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RESEARCH ARTICLE

Gene–environment interactions between DNA repair polymorphisms and exposure to the carcinogen vinyl chloride

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Abstract

We have recently suggested that polymorphisms in metabolism and repair pathways may play a role in modulating the effects of exposure to the carcinogen vinyl chloride in the production of biomarkers of its mutagenic damage. The aim of the present study was to extend these observations by examining gene–environment interactions between several common polymorphisms in the DNA repair genes *XRCC1* and *ERCC2/XPD* and vinyl chloride exposure on the production of vinyl chloride-induced biomarkers of mutation. A cohort of 546 French vinyl chloride workers were genotyped for the *XRCC1* codon 194 (Arg>Trp; rs1799782), 280 (Arg>His; rs25489) and 399 (Arg>Gln; rs25487) polymorphisms and the *ERCC2/XPD* codon 312 (Asp>Asn; rs1799793) and 751 (Lys>Gln; rs13181) polymorphisms. The results demonstrated a statistically significant allele dosage effect of the *XRCC1* 399 variant on the production of the vinyl chloride-induced mutant p53 biomarker, even after controlling for confounders including cumulative vinyl chloride exposure ($p = 0.03$), with a potentially supramultiplicative gene–environment interaction. In addition, the results demonstrate statistically significant allele dosage effects of the *ERCC2/XPD* 312 and 751 variants on the production of the vinyl chloride-induced mutant *ras*-p21 biomarker, even after controlling for confounders including cumulative vinyl chloride exposure ($p < 0.0001$ and $p = 0.0006$, respectively), with a potentially supramultiplicative gene–environment interaction for the codon 751 allele. Finally, the results suggest potential supramultiplicative gene–gene interactions between *CYP2E1* (c2 allele; rs3813867) and *ERCC2/XPD* polymorphisms that are consistent with the proposed carcinogenic pathway for vinyl chloride, which requires metabolic activation by CYP2E1 to reactive intermediates that form DNA adducts that, if not removed by DNA repair mechanisms, result in oncogenic mutations.

Keywords: *Gene–environment interaction; polymorphisms; mutations; biomarkers; cancer*

Introduction

Gene–environment interactions are believed to play important roles in mediating the health effects produced by exposures to exogenous toxins (Miller et al. 2001). In environmental carcinogenesis, gene–environment interaction studies have focused mainly on polymorphic variants in enzymes that generate or eliminate genotoxic intermediates and to a much lesser degree on variations in the repair of genetic damage (Miller et al. 2001). A potential model system for investigation of the latter is provided by the repair of etheno-DNA adducts produced by exposure to the mutagenic carcinogen vinyl chloride (VC).

VC is a known animal and human carcinogen associated with the sentinel neoplasm of angiosarcoma of the liver (ASL) (ATSDR 2006). Following exposure, VC is metabolized principally in the liver by cytochrome P450

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2E1 (CYP2E1), and the resultant electrophilic metabolites chloroethylene oxide and chloroacetaldehyde are believed to be the proximate carcinogens because of their capacity to form the etheno-DNA adducts 1,N^6-ethenoadenine (1,N^6-εA) and N^2,3-ethenoguanine (N^2,3-εG) (ATSDR 2006). These adducts are promutagenic and could account for the mutations seen in ASLs of exposed individuals. For example, the εA adduct can cause A to T transversions in the *TP53* tumor suppressor gene that have been identified in a high proportion of ASLs from individuals with VC exposure (Hollstein et al. 1994). These mutations have also been associated with biomarkers of mutant p53, including mutant p53 protein and anti-p53 antibodies, in VC-exposed individuals with ASL (Brandt-Rauf et al. 1996). Similarly, the εG adduct can cause G to A transitions in the *K-ras* oncogene that have been identified in a high proportion of ASLs from individuals with VC exposure (Marion et al. 1991). These mutations have also been associated with biomarkers of the mutant *ras*-p21 protein in VC-exposed individuals with ASL (DeVivo et al. 1994). In addition, these biomarkers for both mutant p53 and mutant *ras*-p21 have been found in a high proportion of VC-exposed individuals without disease in a highly statistically significant dose–response relationship, reinforcing the idea that generation of the biomarkers is a direct result of VC exposure (Smith et al. 1998; Li et al. 1998). However, at any given exposure level, there are individuals who have none, one or both of these mutant biomarkers. This suggests the possibility of genetic determinants contributing to the susceptibility of individuals to VC mutagenesis and thus explaining different biomarker outcomes with similar exposures.

