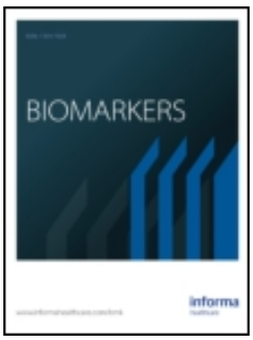

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A common polymorphism in *XRCC1* as a biomarker of susceptibility for chemically induced genetic damage

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We have recently demonstrated a significant dose–response relationship between vinyl chloride exposure and mutant p53 biomarkers in humans. The aim of this study was to examine a common polymorphism in the DNA repair gene *XRCC1* as a potential biomarker of susceptibility modifying this relationship, consistent with the known mechanism of production of p53 mutations via vinyl chloride-induced etheno-DNA adducts, which are repaired by XRCC1. A cohort of 211 French vinyl chloride workers were genotyped for the *XRCC1* codon 399 polymorphism (CGG > CAG; Arg > Gln). Among the homozygous *Arg–Arg* individuals, 34% were biomarker positive compared with 47% in the heterozygous *Arg–Gln* individuals (adjusted odds ratio 1.73, 95% CI0.93–3.22) and 66% in the homozygous *Gln–Gln* individuals (adjusted odds ratio 3.95, 95% CI 1.68–9.28), with a significant trend for increasing *Gln* allele dosage ($p = 0.002$). These preliminary results suggest that a common polymorphism in a DNA repair gene can be an important biomarker of susceptibility for chemically induced genetic damage.

Keywords: vinyl chloride, mutations, p53, DNA repair.

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 variations in enzymes that generate or eliminate reactive genotoxic intermediates and to a 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 mutagen vinyl chloride (VC).

VC is a known animal and human carcinogen associated with the sentinel neoplasm of angiosarcoma of the liver (ASL) (ATSDR 1997). Following exposure, VC is metabolized principally in the liver by 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-εA, 3,N^4-εC and N^2,3-εG (ATSDR 1997). These adducts are pro-mutagenic and could account for the mutations seen in ASLs of exposed individuals (Hollstein *et al.* 1994). For example, the εA adduct can cause the A to T transversions in *TP53* that have been identified in a high proportion of ASLs

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from individuals with VC exposure (Hollstein *et al.* 1994). These mutations have also been associated with overexpression of biomarkers of p53, including mutant p53 protein and anti-p53 antibodies, in VC-exposed individuals with ASL (Trivers *et al.* 1995, Brandt-Rauf *et al.* 1996). In addition, these biomarkers 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 biomarker is the direct result of VC exposure (Smith *et al.* 1998, Luo *et al.* 1999). However, at any given exposure level, some individuals are positive and some negative for these biomarkers, suggesting that there might be some genetically determined susceptibility to VC mutagenesis that could account for different biomarker outcomes with similar exposures. One source for this variability could derive from altered DNA repair capability for the removal of etheno-DNA adducts.

The εA adducts that are produced by VC should be removed by the base excision repair (BER) pathway, starting with methyl purine glycosylase, which recognizes and excises these particular adducts (Lindahl and Wood 1999). The BER machinery contains several other proteins, including an endonuclease, a DNA polymerase, a ligase and poly(ADP-ribose) polymerases (Lindahl and Wood 1999). The activity of this machinery is coordinated by the X-ray cross complementing-1 protein (XRCC1), which acts as a scaffold for the other proteins and is necessary for regulating the steps of BER through interaction with them (Lindahl and Wood 1999). Several polymorphisms of *XRCC1* have been identified in humans (Goode *et al.* 2002). The most common polymorphism results in the substitution of a glutamine for the normally occurring arginine at amino acid residue 399 (Goode *et al.* 2002). Residue 399 occurs in the BRCT1 domain of XRCC1, which is known to interact with poly(ADP-ribose) polymerases, and substitutions in this domain could thus lead to altered DNA repair capability (Vidal *et al.* 2001). Individuals with the 399 polymorphism have been found to have a higher risk for the accumulation of other, non-etheno types of DNA adducts (Lunn *et al.* 1999) and for the occurrence of various types of cancers, although results have been inconsistent (Goode *et al.* 2002). The purpose of the current study was to determine whether *XRCC1* polymorphism could be responsible for variable DNA repair of mutagenic damage in a model population of VC-exposed workers characterized for the presence of biomarkers of *TP53* mutations.

Materials and methods

A group of 211 subjects were selected from a cohort of VC-exposed workers in France who had been characterized for cumulative VC exposure and mutant p53 biomarkers, as described previously (Smith *et al.* 1998). These workers were selected on the basis of having lymphocytes available for DNA extraction for this study, but were otherwise similar to the rest of the workers in the cohort in terms of demographic characteristics and VC exposure. The workers were all French white males. The average age was 56 years (range 35–74 years), and the average cumulative VC exposure was 5871 p.p.m.-years (range 6–46 702 p.p.m.-years); 39% were current or former smokers, 20% were current drinkers, and 44.5% were positive for a mutant p53 biomarker. Positivity for a mutant p53 biomarker was determined by analysis of serum samples for mutant p53 proteins and/or anti-p53 antibodies using an enzyme-linked immunosorbent assay, as previously described (Smith *et al.* 1998).

