XRCC1 and *CYP2E1* Polymorphisms as Susceptibility Factors of Plasma Mutant p53 Protein and Anti-p53 Antibody Expression in Vinyl Chloride Monomer-exposed Polyvinyl Chloride Workers[1]

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Abstract

Mutant p53 protein and anti-p53 antibody in circulating blood can be detected among individuals with mutations of the *p53* tumor suppressor gene. Plasma mutant p53 protein and anti-p53 antibody have also been associated with vinyl chloride monomer (VCM) exposure, although the mechanism of VCM-related carcinogenesis remains unclear. Polymorphisms of metabolic and DNA repair genes have been implicated in chemical exposure-related carcinogenesis. The aim of this study is to explore the association between polymorphisms of metabolic and DNA repair genes with mutant p53 protein and anti-p53 antibody expression induced by VCM. Study subjects comprised 333 male workers occupationally exposed to VCM. Plasma mutant p53 protein and anti-p53 antibody detected with ELISA were grouped together as p53 overexpression. Genotypes of cytochrome P450 2E1 (*CYP2E1*), aldehyde dehydrogenase 2 (*ALDH2*), glutathione *S*-transferase T1 (*GSTT1*), and *X*-ray repair cross-complementing group 1 (*XRCC1*, exon 10) genes were identified by the PCR. High VCM exposure group had significantly higher p53 overexpression as compared with low exposure group [odds ratio (OR), 2.1; 95% confidence interval (CI), 1.1–3.8]. Individuals having experienced a high VCM exposure and displaying a *XRCC1 Gln-Gln* genotype had a highest risk of p53 overexpression among those having different combinations of VCM exposure and *XRCC1* genotypes (OR, 6.5; 95% CI, 1.7–24.2). Interestingly, those subjects reflecting a *CYP2E1 c2c2* genotype among the low VCM-exposure group demonstrated a greater risk of p53 overexpression (OR, 9.8; 95% CI, 1.2–81.6) as compared with those experiencing a low VCM exposure and *CYP2E1 c1c1*/*c1c2* genotypes. Additional analysis revealed that individuals possessing more susceptible *XRCC1 Gln-Gln, CYP2E1 c2c2, ALDH2 1–2/2–2,* and non-null *GSTT1* genotypes were more likely to reveal p53 overexpression. Our results suggest that susceptible *XRCC1* and *CYP2E1* genotypes may modulate the mutation of the *p53* gene among VCM-exposed workers.

Introduction

A greater incidence of liver cancer among workers exposed to VCM[4] (CAS No. 75-01-4) has been demonstrated in previous epidemiological studies (1, 2). Therefore, VCM has been classified by the IARC as a group I carcinogen (3). Previously, our retrospective cohort study revealed that Taiwanese PVC workers reflect a higher standardized mortality ratio for liver cancer as compared with the general male population.[5] Furthermore, we performed a case-control study in a Taiwanese VCM-exposed cohort and also found that VCM may act synergistically with HBV in the development of liver cirrhosis and/or liver cancer.[6] Although the relationship between VCM exposure and liver cancer has been established previously, the mechanism of VCM-related carcinogenesis remains indistinct.

The tumor suppressor gene *p53* has been implicated as the most frequent target for genetic alteration among human cancers (4). These *p53* mutations result in the expression of mutant forms of the encoded p53 protein, which can be detected in circulating blood among individuals with mutant *p53* gene (5). Several previous studies having discussed the association of VCM exposure and the *p53* gene (6, 7). Hollstein *et al.* (6) examined ASL among four VCM-exposed patients, two of whom demonstrated mutations in the *p53* gene in exons 5–8. Previous study also revealed that two of four cases with ASL had overexpression of mutant p53 protein in their tumor tissue

Received 8/4/01; revised 1/11/02; accepted 3/4/02.
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[1] Supported in part by NSC-89-2320-B-002-159, NSC-89-2314-B-002-389, and NSC-90-2320-B-002-125, Taiwan.
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[4] The abbreviations used are: VCM, vinyl chloride monomer; *CYP2E1*, cytochrome P450 2E1; *ALDH2*, aldehyde dehydrogenase 2; GST, glutathione *S*-transferase; *XRCC1*, X-ray repair cross-complementing group 1; OR, odds ratio; CI, confidence interval; PVC, polyvinyl chloride; HBV, hepatitis B virus; ASL, angiosarcoma of the liver; CEO, chloroethylene oxide; CAA, chloroacetaldehyde; BER, base excision repair; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C virus antibody.
[5] Wong, R. H., Chen, P. C., Du, C. L., Wang, J. D., and Cheng, T. J. An increased standardized mortality ratio for liver cancer amongst polyvinyl chloride workers in Taiwan (Occup. Environ. Med., Accepted).
[6] Wong, R. H., Chen, P. C., Wang, J. D., Du, C. L., and Cheng, T. J. Synergistic effects of vinyl chloride monomer exposure and hepatitis B virus infection upon liver cirrhosis and liver cancer, submitted for publication.

and serum, whereas the two cases of ASL and one hepatocellular cancer known not to have *p53* mutations in their tumor were found not to have overexpression of mutant p53 protein in their tumor tissue and serum (7). Smith *et al.* (8) found that overexpression of circulating mutant p53 protein was associated with increasing VCM exposure among French PVC workers, a similar finding being reported for Taiwanese PVC workers (9).

