 and non-drinkers, and 7.51 (95% CI = 0.62–91.34) in workers who were c1/c2 and drinkers (p for trend = 0.062). This is consistent with the fact that both VC and ethanol metabolism are mediated by CYP2E1. Similarly, the effect of smoking on the relationship between CYP2E1 status and p53 biomarker positivity was also examined. Assigning odds ratio of 1 to those workers who were c1/c1 and non-smokers, the odds ratios for the presence of either biomarker were 1.03 (95% CI = 0.58–1.86) in workers who were c1/c1 and smokers, 2.03 (95% CI = 0.47–8.74) in workers who were c1/c2 and non-smokers, and 10.17 (95% CI = 0.95–108.99) in workers who were c1/c2 and smokers (p for trend = 0.088).

As noted above, in Taiwanese workers the presence of ALDH2-2 in combination with CYP2E1-c2 alleles was found to be significantly associated with an elevation of SCE frequency [2], another indicator of genetic damage. In the present study, no ALDH2-2 variants were identified and a significant, but small effect was found for the CYP2E1-c2 allele on mutant *ras*-p21 or mutant p53 biomarker status. This partial discrepancy between the two different worker groups could be due to several things. For example, it is possible that SCE frequency is unrelated to markers of specific genetic mutation. However, Taiwanese VC workers have also been identified with the same serum biomarkers of specific mutant proteins as in this cohort and with an approximately similar dose-response in terms of cumulative VC exposure [8,9]. It seems unlikely that markers of gross chromosomal damage such as SCEs are unrelated to more specific mutant markers, since both would result from DNA damage induced by the same reactive intermediates.

A more likely explanation is that there is a basic difference in the genetics of the Taiwanese and French populations. The ALDH2-2 allele has been described as relatively common in Asian populations, occurring in 24–32% of Taiwanese [10,11], but has only occasionally been identified in other populations such as Caucasians [12]. Although we had anticipated finding at least a few ALDH2 polymorphic individuals within a cohort of this size, the absence of the variant allele in this study is entirely consistent with some reported low allele frequencies. Similarly, the CYP2E1-c2 allele is more common in Asian populations (identified in 19.5% of the Taiwanese workers) compared to Caucasian populations (2–9%) [13,14], like in this study (7.6%).

Finally, phenotypic expression of these metabolic enzymes, in addition to genotypic differences, may vary in different populations. For example, CYP2E1 expression is inducible by ethanol [15]. If ethanol consumption or other

Table 3. Association between CYP2EI polymorphism and mutant p53 and mutant *ras*-p21 biomarkers in VC workers

CYP2E1 status	p53 and p21 biomarker		Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
	Both –	Either +		
c1c1 (n = 195)	78 (40%)	117 (60%)	1.00	1.00
c1c2 (n = 16)	2 (12.5%)	14 (87.5%)	4.67 (1.16–18.71)	5.05 (1.10–23.25)
c2c2 (n = 0)	0	0	–	–

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

inducing exposures (such as drugs or diet) varies between populations, then the level of expression in terms of enzymatic activity could vary altering the risk from exposures independent of genotype.

These results serve to underscore the fact that different populations can differ substantially in their metabolic processing of similar genotoxic workplace exposures. If genetic metabolic susceptibilities are to be used in refining risk assessments for such exposures, as has been suggested [16], these metabolic differences between populations will have to be taken into account. Exposure levels that may be deemed acceptable in one population cannot necessarily be translated directly to another population with a different basis for their susceptibility.

Finally, although ALDH2 and CYP2E1 polymorphisms do not appear to contribute greatly to altered susceptibility to VC in French workers, it is possible that polymorphisms in other genes can be identified, which can explain the differences observed. For example, polymorphisms in the glutathione-S-tranferases are known to be relatively common in Caucasian populations and thus could contribute to the variability seen, particularly since they have been implicated in the metabolism of the CEO intermediate. Besides metabolism of VC to reactive intermediates, repair of resultant DNA adducts also likely contributes to the accumulation of cancer-related mutations, as has recently been reported among the Taiwanese VC workers [17]. Preliminary results from analysis of DNA repair polymorphisms in these workers suggest that this may in fact be a highly significant and major contributor to altered susceptibility for the occurrence of these VC-induced mutations.

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