Some of this genetic variability is attributable to polymorphisms in enzymes involved in VC metabolism. For example, in a cohort of French VC workers, we have recently shown that individuals with the variant *c2 CYP2E1* allele (rs3813867) have a significantly increased risk for the occurrence of the VC-related mutant biomarkers compared with individuals who are homozygous for the normal *c1* allele even after controlling for various confounders including cumulative VC exposure (Schindler et al. 2007). However, the *c2* allele occurs infrequently among these workers so it cannot explain a large amount of the genetic attributable risk in this population. However, genetic polymorphisms in proteins involved in DNA repair pathways that are responsible for the removal of VC-induced damage tend to be more prevalent in this type of population and thus may account for a much greater proportion of the genetic variability. In fact, we have recently shown in a subset of the cohort of French VC workers that the Arg399Gln polymorphism (rs25487) in the base excision repair x-ray cross-complementing-1 (XRCC1) protein was not only quite common (only 41% of the population was homozygous for the normal Arg allele) but also had a significant effect on the occurrence of the mutant p53 biomarker even after controlling for appropriate confounders including cumulative VC exposure (Li et al. 2003). Interestingly, this *XRCC1* polymorphism had no effect on the occurrence of the mutant *ras*-p21 biomarker. Thus, the purpose of the present study was two-fold: to confirm the effect of the *XRCC1* 399 polymorphism on the occurrence of the p53 biomarker in the full cohort; and to examine the effect of other polymorphisms in *XRCC1* (rs1799782 and rs25489) as well as common polymorphisms in other DNA repair pathways, such as the codon 312 and 751 polymorphisms (rs1799793 and rs13181, respectively) in the excision repair cross-complementing group 2/xeroderma pigmentosum D (ERCC2/XPD) protein of the transcription repair and nucleotide excision repair pathways, on the biomarkers in the full cohort, in particular to see if they could account for variability in the occurrence of the *ras*-p21 biomarker.

Materials and methods

Study subjects

Subjects for study were derived from a previously described population of more than 650 workers employed in VC polymerization plants in France since 1950 and followed by INSERM in Lyon (Schindler et al. 2007). A subset of 597 of these workers (including 546 workers with probable exposure to VC and 51 unexposed controls) had blood samples available for analysis. The cohort of 546 of these workers with both VC exposure and with available serum samples and lymphocytes for DNA extraction were appropriate for this study, as we were interested in examining the potential for a gene–environment interaction. All the workers in the study were white males with the following characteristics: average age 60 years (range 33–89); average cumulative VC exposure 3184 ppm-years (range 2–46 702), where 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 previously described (Schindler et al. 2007): 50% current or former smokers; and 26% current drinkers. Additional available information on these workers includes other chronic medical conditions and related evaluations and/or treatments, but generally this is a relatively healthy worker cohort without significantly different exposures or medical conditions compared with the general population other than those from their VC work. This research was approved by the Columbia University Institutional Review Board.

Laboratory analyses

From blood samples collected by venipuncture from each worker, serum and lymphocytes were separated by standard procedures, and lymphocyte DNA extracted by routine techniques. Serum samples from all 597 workers (546 VC-exposed and 51 unexposed controls) had already been assayed for the mutant *ras*-p21 and mutant p53 biomarkers as previously described (Smith et al. 1998, Li et al. 1998).