For some individuals, the expression of these conformationally altered proteins induces an immune response, thus leading to the presence of circulating anti-p53 antibodies in cancer patients (10). Trivers *et al.* (11) used anti-p53 antibody as a marker noting a greater level of anti-p53 antibody among five cases of VCM-induced ASL, the most notable result from this study being that two of the study subjects revealed detectable anti-p53 antibodies before the diagnosis of ASL. These results suggest that the plasma mutant p53 protein and the anti-p53 antibody may be useful biological-effect markers for the investigation of VCM- induced carcinogenesis among occupationally exposed workers.

Metabolic polymorphism has been implicated previously in chemical exposure-related health effects. An earlier animal study has noted that VCM is primarily metabolized in the liver by CYP2E1 into active CEO and CAA (12), both of which may be reactive with DNA to form DNA adducts (13). Furthermore, those detoxifying enzymes for reactive metabolites of VCM such as ALDH2 and GSTs may also modulate the formation of these DNA adducts (14). Our previous studies have revealed that *CYP2E1 c1c2/c2c2* and *ALDH2 1–2/2–2* were associated with an increased frequency of sister chromatid exchange (15), and that *CYP2E1 c2c2* and *GSTT1* non-null was associated with abnormal liver function (16) among VCM-exposed workers, although the association between VCM exposure-related metabolic traits with mutant p53 protein and anti-p53 antibody remains unclear.

DNA repair is a universal process occurring in living cells. This process is responsible for the maintenance of the structural integrity of DNA in the face of damage arising from environmental insults, as well as from normal metabolic processes. Chinese hamster ovary cell lines (EM9 and EM-C11) with *XRCC1* mutant reveal an unusually high frequency of sister chromatid exchange induced by alkylating agents (17), such cells reverting subsequent to the transfection of human *XRCC1* (18). The XRCC1 protein has also been found to be responsible for BER (19, 20). The probable participation of XRCC1 in the BER process has been demonstrated previously by interaction with proteins known to function in this pathway (19, 20). Evidence also indicates that the VCM derivatives etheno DNA adducts can be repaired through the BER pathway (21). Thus, it would seem likely that susceptible *XRCC1* might play a critical role in VCM-related carcinogenesis.

The present study was undertaken to examine whether mutant p53 protein and anti-p53 antibody expression is associated with VCM exposure; the effect of inherited polymorphisms of metabolic and DNA repair genes on mutant p53 protein and anti-p53 antibody expression is also evaluated.

Materials and Methods

Study Subjects and Epidemiological Data. On the basis of employment records, we retrospectively established a cohort in 1998 consisting of 3293 Taiwanese male workers from six PVC-manufacturing factories.[5] Medical surveillance has been conducted periodically for PVC workers in Taiwan and focuses on currently used and retired workers. Information was collected from workers at a number of PVC-manufacturing plants by use of interviewer-administered questionnaires subsequent to informed consent having been obtained during the medical surveillance process. The structured questionnaire contained questions that covered demographic characteristics, lifestyles including cigarette-smoking habits and alcohol consumption, as well as compiling a detailed occupational history. A smoking history of a subject included the number of cigarettes smoked daily and also the duration of smoking habit. A parameter termed "pack-years" was coined as an indicator of cumulative smoking dose of a subject and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. Because the average quantity of alcohol consumed by Taiwanese people is much less than people in Western countries, habitual alcohol drinking was defined as alcohol consumption on at least one occasion weekly and also as a subject consuming >80 grams of alcohol weekly.

Previously, we conducted a study to explore the expression of mutant p53 protein and anti-p53 antibody among 251 PVC workers (9). To acquire sufficient statistical discriminatory power to detect the difference between mutant p53 protein and anti-p53 antibody expression among various inherited polymorphisms involved in metabolic and DNA repair, herein, we additionally collected a larger pool of study subjects including retired PVC workers. Study subjects exposed to VCM for a period of >1 year were selected if the following criteria were met: detailed questionnaires had been completed, the HBsAg and anti-HCV status was known, and a blood sample could be provided. At final tally, a total of 333 male subjects who had worked in the PVC industry and who had been occupationally exposed to VCM were included for analysis.

Assessment of Vinyl Chloride Exposure. Cumulative VCM exposure levels for study subjects were calculated according to the job exposure matrix model. Because environmental monitoring data were not available before 1985, a mathematical model was developed for estimating the historical mean VCM exposure for 24 job categories for different calendar years in the PVC industry before 1985 (22).

Ambient VCM in the working environment is emitted from the PVC polymerization process including opening of polymerization tank, PVC unloading, stripping, and drying. Our previous study indicated that polymerization tank was the major source of VCM emission, and workers experienced higher levels of VCM exposure when their working areas were close to the polymerization tank (23). Thus, a mass balance model was constructed to estimate VCM emission from polymerization tanks. The amount of VCM emission from polymerization tank was estimated by subtracting the amount of PVC production, PVC loss including PVC scale and cake, and VCM vapor released from dryers and incinerators from the total weight of VCM raw material added to the tank. Changes in operation including polymerization efficiency and recycling of VCM from reaction tank over time based on expert opinions from manufacturing engineers, industrial hygienists, and field directors were also considered in the model. Subsequently, the model were additionally expanded to predict the field VCM exposure for each job category by considering the variables of distance from polymerization tank, working areas close to dryers or stripping operation, and working indoor or outdoor, as well as wind direction and velocity. This reconstructed model revealed that high exposure jobs might have median VCM exposure of 120 ppm before 1970 and 180 ppm before 1985.