These studies were approved by our Institutional Review Board for conformance with human subject protections. DNA was isolated from lymphocytes from blood samples, amplified by polymerase chain reaction (PCR) and analysed for the *XRCC1-MspI* polymorphism, as described previously (Lunn *et al.*

1999). Briefly, the primers used were 5′-TTGTGCTTTCTCTGTGTCCA-3′ and 5′-TCCTCCAGCCTTTTCTGCTA-3′, and the PCR conditions consisted of 20 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.25 units Taq polymerase, and 25 ng of the primer pair. Reaction mixtures underwent a 5 min denaturation step at 94°C followed by 32 cycles of 30 s at 94°C, 90 s at 61°C, and 60 s at 72°C. The PCR products were digested with 2 units of *Msp*I at 37°C for 2 h and resolved on 1.6% agarose gels, with controls for the *Arg–Arg*, *Arg–Gln* and *Gln–Gln* codon 399 variations included on each gel. Homozygous *Arg–Arg* individuals demonstrated 240 and 375 bp fragments, heterozygous *Arg–Gln* individuals demonstrated 240, 375 and 615 bp fragments, and homozygous *Gln–Gln* individuals demonstrated a single 615 bp fragment. These workers had already been genotyped for the *CYP2E1-PstI* polymorphism (Li *et al.* 2003).

The cohort was stratified by *XRCC1* genotype as homozygous *Arg–Arg*, heterozygous *Arg–Gln*, or homozygous *Gln–Gln* at codon 399 for comparison of the prevalence of the p53 biomarker. Assigning an odds ratio (OR) of 1 to the homozygous *Arg–Arg* stratum, unadjusted and adjusted (for age, smoking, drinking, *CYP2E1* genotype and cumulative VC exposure) OR and 95% confidence intervals (CIs) for the p53 biomarker were calculated for the other strata.

Results and discussion

Of the 211 workers studied, 86 (40.8%) were homozygous *Arg–Arg*, 90 (42.7%) were heterozygous *Arg–Gln*, and 35 (16.6%) were homozygous *Gln–Gln* for *XRCC1* codon 399. The distribution of genotypes was consistent with a Hardy–Weinberg equilibrium ($\chi^2 = 1.86$). The *Gln* allele frequency was 0.38, similar to that reported in other Caucasian populations (Lunn *et al.* 1999). There was no statistically significant difference in terms of the distribution of VC exposure levels among the different genotypes ($\chi^2 = 3.18$, $p = 0.53$). Assigning an OR of 1 to the lowest exposure group (≤ 1000 p.p.m.-years) and adjusting for genotype, age, smoking and drinking, there was a statistically significant trend for increasing prevalence of the p53 biomarker in the medium (1001–4000 p.p.m.-years; OR 1.72, 95% CI 0.77–3.82) and high (>4000 p.p.m.-years; OR 2.59, 95% CI 1.10–6.11) exposure groups ($p = 0.033$), as has been previously observed (Smith *et al.* 1998).

Table 1 shows the difference in the prevalence of the p53 biomarker by genotype. With an OR of 1 for homozygous *Arg–Arg* individuals, the adjusted ORs for the prevalence of the p53 biomarker were 1.73 (95% CI 0.93–3.22) for the heterozygous *Arg–Gln* individuals and 3.95 (95% CI 1.68–9.28) for the homozygous *Gln–Gln* individuals. A highly statistically significant trend was observed for increasing *Gln* allele dosage ($p = 0.002$).

Table 1. Association between *XRCC1* polymorphism and p53 biomarker in VC workers.

		p53 biomarker							
		−		+		Unadjusted OR		Adjusted OR[a]	
XRCC1 polymorphism	*n*	No.	%	No.	%	OR	95% CI	OR	95% CI
Arg–Arg	86	57	66	29	34	1.00	—	1.00	—
Arg–Gln	90	48	53	42	47	1.72	0.92–3.16	1.73	0.93–3.22
Gln–Gln	35	12	34	23	66	3.77	1.68–8.47	3.95	1.68–9.28

[a] Adjusted for age, smoking, drinking, *CYP2E1* genotype and cumulative VC exposure.
p for trend = 0.002.

Table 2 shows the joint effect of *XRCC1* genotype and cumulative VC exposure on the occurrence of the p53 biomarker, demonstrating a potential gene–environment interaction, particularly in individuals with the *Gln* allele at high exposure levels. A common method for estimating the strength of this interaction is comparison of the product of the unadjusted ORs from the homozygous *Arg–Arg*, high-exposure group and the homozygous *Gln–Gln*, low-exposure group (1.65×2.5) with the OR from the homozygous *Gln–Gln*, high-exposure group (12), yielding an interaction term of 2.9. Alternately, risk ratios can be used to assess the interaction, since it has been suggested that ORs overestimate the interaction effect, particularly when the incidence of the outcome of interest is high (Morabia *et al.* 1997). In this case, comparisons of corresponding risk ratios (1.42×1.82 compared with 3.2) yields an interaction term that is reduced but is still greater than 1 (1.24), although the interaction terms are not statistically significant with either method.