Lymphocyte DNA isolated from the blood samples of the 546 VC-exposed workers had already been genotyped for the *CYP2E1 c2* polymorphism (rs3813867), as previously described (Schindler et al. 2007), and were now genotyped for *XRCC1* polymorphisms at codons 194, 280 and 399 (rs1799782, rs25489 and rs25487, respectively) and for *ERCC2/XPD* polymorphisms at codons 312 and 751 (rs1799793 and rs13181, respectively). For the *XRCC1* 399 and *ERCC2/XPD* 312 and 751 polymorphisms, genotyping analysis was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP), as previously described (Li et al. 2003, Lunn et al. 1999). In all cases, the PCR reaction mixture consisted of 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 U Taq polymerase and 25 ng of the primer pairs. The *XRCC1* 399 primers were: 5′-TTGTGCTTTCTCTGTGTCCA-3′ and 5′-TCCTCCAGCCTTTTCTGATA-3′. The *ERCC2/XPD* 312 primers were: 5′-CTGTTGGTGGGTGCCCGTATCTGTTGGTCT-3′ and 5′-TAATATCGGGGCTCACCCTGCAGCACTTCCT-3′. The *ERCC2/XPD* 751 primers were: 5′-TCAAACATCCTGTCCCTACT-3′ and 5′-CTGCGATTAAAGGCTGTGGA-3′. Reaction mixtures underwent a 5 min denaturation step at 94°C followed by 32 cycles of 30 s at 94°C, 30 s at 60–63°C (60°C for *ERCC2/XPD* 312, 61°C for *XRCC1* 399 and 63°C for *ERCC2/XPD* 751), and 60 s at 72°C, and a final step at 72°C for 5–7 min (5 min for *ERCC2/XPD* 312 and 7 min for the others). For the *XRCC1* 399 polymorphism, PCR products were digested with 2 U MspI at 37°C for 2 h and resolved on 1.6% agarose gels. Homozygous Arg-Arg individuals demonstrated 240 and 375 bp fragments, heterozygous Arg-Gln individuals demonstrated 240, 375 and 640 bp fragments, and homozygous Gln-Gln individuals demonstrated a single 615 bp fragment. For the *ERCC2/XPD* 312 polymorphism, PCR products were digested with 4 U StyI at 37°C for 3 h and resolved on 3% agarose gels. Homozygous Asp-Asp individuals demonstrated 244 and 507 bp fragments, heterozygous Asp-Asn individuals demonstrated 33, 244, 474 and 507 bp fragments, and homozygous Asn-Asn individuals demonstrated 33, 244 and 474 bp fragments. For the *ERCC2/XPD* 751 polymorphism, PCR products were digested with 4 U PstI at 37°C for 3 h and resolved on 3% agarose gels. Homozygous Lys-Lys individuals demonstrated 110 and 234 bp fragments, heterozygous Lys-Gln individuals demonstrated 63, 110, 171 and 234 bp fragments, and homozygous Gln-Gln individuals demonstrated 63, 110 and 171 bp fragments.

For the *XRCC1* 194 and 280 polymorphisms, genotype analysis was modified to incorporate high-throughput methodology based on fluorescence polarization analysis (Shen et al. 2005). In this case, the PCR mixture as above contained 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 U Taq polymerase and 2 pM of the primer pairs (for *XRCC1* 194, 5′-ATGAGAGCGCCAACTCTCTG-3′ and 5′-CTACCCTCCTCCCTCAGACC-3′; for *XRCC1* 280, 5′-CCCCAGTGGTGCTAACCTAAT-3′ and 5′-GGTCCAGTCTGGCCGATACCT-3′). The reaction mixtures were denatured at 94°C for 5 min followed by 34 cycles of 30 s at 94°C, 30 s at 60–61°C (60°C for *XRCC1* 194 and 61°C for *XRCC1* 280), and 50–60 s (50 s for *XRCC1* 280 and 60 s for *XRCC1* 194) at 72°C, and a final step at 72°C for 5 min. After amplification, the primers were digested with 1 U shrimp alkaline phosphatase and 1 U *Escherichia coli* exonuclease I at 37°C for 45 min and 95°C for 15 min. The resulting cleaned PCR products were added to AcycloProme-FP mixture (Perkin-Elmer, Boston, MA, USA) with 5 pM *XRCC1* probes (for *XRCC1* 194, 5′-CGGGGGCTCTCTTCTTCAGC-3′; for *XRCC1* 280, 5′-ACTGGGGCTGTGGCTGGGGTA-3′), according to the instructions of the manufacturer (Perkin-Elmer). This mixture was denatured at 95°C for 2 min followed by 50–70 cycles of 95°C for 15 s and 55°C for 30 s. Fluorescence polarization was measured on a VICTOR 2 fluorescence polarization microplate reader (Perkin-Elmer), which provided clear separate clusterings of samples by homozygous wild-type (Arg-Arg for both codons), heterozygous (Arg-Trp for codon 194 and Arg-His for codon 280) and homozygous variant (Trp-Trp for 194 and His-His for 280) genotypes.