Exposure levels of VCM after 1985 were based on existing environmental monitoring data (23). Subsequently, an 8-h time-

weighted job-exposure matrix for PVC workers was constructed, and the cumulative VCM exposure dose for each worker was estimated. In this study, subjects were then divided into a high and a low VCM-exposure group. Individuals placed into the high-exposure group had been exposed to a cumulative VCM-in-air concentration of >40 ppm-years. Those included in the low-exposure group had been exposed to a cumulative VCM concentration level of <40 ppm-years. Forty ppm-years is equivalent to 1 ppm of VCM exposure for 40 working years, which is the current permissible exposure limit for most developed countries (24).

Determination of Hepatitis Status. The HBsAg titer was assayed with radioimmunoassay (Abbott Laboratories, Chicago, IL) or ELISA (Austria-II; Abbott Laboratories). The anti-HCV antibody titer was also assayed with an ELISA technique (Abbott Laboratories). Because the number of subjects with anti-HCV was small, HBsAg and anti-HCV were grouped together. When either HBsAg or anti-HCV was positive, the hepatitis viral infection was defined as being positive.

Plasma Mutant p53 Protein and Anti-p53 Antibody Analysis. The plasma p53 protein assay was performed with an ELISA assay (Oncogene Science, Cambridge, MA) based on the mouse monoclonal antibody PAb240, which is specific for the mutant conformation of the p53 protein (25). Diluted (1:1) plasma (100 μl) was loaded on a microtiter plate, which had been coated with PAb240, and incubated overnight at 4°C. After plate washing, 100 μl of a rabbit polyclonal reporter antibody for p53 was added and incubated for 2 h at room temperature. After an additional washing, the remaining reporter antibody was bound to horseradish peroxidase-conjugated goat antirabbit IgG, and the resultant color was developed by incubation with the peroxidase substrate. The absorbance of each well was measured on a spectrophotometric plate reader at 405 nm, and the concentration of mutant p53 protein was then determined by comparison with the absorbance of purified recombinant mutant human p53 protein. A positive result was defined by any level of absorbance greater than the mean plus 2 SDs (*i.e.*, 0.878 ng/ml) of mutant p53 protein in 36 unexposed controls as specified in our previous study (9).

The detection of plasma anti-p53 antibody was carried out under conditions similar to those described for the detection of mutant p53 protein. Diluted (1:100) plasma and controls were loaded at 100 μl/well into a microtiter plate (Oncogene Science), which had been coated with recombinant human p53 antigen and incubated for 1 h at room temperature. The addition of a 3,3′,5,5′-tetramethylbenzidine substrate resulted in the development of color in proportion to the quantity of antibody bound to the plate. The color reaction was terminated by the use of stop solution (hydrochloric acid), and the absorbance was measured on a spectrophotometric plate reader at a wavelength of 450 nm. The absorbance of subject plasma above the cutoff point designated by the assay manufacturer was treated as being positive for anti-p53 antibody.

Because the number of positive anti-p53 antibody test subjects was small, mutant p53 protein and anti-p53 antibody were subsequently combined as a p53 expression class. When either mutant p53 protein or anti-p53 antibody was positive for a subject, the p53 overexpression was defined as being positive.