As noted, this cohort had also been genotyped for the presence of the *CYP2E1 c2* allele, which is a high-activity variant that could contribute to the generation of greater amounts of reactive intermediates at any given VC exposure level, leading to increased etheno-DNA adducts, increased *TP53* mutations and increased mutant p53 biomarkers (Li *et al.* 2003). In this population, however, the *CYP2E1 c2* allele frequency was quite low (0.038), and thus the independent effect on the prevalence of the p53 biomarker was not statistically significant (Li *et al.* 2003). However, a potential gene–gene interaction between the *XRCC1* and the *CYP2E1* genotypes on the prevalence of the p53 biomarker was observed (Table 3). In this case, the heterozygous *Arg–Gln* and homozygous *Gln–Gln* individuals for *XRCC1* were grouped together for analysis because of the small numbers of *CYP2E1* heterozygotes. A similar analysis for interaction as above yielded an interaction term greater than 1 (2.3 using ORs and 1.4 using risk ratios), although again these were not statistically significant.

This study provides an interesting model linking exposure to a known mutagenic/carcinogenic agent to a biomarker of effect (mutant p53) and a biomarker of susceptibility (*XRCC1* polymorphism). The results observed are entirely consistent with the proposed carcinogenic mechanism for VC, in that VC is known to generate pro-mutagenic etheno-DNA adducts that can produce the types of mutations in *TP53* seen in VC-exposed individuals, and XRCC1 is known to participate in the process of BER of such DNA adducts. The results are consistent with other studies suggesting that the 399 polymorphism in *XRCC1* is associated with diminished DNA repair capability (Goode *et al.* 2002), and with studies on the effects of polymorphisms on biomarkers of genetic damage in VC-exposed workers in Taiwan (Wong *et al.* 1998, 2002).

The results in this study also suggest the existence of potential gene–environment and gene–gene interactions such that the combined effects in both instances are greater than the product of the individual effects alone; however, these results must be considered preliminary until they can be confirmed in a larger sample of VC-exposed individuals. Nevertheless, it should be emphasized that this model could have much broader implications, since etheno-DNA adducts are known to be generated by other carcinogenic exposures, XRCC1 participates in

Table 2. Effect of interaction of *XRCC1* polymorphism with VC exposure on p53 biomarker status.

			p53 biomarker							
			−		+		Unadjusted OR		Adjusted OR[a]	
XRCC1 genotype	VC exposure (p.p.m.-years)	*n*	No.	%	No.	%	OR	95% CI	OR	95% CI
Arg–Arg	≤1000	24	18	75	6	25	1.00	—	1.00	—
	1001–4000	31	19	61	12	39	1.89	0.58–6.14	2.19	0.65–7.40
	>4000	31	20	65	11	35	1.65	0.50–5.41	1.96	0.56–6.80
Arg–Gln	≤1000	26	16	62	10	38	1.88	0.55–6.36	1.90	0.55–6.52
	1001–4000	25	16	64	9	36	1.69	0.49–5.84	1.89	0.53–6.69
	>4000	39	16	41	23	59	4.31	1.44–12.94	5.43	1.57–18.83
Gln–Gln	≤1000	11	6	55	5	45	2.50	0.55–11.27	2.50	0.54–11.51
	1001–4000	14	4	29	10	71	7.50	1.79–31.38	8.84	1.87–41.70
	>4000	10	2	20	8	80	12.00	2.27–63.43	12.17	1.88–78.67

[a] Adjusted for age, smoking, drinking and *CYP2E1* genotype.
p for trend = 0.0004.

Table 3. Effect of interaction of *XRCC1* and *CYP2E1* polymorphisms on p53 biomarker status.

Genotype			p53 biomarker				Unadjusted OR		Adjusted OR[a]	
			−		+					
XRCC1	CYP2E1	*n*	No.	%	No.	%	OR	95% CI	OR	95% CI
Arg–Arg	*c1c1*	80	53	66	27	34	1.00	—	1.00	—
Arg–Arg	*c1c2*	6	4	67	2	33	0.98	0.17–5.76	1.08	0.18–6.42
Arg–Gln or *Gln–Gln*	*c1c1*	115	57	50	58	50	2.00	1.11–3.60	2.06	1.13–3.75
Arg–Gln or *Gln–Gln*	*c1c2*	10	3	30	7	70	4.58	1.19–17.59	4.33	1.01–18.60

[a]Adjusted for age, smoking, drinking and cumulative VC exposure.
p for trend = 0.006.

BER with many glycosylases for a range of other DNA damage, and this polymorphism is quite common in many populations, so its contribution to susceptibility differences could be substantial.

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