Data analysis

In the analysis of the data, for each polymorphism the cohort was stratified by genotype as homozygous normal, heterozygous or homozygous variant for comparison of the prevalence of the VC-related mutant biomarkers. Assigning an odds ratio (OR) of 1 to the homozygous wild-type stratum, unadjusted and adjusted (for age, smoking, drinking and cumulative VC exposure) ORs and 95% confidence intervals (CIs) for the biomarkers were calculated for the other strata by multiple logistic regression analysis.

Potential gene–environment and gene–gene interaction effects were examined by further substratification for each polymorphism either by VC exposure or by the presence of another polymorphism, as previously described (Li et al. 2003). For example, for gene–environment effects, one method for estimating the strength of the interaction is calculating the ratio of the unadjusted OR

from the homozygous variant, high-exposure group to the product of the ORs from the homozygous normal, high-exposure group and the homozygous variant, low-exposure group; a ratio greater than 1 would suggest a supramultiplicative interaction. Similarly, for gene–gene effects, the strength of the interaction is calculated from the ratio of the unadjusted OR for the variant allele, variant allele group with the product of the ORs from the two variant allele, normal allele groups; once again, a ratio greater than 1 suggests a supramultiplicative interaction.

Results

Among the 546 VC-exposed workers in this study, 266 (49%) were negative for both biomarkers, 217 (40%) were positive for at least one biomarker and 63 (11%) were positive for both biomarkers, whereas among the 51 unexposed control workers, 47 (92%) were negative for both biomarkers, four (8%) were positive for one biomarker and none (0%) were positive for both biomarkers. When the 546 VC-exposed workers in this study were stratified into tertiles of approximately equal size (2–1705 ppm-years, $n = 179$; 1706–5703 ppm-years, $n = 186$; and >5703 ppm-years, $n = 181$) and compared with the 51 unexposed controls, the adjusted ORs for the occurrence of either or both biomarkers increased with a highly statistically significant trend with increasing exposure ($p < 0.0001$), as described previously (Schindler et al. 2007).

However, within each tertile of exposure there were workers who were comparable in terms of age, smoking, drinking, medical history and cumulative VC exposure, but who were positive for none, one or both mutant biomarkers. To determine whether or not this could be attributable to genetic variations in DNA repair, the workers were genotyped for the *XRCC1* and *ERCC2/XPD* polymorphisms, as noted. All the polymorphisms tested were in Hardy–Weinberg equilibrium. There were no statistically significant differences in the distribution of VC exposure levels among the different genotypes for any of the polymorphisms.

The results confirmed that the *XRCC1* 399 polymorphism is quite common in this cohort with 37% (202/546) being homozygous normal, 48% (264/546) being heterozygous, and 15% (80/546) being homozygous variant. Furthermore, the results confirmed the prior preliminary finding that the *XRCC1* 399 polymorphism significantly affects the occurrence of the mutant p53 biomarker. As shown in Table 1, assigning an OR of 1 to the homozygous Arg-Arg individuals, the adjusted ORs for the prevalence of the p53 biomarker increases to 1.3 (95% CI 0.8–1.9) in the heterozygous Arg-Gln individuals and to 1.9 (95% CI 1.1–3.3) in the homozygous Gln-Gln individuals after controlling for age, smoking, drinking and cumulative VC exposure. Thus, a statistically significant trend was observed for increasing Gln allele dosage ($p = 0.03$). Furthermore, the joint effects of VC exposure and *XRCC1* genotype from our previous results had suggested a potentially supramultiplicative gene–environment interaction effect on the occurrence of the p53 biomarker. Table 2 shows the analysis for this joint effect for the full cohort. In this case, comparison of the product of the unadjusted ORs from the homozygous Arg-Arg, high-exposure group and the homozygous Gln-Gln, low-exposure group (1.2×1.2) with the OR from the homozygous Gln-Gln, high-exposure group (1.8), yields an interaction term of 1.3, once again indicating a potentially supramultiplicative interaction.

As with our prior results, the *XRCC1* 399 polymorphism was found to have no significant effect on the occurrence of the mutant *ras*-p21 biomarker (data not shown). Furthermore, the *XRCC1* 194 and 280 polymorphisms were found to be much less common in this cohort (88% homozygous normal for 194 and 91% homozygous normal for 280), and these polymorphisms had no significant effects on the occurrence of either of the biomarkers (data not shown).