Genotyping of Polymorphic Metabolic and DNA Repair Traits. The determination of *CYP2E1*, *ALDH2*, and *GSTT1* genotypes was performed as we have indicated previously (15). Briefly, for the CYP450 2E1 gene analysis, any restriction fragment length polymorphism was detected by differences in *Pst*I sites in the 5′-flanking region after PCR amplification using methods described in 1991 by Hayashi *et al.* (26). Primers used for the amplification of *CYP2E1* gene were 5′-CCA GTC GAG TCT ACA TTG TCA-3′ and 5′-TTC ATT CTG TCT TCT AAC TGG-3′. Amplification was carried out under conditions that the denaturing step was conducted at 95°C, annealing at 55°C, and extension at 72°C. The PCR products were digested with *Pst*I. Homozygous *c1c1* individuals exhibited a product fragment of 410 bp, whereas homozygous *c2c2* individuals revealed a 290-bp and a 120-bp fragment, and heterozygous *c1c2* individuals demonstrated all three of the fragments. The *ALDH2-Mbo*II polymorphism was determined by a modification of the methods developed by Harada and Zhang (27). The sequences of *ALDH2* primers were 5′-CAA ATT ACA GGG TCA ACT GCT ATG-3′ and 5′-CCA CAC TCA CAG TTT TCT CTT-3′. Amplification was carried out under conditions that the denaturing step was conducted at 94°C, annealing at 52°C, and extension at 65°C. The PCR products were digested with *Mbo*II and analyzed with 6% PAGE. Homozygous *2–2* individuals demonstrated a single product fragment of 135 bp, whereas homozygous *1–1* individuals revealed both 125- and 10-bp fragments, and heterozygous *1–2* individuals exhibited all three of the fragments. The *GSTT1* genotype was determined as described by Pemble *et al.* (28). Primers used for the *GSTT1* gene were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′. The amplification of human β-globin (110-bp) was also performed as a positive control in each reaction to confirm the presence of amplifiable DNA in the samples. The primers used for β-globin were 5′-ACA CAA CTG TGT TCA CTA GC-3′ and 5′-CAA CTT CAT CCA CGT TCA CC-3′. The amplification procedure was carried out under conditions that denaturing was conducted at 94°C, annealing at 52°C, and extension at 65°C. The reaction product then underwent electrophoresis in a 2% agarose gel. Individuals with *GSTT1* alleles demonstrated a 480-bp fragment. The *XRCC1- MspI* polymorphism was determined using a PCR-restriction fragment length polymorphism technique (29). An *Arg* to *Gln* substitution in exon 10 (codon 399) was amplified to form an undigested fragment of 242 bp using the primer pair 5′-CCC CAA GTA CAG CCA GGT C-3′ and 5′-TGT CCC GCT CCT CTC AGT AG-3′. The amplification was carried out under conditions that denaturing was conducted at 94°C, annealing at 59°C, and extension at 72°C. The PCR products were digested with *Msp*I and analyzed in a 2% agarose gel. Homozygous *Gln-Gln* individuals reflected a single product fragment of 242 bp, whereas homozygous *Arg-Arg* individuals demonstrated both 94- and 148-bp fragments, and heterozygous *Arg-Gln* individuals revealed all three of the fragments.

Statistical Analysis. Comparisons between low and high VCM-exposure groups for age, duration of employment, smoking habit, alcohol-drinking habit, and the status of hepatitis viral infection, as well as genotypes were made using a Student's *t* test or nonparametric test for continuous variables and a χ^2 test for discrete variables. Subsequently, adjusted ORs and a 95% CI on p53 overexpression (positive and negative) were evaluated for age (<50, 50–59, and ≥60), VCM exposure (high and low), cumulative VCM dose (<5, 5–39, 40–79, and ≥80 ppm-years), cigarette smoking (yes and no), alcohol drinking (yes and no), hepatitis viral infection (yes and no), and genotypes of *XRCC1* (*Arg-Arg, Arg-Gln*, and *Gln-Gln*), *CYP2E1* (*c1c1, c1c2*, and *c2c2*), *ALDH2* (*1–1, 1–2*, and *2–2*), and *GSTT1* (non-null and null) using a multiple logistic regression model. Furthermore, an adjusted OR and a 95% CI on p53 overexpression (positive and negative) were presented for the

Table 1 Basic characteristics of vinyl chloride-exposed workers

Characteristic	VCM exposure		All (n = 333)
	Low (n = 133)	High (n = 200)	
Mutant p53 protein positive	12	36[a]	48 (14.4%)[b]
Anti-p53 antibody positive	4	11	15 (4.5%)
p53 overexpression	16	44[a]	60 (18.0%)
Age (years)	38.4 ± 9.9[c]	45.3 ± 10.4[d]	42.5 ± 10.7
≥60	0	14[d]	14 (4.2%)
50–59	21	61	82 (24.6%)
<50	112	125	237 (71.2%)
Duration of employment (years)	8 (1–33)[e]	19 (1–37)[d]	16 (1–37)
VCM exposure (ppm-years)	8.0 (0.4–38.7)[e]	138.5 (40.4–1116.5)[d]	65 (0.4–1116.5)
Smoking	51	112[d]	163 (49.0%)
Habitual alcohol drinking	12	27	39 (11.7%)
HBsAg positive	26	43	69 (20.7%)
Anti-HCV positive	4	6	10 (3.0%)

[a] $P < 0.05$.
[b] Number (%).
[c] Mean ± SD.
[d] $P < 0.01$.
[e] Median (range).

genotypes of *XRCC1* (*Arg-Arg/Arg-Gln* and *Gln-Gln*), *CYP2E1* (*c1c1/c1c2* and *c2c2*), *ALDH2* (*1–1* and *1–2/2–2*), and *GSTT1* (non-null and null) stratified by VCM exposure (high and low). The trend of the association between genotypes and p53 overexpression by VCM exposure was also assessed. Additionally, numbers of susceptible *XRCC1 Gln-Gln*, *CYP2E1 c2c2*, *ALDH2 1–2/2–2*, and non-null *GSTT1* genotypes together were also taken into multiple logistic regression model to determine their association with p53 overexpression.

Results

The demographic characteristics of the study subjects are summarized in Table 1. Among these 333 VCM-exposed workers, mutant p53 protein and positive anti-p53 antibody were detected among 48 (14.4%) and 15 (4.5%) of the subjects, respectively. Three subjects in high exposure group were found to experience both the overexpression of mutant p53 protein and anti-p53 antibody. Forty-four of 200 subjects experiencing a high VCM exposure demonstrated p53 overexpression. This prevalence is higher than that for those subjects subjected to a low VCM exposure (22.0% *versus* 12.0%; $P = 0.02$). The mean age of study subjects was 42.5 ± 10.7 (SD) years, 163 (49%) of the subjects being smokers. Differences in age ($P < 0.01$; t test), the duration of employment ($P < 0.01$), and cigarette smoking ($P < 0.01$) were statistically significant when comparing the low and high VCM-exposure groups. However, habitual alcohol drinking, HBsAg, and anti-HCV status were not significantly different when comparing these two groups.

The genotype prevalence of *XRCC1, CYP2E1, ALDH2,* and *GSTT1* among the study subjects is indicated in Table 2. The frequencies of the *399Arg* and *399Gln* alleles of *XRCC1* were 73.6% and 26.4%, respectively, although a slightly lower prevalence of the *399Gln* was observed for individuals with a low VCM-exposure rating. The frequencies of occurrence of the *c1* and *c2* alleles of *CYP2E1* were 75.1% and 24.9%, respectively. The frequencies of the *ALDH2* allele 1 and *ALDH2* allele 2 were 70.7% and 29.3%, respectively, whereas the prevalence of GSTT1 null-type was 46.2% and the non-null type was 53.8%.

Table 2 Prevalence of genotypes of *XRCC1, CYP2E1, ALDH2,* and *GSTT1* among VCM-exposed workers stratified by VCM exposure

Genotype	VCM exposure		All (n = 333)
	Low (n = 133)	High (n = 200)	
XRCC1-exon10			
Arg-Arg	76 (57.1%)[a]	97 (48.5%)	173 (52.0%)
Arg-Gln	54 (40.6%)	90 (45.0%)	144 (43.2%)
Gln-Gln	3 (2.3%)	13 (5.5%)	16 (4.8%)
CYP2E1			
c1c1	73 (54.9%)	111 (55.5%)	184 (55.3%)
c1c2	56 (42.1%)	76 (38.0%)	132 (39.6%)
c2c2	4 (3.0%)	13 (6.5%)	17 (5.1%)
ALDH2			
1-1	78 (58.7%)	94 (47.0%)	172 (51.7%)
1-2	43 (32.3%)	84 (42.0%)	127 (38.1%)
2-2	12 (9.0%)	22 (11.0%)	34 (10.2%)
GSTT1			
Null	63 (47.4%)	91 (45.5%)	154 (46.2%)
Non-null	70 (52.6%)	109 (54.5%)	179 (53.8%)

[a] Number (%).

Adjusted ORs of p53 overexpression for age, cigarette smoking status, alcohol drinking status, VCM exposure, hepatitis viral infection, and genotypes of *XRCC1, CYP2E1, ALDH2,* and *GSTT1* are shown in Table 3. Those with cumulative VCM exposure ≥40 ppm-years had significantly higher p53 overexpression than those with VCM exposure <40 ppm-years (OR, 2.1; 95% CI, 1.1–3.8), Additional analysis also revealed that overexpression of p53 was associated with cumulative VCM exposure ($P = 0.07$; test for trend). The p53 overexpression was associated with an age of >60 years as compared with those with age <50 years (OR, 4.9; 95% CI, 1.6–14.8). No obvious association was found between p53 overexpression and smoking status. Pack-years of cigarette smoked were also not associated with p53 overexpression. Those who consumed alcohol had lower OR of p53 overexpression than those who did not consume alcohol, although the association did not reach a significance. Analysis also revealed that hepatitis viral infection was not associated with p53 overexpression.

Interestingly, those subjects revealing the *XRCC1 Gln-Gln* genotype had higher OR of p53 overexpression as compared with those who exhibited the *Arg-Gln or Arg-Arg* genotypes (OR, 2.9, 0.8, and 1.0, respectively), and those possessing the *CYP2E1 c2c2* genotype also had higher OR of p53 overexpression as compared with those demonstrating the *c1c2 or c1c1* genotypes (OR, 1.7, 0.5, and 1.0, respectively). Again, those individuals exhibiting the *GSTT1* non-null genotype also had higher OR of p53 overexpression as compared with those possessing *GSTT1* null genotypes (OR, 1.0, 0.6, respectively). However, overexpression of p53 was not different among the *ALDH2 1–2, 2–2, or 1–1* genotype. Thus, in the subsequent analysis, those possessing *XRCC1 Arg-Arg* and *Arg-Gln* genotypes were grouped together. Similarly, those individuals possessing *CYP2E1 c1c1* or *c1c2* were grouped together. It has been reported previously that PVC workers displaying at least one *ALDH2* allele 2 reveal much lower enzyme activity than *ALDH2 1–1* (30), thus those subjects in our study presenting with at least one *ALDH2* allele 2 being grouped as *ALDH2 1–2/2–2.*