However, both of the *ERCC2/XPD* polymorphisms proved to be not only common in this cohort but also to have effects on the occurrence of both biomarkers which were particularly significant for the mutant *ras*-p21 biomarker. For the *ERCC2/XPD* 312 polymorphism, 41% (223/546) were homozygous normal, 48% (260/546)

Table 1. Association between *XRCC1* 399 polymorphism and p53 biomarker in vinylchloride (VC) workers.

XRCC1	p53 Biomarker		Crude OR		Adjusted OR[a]	
genotype	-	+	OR	95% CI	OR	95% CI
Arg-Arg	146	56	1.0		1.0	
Arg-Gln	180	84	1.2	0.8–1.8	1.3	0.8–1.9
Gln-Gln	48	32	1.7	1.0–3.0	1.9	1.1–3.3

[a]Adjusted for age, smoking, drinking and cumulative VC exposure; p for trend = 0.03.

Table 2. Effect of interaction of *XRCC1* 399 polymorphism with vinylchloride (VC) exposure on p53 biomarker.

XRCC1	VC exposure	p53 Biomarker		Crude OR		Adjusted OR[a]	
genotype	(ppm-years)	–	+	OR	95% CI	OR	95% CI
Arg-Arg	<1706	48	18	1.0		1.0	
	1706–5704	48	15	0.8	0.4–1.7	1.1	0.5–2.4
	>5704	50	23	1.2	0.6–2.6	1.8	0.8–3.8
Arg-Gln	<1706	67	21	0.8	0.4–1.7	0.8	0.4–1.7
	1706–5704	66	32	1.3	0.7–2.6	1.6	0.8–3.2
	>5704	47	31	1.8	0.9–3.6	2.8	1.3–6.2
Gln-Gln	<1706	17	8	1.2	0.5–3.4	1.3	0.5–3.5
	1706–5704	13	12	2.5	1.0–6.3	3.3	1.2–8.5
	>5704	18	12	1.8	0.7–4.4	3.1	1.2–82

[a]Adjusted for age, smoking and drinking; p for trend = 0.002.

were heterozygous, and 11% (63/546) were homozygous variant. For the *ERCC2/XPD* 751 polymorphism in this cohort, 41% (222/546) were homozygous normal, 47% (256/546) were heterozygous, and 12% (68/546) were homozygous variant. Table 3 shows the results for the effects of these polymorphisms on the mutant p53 biomarker. Although for both alleles trends are apparent for an effect of increasing allele dosage on increasing prevalence of the biomarker, the results are not statistically significant. However as shown in Table 4, the effects of both polymorphisms on the mutant *ras*-p21 biomarker are quite significant. For example, for the *ERCC2/XPD* 312 polymorphism where an OR of 1 is assigned to homozygous Asp-Asp individuals, the adjusted OR for the prevalence of the *ras*-p21 biomarkers increases to 1.8 (95% CI 1.2–2.7) in the heterozygous Asp-Asn individuals and to 3.0 (95% CI 1.6–5.4) in the homozygous Asn-Asn individuals after controlling for age, smoking, drinking and cumulative VC exposure with a highly statistically significant trend for increasing allele dosage ($p < 0.0001$). Similarly for the *ERCC2/XPD* 751 polymorphism where an OR of 1 is assigned to the homozygous Lys-Lys individuals, the adjusted OR for the prevalence of the *ras*-p21 biomarker increases to 1.6 (95% CI 1.1–2.5) in the heterozygous Lys-Gln individuals and to 2.6 (95% CI 1.5–4.6) in the homozygous Gln-Gln individuals with a highly statistically significant trend for increasing allele dosage ($p = 0.0006$).

Table 5 shows the joint effect of the *ERCC2/XPD* genotypes and cumulative VC exposure on the occurrence of the *ras*-p21 biomarker, demonstrating a potential gene–environment interaction for individuals with the 751 Gln allele particularly at high-exposure levels. Once again, we can estimate the strength of this interaction by comparing the product of the unadjusted ORs for the homozygous Lys-Lys, high-exposure group and the homozygous Gln-Gln, low-exposure group (3.2×1.4) with the OR from the homozygous Gln-Gln, high-exposure group (9.6), yielding a supramultiplicative interaction term of 2.1. In this case, no such interaction is apparent for the *ERCC2/XPD* 312 polymorphism.