Table 3 p53 overexpression status according to genotypes and variables of interest

	p53 overexpression		Adjusted OR[a] (95% CI)
	Yes (n = 60)	No (n = 273)	
XRCC1-exon10			
Arg/Arg	33 (55.0%)	140 (51.3%)	1.0 (reference)
Arg/Gln	21 (35.0%)	123 (45.0%)	0.8 (0.4–1.4)
Gln/Gln	6 (10.0%)	10 (3.7%)	2.9 (1.0–9.0)[b]
CYP2E1			
c1c1	38 (63.3%)	146 (53.5%)	1.0 (reference)
c1c2	16 (26.7%)	116 (42.5%)	0.5 (0.3–0.9)
c2c2	6 (10.0%)	11 (4.0%)	1.7 (0.6–5.0)
ALDH2			
1-1	28 (46.7%)	144 (52.8%)	1.0 (reference)
1-2	27 (45.0%)	100 (36.6%)	1.3 (0.7–2.4)
2-2	5 (8.3%)	29 (10.6%)	0.7 (0.3–2.1)
GSTT1			
Non-null	37 (61.7%)	142 (52.0%)	1.0 (reference)
Null	23 (38.3%)	131 (48.0%)	0.6 (0.4–1.1)
VCM exposure			
Low (<40 ppm-years)	16 (26.7%)	117 (42.9%)	1.0 (reference)
High (≥40 ppm-years)	44 (73.3%)	156 (57.1%)	2.1 (1.1–3.8)[c]
Cumulative exposure dose (ppm-years)			
<5	4 (6.7%)	42 (15.4%)	1.0 (reference)
5–39	12 (20.0%)	75 (27.5%)	1.8 (0.6–6.0)
40–79	15 (25.0%)	36 (13.2%)	5.0 (1.5–16.9)[c]
≥80	29 (48.3%)	120 (44.0%)	2.6 (0.8–8.2)[d]
Age (years)			
<50	40 (66.7%)	197 (72.1%)	1.0 (reference)
50–59	13 (23.3%)	69 (25.3%)	0.9 (0.5–1.8)
≥60	7 (10.0%)	7 (2.6%)	4.9 (1.6–14.8)[c]
Smoking			
No	32 (53.3%)	138 (50.5%)	1.0 (reference)
Yes	28 (46.7%)	135 (49.5%)	0.9 (0.5–1.5)
Alcohol drinking			
No	58 (96.7%)	236 (86.4%)	1.0 (reference)
Yes	2 (3.3%)	37 (13.6%)	0.2 (0.1–1.0)
Hepatitis infection			
Negative	49 (81.7%)	209 (76.6%)	1.0 (reference)
Positive	11 (18.3%)	64 (23.4%)	0.7 (0.4–1.5)

[a] Adjusted for smoking, age, drinking, and genotypes.
[b] $P = 0.07$, compared with reference.
[c] $P < 0.05$, compared with reference.
[d] $P = 0.07$, test for trend in dose-response.

Subsequently, adjusted ORs of p53 overexpression were calculated to investigate the joint effect of VCM exposure and genotypes. After adjusting for the effects of age, smoking status, and presence/absence of genotypes of *CYP2E1*, *ALDH2*, and *GSTT1*, using a low VCM exposure and *XRCC1 Arg-Arg/Arg-Gln* genotypes as a reference (OR, 1.0), a prominent risk of p53 overexpression was observed for those individuals having experienced a high VCM exposure and possessing the *XRCC1 Gln-Gln* genotype (OR, 6.5; 95% CI, 1.7–24.2; Table 4). When using a high VCM exposure and *XRCC1 Arg-Arg/Arg-Gln* genotypes as a reference (OR, 1.0), a prominent risk of p53 overexpression was observed for those individuals having experienced a high VCM exposure and possessing the *XRCC1 Gln-Gln* genotype (OR, 3.6; 95% CI, 1.1–11.8). Similarly, when *XRCC1* was replaced by *CYP2E1* in the statistical analysis, those having experienced a low VCM exposure and who revealed the *CYP2E1 c2c2* genotype were found to experience a greater risk of p53 overexpression than those exhibiting a low VCM exposure and demonstrating the presence of the *CYP2E1 c1c1/c1c2* genotypes (OR, 9.8; 95% CI, 1.2–81.6). When *ALDH2* replaced *CYP2E1* in the low exposure group, those subjects with *ALDH2 1–2/2–2* genotypes also experienced a greater risk of p53 overexpression as compared with those with *ALDH2 1–1* (OR, 1.6; 95% CI, 0.5–4.6). Again, in the low exposure group, *GSTT1* non-null was associated with an increased risk of p53 overexpression compared with *GSTT1* null genotype (OR, 2.4; 95% CI, 0.8–7.6). In the high exposure groups, p53 overexpression was not different between *CYP2E1 c2c2* and *c1c2/c1c1*, *ALDH21–1* and *1–2/2–2*, or *GSTT1* non-null and null genotypes, respectively, although they had higher risk of p53 overexpression as compared with their corresponding less susceptible genotypes in the low exposure group.

Subsequently, we also evaluate the combined effects of *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* genotypes adjusted for potential confounders (Fig. 1). When workers who had experienced a low VCM exposure and who carried none of susceptible genotypes were used as a reference, those workers expressing more susceptible genotypes of *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* experienced a greater risk of p53 overexpression, and the trend was obvious both for subjects in the high VCM-exposure group (test for trend; $P = 0.06$) and for those in the low VCM-exposure group ($P = 0.03$).