Finally, in this cohort there is the potential for important gene–gene interactions between the previously determined *CYP2E1* polymorphism in the VC metabolism step (Schindler et al. 2007) and the *XRCC1* or *ERCC2/XPD* polymorphisms in the DNA repair step. In this case, the most striking interactions were between the *CYP2E1* and *ERCC2/XPD* polymorphisms on the occurrence of the *ras*-p21 biomarker, as shown in Table 6. For these analyses, the *CYP2E1* heterozygotes and homozygous variants were collapsed into one *c2* variant allele group due to the small numbers involved. The product of the unadjusted ORs for the *ERCC2/XPD* 312 homozygous variant, *CYP2E1* homozygous normal group and the *ERCC2/XPD* 312 homozygous normal, *CYP2E1 c2* variant group ($2.7 \times 2.6 = 7.0$) yielded an approximately multiplicative interaction when compared with the *ERCC2/XPD* 312 homozygous variant, *CYP2E1 c2* variant group (7.2). The product of the unadjusted ORs for the *ERCC2/XPD* 751 homozygous variant, *CYP2E1* homozygous

Table 3. Association between *ERCC2/XPD* 312 and 751 polymorphisms and p53 biomarker in vinylchloride (VC) workers.

ERCC2/XPD	p53 Biomarker		Crude OR		Adjusted OR[a]	
genotype	–	+	OR	95% CI	OR	95% CI
312 *Asp-Asp*	159	64	1.0		1.0	
Asp-Asn	179	81	1.1	0.8–1.7	1.2	0.8–1.7
Asn-Asn	36	27	1.9	1.1–3.3	1.9	1.0–3.4
751 *Lys-Lys*	155	67	1.0		1.0	
Lys-Gln	179	77	1.0	0.7–1.5	1.0	0.7–1.6
Gln-Gln	40	28	1.6	0.9–2.8	1.6	0.9–2.9

[a]Adjusted for age, smoking, drinking and cumulative VC exposure; *p* for trend >0.05.

Table 4. Association between *ERCC2/XPD* 312 and 751 polymorphisms and p21 biomarker in vinylchloride (VC) workers.

ERCC2/XPD	p21 Biomarker		Crude OR		Adjusted OR[a]	
genotype	–	+	OR	95% CI	OR	95% CI
312 *Asp-Asp*	172	51	1.0		1.0	
Asp-Asn	169	91	1.8	1.2–2.7	1.8	1.2–2.7
Asn-Asn	34	29	2.9	1.6–5.1	3.0	1.6–5.4
751 *Lys-Lys*	169	53	1.0		1.0	
Lys-Gln	168	88	1.7	1.1–2.5	1.6	1.1–2.5
Gln-Gln	38	30	2.5	1.4–4.4	2.6	1.5–4.6

[a]Adjusted for age, smoking, drinking and cumulative VC exposure; *p* for trend <0.0006.

Table 5. Effect of interaction of *ERCC2/XPD* 312 and 751 polymorphisms with vinylchloride (VC) exposure on p21 biomarker in VC workers.

ERCC2/XPD	VC exposure	p21 Biomarker		Crude OR		Adjusted OR[a]	
genotype	(ppm-years)	–	+	OR	95% CI	OR	95% CI
312 *Asp-Asp*	<1706	59	15	1.0		1.0	
	1706–5704	69	8	0.5	0.2–1.1	0.5	0.2–1.2
	>5704	44	28	2.5	1.2–5.2	2.6	1.2–5.7
Asp-Asn	<1706	63	21	1.3	0.6–2.8	1.3	0.6–2.7
	1706–5704	52	35	2.7	1.3–5.3	2.7	1.3–5.7
	>5704	54	35	2.6	1.3–5.1	2.7	1.3–5.8
Asn-Asn	<1706	13	8	2.4	0.9–6.8	2.4	0.8–6.9
	1706–5704	11	11	3.9	1.5–10.5	4.1	1.5–11.3
	>5704	10	10	3.9	1.4–10.8	4.1	1.4–11.8
751 *Lys-Lys*	<1706	67	14	1.0		1.0	
	1706–5704	60	11	0.9	0.4–2.1	0.9	0.4–2.1
	>5704	42	28	3.2	1.5–6.7	3.3	1.5–7.1
Lys-Gln	<1706	51	25	2.4	1.1–4.9	2.3	1.1–4.9
	1706–5704	58	32	2.6	1.3–5.4	2.7	1.3–5.6
	>5704	59	31	2.5	1.2–5.1	2.5	1.2–5.5
Gln-Gln	<1706	17	5	1.4	0.4–4.5	1.4	0.4–4.4
	1706–5704	14	11	3.8	1.5–9.7	3.8	1.4–10.4
	>5704	7	14	9.6	3.6–25.7	9.7	3.2–29.0

[a]Adjusted for age, smoking and drinking; *p* for trend <0.0001.