Discussion

The aim of this study is to explore the association between polymorphisms of metabolic and DNA repair genes with mutant p53 protein and anti-p53 antibody expression induced by VCM. Our results revealed that VCM exposure was associated with the expression of mutant p53 protein and anti-p53 antibody. This study also observed that PVC workers who had experienced a low VCM exposure and who possessed the *CYP2E1 c2c2* genotype or those reflecting a high VCM exposure and the *XRCC1 Gln-Gln* genotype faced a significantly higher risk of mutant p53 protein and anti-p53 antibody overexpression. Furthermore, those workers with more genotypes that were susceptible to VCM-related metabolism and DNA repair revealed a high prevalence of mutant p53 protein and anti-p53 antibody overexpression.

Previous studies have reported that prolonged exposure to VCM can increase the accumulation of mutant p53 protein (8, 9) and anti-p53 antibody (9, 11). Active metabolites of VCM are known to form etheno-adenosine adducts in DNA, these adducts possibly resulting in *A* to *T* transversions (31), with such *A* to *T* transversions having been found in the DNA from some ASL cases for VCM-exposed workers (6). It is likely that these mutations would result in amino acid substitutions in the encoded p53 proteins (Arg to Trp at codon 249 and Ile to Phe at codon 255). These amino acid substitutions occur in a highly conserved region of p53 that is believed to be critical to its tumor-suppressor function (32). A previous study has demonstrated that the conformation of mutant protein can be detected by using PAb 240.

Although the association between the *p53* gene mutation in tumor tissue with mutant p53 protein and anti-p53 antibody in plasma has already been demonstrated (6, 11), some evidence suggests that not all *p53* gene mutations lead to increased p53 protein levels (11). Because there was no opportunity to acquire VCM-elicited tumor tissue, mutant p53 protein and anti-p53 antibody were used as surrogates in this study. Non-p53 cross-reacting proteins for PAb 240 have been reported previously to exist (33), thus the possibility of a misclassification of *p53* mutation by a false-positive ELISA assay in this study cannot be ruled out.

Table 4 Adjusted OR of p53 overexpression for the joint effect of exposure (ppm-years) and genotypes

Genotype	Cumulative VCM exposure					
	>40 ppm-years			≤40 ppm-years		
	p53 overexpression		Adjusted OR (95% CI)[a]	p53 overexpression		Adjusted OR (95% CI)[a]
	Yes	No		Yes	No	
XRCC1-exon10[b]						
Arg-Arg/Arg-Gln	38	149	1.8 (0.9–3.6)	16	114	1.0 (reference)
Gln-Gln	6	7	6.5 (1.7–24.2)[c,d]	0	3	—
CYP2E1[d]						
c1c1/c2c2	40	147	2.8 (0.7–11.6)	14	115	1.0 (reference)
c2c2	4	9	2.3 (1.1–4.6)[c]	2	2	9.8 (1.2–81.6)[c]
ALDH2						
1-1	20	74	2.4 (1.0–5.9)[c]	8	70	1.0 (reference)
1-2/2-2	24	82	2.5 (1.0–6.2)[c]	8	47	1.6 (0.5–4.6)
GSTT1						
Non-null	26	83	3.8 (1.3–10.9)[c]	11	59	2.4 (0.8–7.6)
Null	18	73	3.0 (1.0–8.9)[c]	5	58	1.0 (reference)

[a] Adjusted for smoking, drinking, age, and genotypes.
[b] $P < 0.01$, test for trend for the joint effects of genotypes and VCM exposure on p53 overexpression.
[c] $P < 0.05$, compared with reference.
[d] $P < 0.05$, compared with *Arg-Arg/Arg-Gln.*

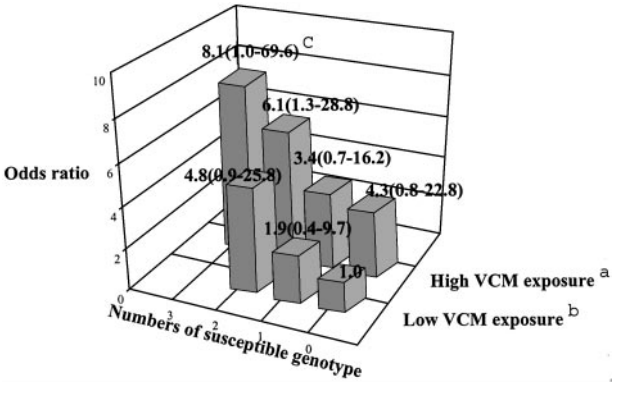

Fig. 1. OR of p53 overexpression by VCM exposure and the number of susceptible *XRCC1*, *CYP2E1*, *ALDH2*, and *GSTT1* genotypes after adjusting for potential confounders. [a], $P = 0.06$, test for trend in high exposure group. [b], $P = 0.03$, test for trend in low exposure group. [c], OR (95% CI).

The dose-response relationship between p53 overexpression and VCM cumulative dose was less prominent in the high dose range. The possible explanation is that cumulative VCM dose of high exposure jobs may be underestimated in our reconstruction model, which could lead to the misclassification of VCM dose. The job-exposure matrix is required for additional modification.