Table 6. Effect of interaction of *CYP2E1* polymorphism and *ERCC2/XPD* 312 and 751 polymorphisms on p21 biomarker in vinylchloride (VC) workers.

CYP2E1 genotype	*ERCC2/XPD* genotype	p21 Biomarker –	+	Crude OR OR	-	Adjusted OR[a] +	OR
c1ci	312 *Asp-Asp*	165	46	1.0		1.0	
	Asp-Asn	161	79	1.8	1.2–2.7	1.8	1.2–2.7
	Asn-Asn	31	23	2.7	1.4–4.9	2.7	1.4–5.2
c1c2/c2c2	312 *Asp-Asp*	7	5	2.6	0.8–8.2	2.5	2.0–13.3
	Asp-Asn	8	12	5.4	2.2–12.9	5.1	2.0–13.3
	Asn-Asn	3	6	7.2	2.1–25.0	7.5	1.8–31.6
c1c1	751 *Lys-Lys*	160	50	1.0		1.0	
	Lys-Gln	162	77	1.5	1.0–2.3	1.5	1.0–2.3
	Gln-Gln	35	21	1.9	1.0–3.6	1.9	1.0–3.7
c1c2/c2c2	751 *Lys-Lys*	9	3	1.1	0.3–4.1	1.0	0.3–3.8
	Lys-Gln	6	11	5.9	2.3–15.2	5.6	1.9–16.2
	Gln-Gln	9	12	9.6	3.1–30.0	10.4	2.7–40.5

[a]Adjusted for age, smoking, drinking and cumulative VC exposure; p for trend <0.0001.

normal group and the *ERCC2/XPD* 751 homozygous normal, *CYP2E1 c2* variant group (1.9 × 1.1 = 2.1) yielded a supramultiplicative interaction term of 4.6 when compared with the *ERCC2/XPD* 751 homozygous variant, *CYP2E1 c2* variant group (9.6).

Discussion

This study provides an interesting model linking exposure to a known mutagenic/carcinogenic agent (VC) to biomarkers of effect (mutant p53 and mutant *ras*-p21) and to biomarkers of susceptibility (polymorphic *XRCC1* and *ERCC2/XPD*). The results observed are entirely consistent with the proposed carcinogenic mechanism for VC in that VC is known to generate promutagenic etheno-DNA adducts that can produce the types of mutations in *TP53* and K-*ras* seen in VC-exposed individuals, and XRCC1 and ERCC2/XPD could participate in the process of DNA repair of such etheno-DNA adducts (Brandt-Rauf et al. 2000).

For example, it is known that XRCC1 participates in the base excision repair pathway which is particularly efficient in repairing the εA adducts that are responsible for the generation of the *TP53* mutations (Dosanjh et al. 1994). XRCC1 has no enzymatic activity of its own but is thought to function as a platform protein that interacts with and coordinates the activity of the other proteins in the base excision repair machinery (Campalans et al. 2005, Vidal et al. 2001). The 399 polymorphism in *XRCC1* has been associated with diminished DNA repair capability and an increased risk of cancer (Goode et al. 2002, Hung et al. 2005, Bolufer et al. 2006, Kiyohara et al. 2006). In fact, in our own prior work, we studied the effect in cell culture of chloroacetaldehyde exposure on the formation of εA adducts in lymphoblastoid cell lines from individuals who were homozygous normal or homozygous variant for the *XRCC1* 399 polymorphism; the efficiency of repair of adducts in the homozygous normal cells was approximately four times greater than the efficiency of repair in the homozygous variant cells, analogous to the prior and current results of increased risk for the occurrence of the mutant p53 biomarker from the epidemiological study of exposed workers (Li et al. 2006). Amino acid residue 399 of XRCC1 occurs in the so-called central BRCT1 domain of the protein which is responsible for interacting with other base excision repair proteins such as PARP-1 (Caldecott 2003). Preliminary evidence from our laboratory on the molecular modelling of the normal and polymorphic XRCC1 proteins suggests that the 399 substitution produces significant conformational changes in the BRCT1 domain, including loss of secondary structural features such as α-helices that can be critical for protein–protein interactions (Monaco et al. 2007). These changes could disrupt XRCC1 interactions with other BER proteins and the coordination of the base excision repair machinery leading to the diminished repair capacity noted in cell culture.

The lack of effect of the 399 polymorphism on the mutant *ras*-p21 biomarker is also consistent with available data. Some studies have suggested that, in comparison to the εA adduct, the εG adduct that is responsible for the generation of the *ras*-p21 mutations is relatively poorly removed by base excision repair (Dosanjh et al. 1994). Thus, this adduct may be repaired by some other mechanism. Other major mechanisms for DNA repair include transcription-coupled repair and nucleotide excision repair, both of which are dependent upon ERCC2/XPD. ERCC2/XPD is a DNA helicase that functions as a subunit of the transcription factor IIH complex (TFIIH) to promote DNA bubble formation at the damaged site by unwinding the DNA (Chen & Suter 2003). Amino acid residue 751 might be critical for ERCC2/XPD function as it occurs in the C-terminal domain which may interact with the p44 helicase activator protein of the TFIIH complex (Bienstock et al. 2003); an *ERCC2/XPD* mutation that results in the loss of the final 17 C-terminal amino acids, including residue 751, is known to result in the clinical phenotype of trichothiodystrophy (Botta et al. 1998). Although the results have not been entirely consistent, in some other studies *ERCC2/XPD* polymorphisms at both amino acid residues 312 and 751 have been associated with diminished DNA repair capability and an increased risk of cancer (Benhamou & Sarasin 2002). Although it is clear that other etheno-DNA adducts, such as εA and εC, are primarily removed by base excision repair, it has been suggested that etheno-DNA adducts can also be removed by different DNA repair mechanisms such as nucleotide excision repair and transcription-coupled

repair (Przybyszewski et al. 2005), which as noted are dependent on ERCC2/XPD. In fact, in other recent studies of VC-exposed workers in China, the *ERCC2/XPD* 751 Lys/Gln and Gln/Gln genotypes have been shown to be statistically significantly associated with increases in non-specific markers of DNA damage as measured by the single cell gel electrophoresis (SCGE) assay (Zhu et al. 2005, 2008). Thus, it is possible that the presence of *ERCC2/XPD* polymorphisms may also be associated with an increased risk of a specific *ras*-p21 mutation induced by VC exposure, as we have seen. We are pursuing further biochemical studies to elucidate the biological plausibility of this association, as we have done with XRCC1. In fact, preliminary results from our laboratory of molecular modelling of the normal and polymorphic forms of the C-terminus of the ERCC2/XPD protein suggest that the 751 substitution produces significant conformational changes that could result in altered activity and diminished DNA repair capacity.

In summary, the results of this study support the existence of potential gene–environment interactions between *XRCC1* and *ERCC2/XPD* polymorphisms and VC exposure and potential gene–gene interactions between *CYP2E1* and *ERCC2/XPD* polymorphisms such that the combined effects in both instances are greater than the product of the individual effects alone. In addition to the caveats noted above, it should be emphasized that in some cases these interactions are based on substratifications with small numbers of individuals, and so they should be interpreted cautiously until confirmed in further studies and supported by a biochemical rationale. Furthermore, in this study, we have examined the effects of five different polymorphisms on VC biomarkers so that there is the possibility that statistically significant results could occur by chance due to the multiple comparisons. However, even applying the stringent Bonferroni correction for multiple comparisons to the p-value for statistical significance ($p = 0.05/5 = 0.01$), all of the results remain significant except for the allele dosage effect for the *XRCC1* 399 polymorphism on the mutant p53 biomarker (Table 1), but, in that case, the correction is arguably not relevant anyway as this particular evaluation was hypothesis-driven based on follow-up of the previous results. More importantly, the more refined substratification analysis for the *XRCC1* 399 polymorphism with VC exposure (Table 2) would remain significant even with the correction. Thus, consideration of multiple comparisons does not have a major impact on the conclusions of this study**.** Finally, it should also be realized that the results in this model system could have much broader implications, as etheno-DNA adducts are known to be generated by other carcinogenic exposures, XRCC1 and ERCC2/XPD participate in the repair of a range of other types of DNA damage, and these polymorphisms are quite common in many populations, so their overall contributions to susceptibility differences could be substantial.

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