In this study, the frequencies of the *XRCC1 399Gln* allele (26.4%) was close to that noted in a previous study pertaining to Taiwanese normal subjects (26.0%; Ref. 34). The figure of 24.9% for the prevalence of the *CYP2E1 c2* allele found in this study was similar to that found in a previous study conducted among people of Taiwanese descent (20.3%, Ref. 35). The frequency of the *ALDH2* allele 1 (70.7%) was also comparable with the corresponding value for the control group of other alcoholism studies for the Taiwanese population (68–76%; Refs. 36, 37). The prevalence of the *GSTT1* null-type (46.2%) was likewise consistent with the analogous figure as revealed by previous studies: 64.4% for Chinese (38) and 51.4% for Taiwanese populations (39). These findings, to some extent, validate the technique of our genotyping.

Importantly, an animal study has demonstrated previously that VCM is metabolized by CYP2E1 to become CEO (12), and CEO may spontaneously transform into CAA, which may be subsequently metabolized by ALDH2 (14). Previous studies have also reported that GSTs may act as detoxification enzymes by reacting with the epoxide product(s) of many different environmental chemicals (39, 40). Both CEO and CAA are electrophiles capable of reacting with DNA bases to yield etheno adducts (13). In our study, VCM-exposed workers featuring the *CYP2E1 c2c2* genotype revealed a higher risk of p53 overexpression than those lacking that genotype. Workers with the *CYP2E1 c2c2* genotype demonstrate a higher metabolic activity than those expressing the *CYP2E1 c1c1/c1c2* genotypes (26), thus they may experience elevated CEO and CAA levels. Furthermore, *CYP2E1 c2c2* carriers from the low VCM-exposure group experienced a more prominent risk of p53 overexpression compared with other combinations of the *CYP2E1* genotype and VCM exposure, although there was limited statistical power to note the effect of the *CYP2E1 c2c2* genotype. This appears to be supported by a previous study in rats (41) in which the metabolism of VCM at low concentration follows first-order kinetics and is perfusion-limited. When the VCM level exceeded 250 ppm in the study by these authors, the kinetic became zero-order (41). Furthermore, an earlier study has also reported that high concentrations of VCM can degrade cytochrome P450 (42).

Similar to the case for *CYP2E1*, our model analysis also revealed the risk of p53 overexpression increased with the presence of *GSTT1 non-null* genotype in the low exposure group. This result was consistent with our previous finding that workers experiencing a low VCM exposure and who revealed the presence of *GSTT1 non-null* genotype exhibited a higher proportion of abnormal serum alanine aminotranferase (16). This phenomenon also implies that VCM may be metabolized by GSTs to conjugate with glutathione and that these conjugates may demonstrate a more substantial mutagenic activity, as observed in ethylene dibromide (43). We also observed that PVC workers experiencing a low VCM exposure and for whom the *ALDH2 1–2/2–2* genotypes were present demonstrated a greater risk of p53 overexpression than low VCM-exposed workers who carried *ALDH2 1–1* genotype. Those who fea-

tured the *ALDH2 1–2* demonstrate only 6% of metabolic activity as those possessing the *ALDH2 1–1*, whereas those with *ALDH2 2–2* did not demonstrate any *ALDH2* activity (30). Thus, those having at least one ALDH2 allele 2 may have accumulated CAA in their bodies and caused more damage. It is interesting to observe that the effects of susceptible *ALDH2 and GSTT1* on p53 overexpression were more prominent in the low exposure group. The possible explanation is the limited capacity of VCM metabolism through these enzymes (41).

In our current study, we first highlighted the association between XRCC1 and p53 overexpression, especially for those experiencing a high VCM exposure. Lunn *et al.* (34) reported in 1999 that the *XRCC1 Gln-Gln* genotype was associated with higher levels of both aflatoxin B1-DNA adducts and glycophorin A variants. *XRCC1 Gln-Gln* carriers from the high VCM-exposure group revealed a significantly higher risk of p53 overexpression compared with other combinations of the *XRCC1* genotype in this specific VCM-exposure group. A possible explanation for this result is that workers from the high VCM-exposure group experienced a greater chance of developing DNA damage than those subjects from the low VCM-exposure group, and we speculate that an increased mutation frequency for the *p53* gene among *XRCC1 Gln-Gln* carriers might additionally prompt the persistence of DNA damage.

Interestingly, those individuals who demonstrated with more susceptible genotypes of *XRCC1, CYP2E1, ALDH2,* and *GSTT1* were more likely to experience p53 overexpression. *CYP2E1* and *GSTT1* are involved in the activation of VCM, and *ALDH2* acts as detoxifying enzymes for the reactive metabolites of VCM, whereas *XRCC1* is involved in the subsequent DNA-repair process. This indicates that each susceptible genotype may not generate a significant risk for p53 overexpression; however, when they are combined together, a more prominent risk may develop. It seems that subjects who carry susceptible genotypes of metabolic and/or DNA repair traits are more likely to express *p53* mutation when they are exposed to VCM regardless of high or low VCM cumulative dose. Because of the relatively few subjects with p53 overexpression in this study, it is not clear if similar effects will be observed in much lower dose range. Additional study including more subjects may shed light on this question.

In our study, only three healthy subjects were found to experience both the overexpression of mutant p53 protein and anti-p53 antibody. It is possible that the presence of such antibodies could accelerate the clearance of p53 protein from plasma (10). Additionally, it has been demonstrated previously that the development of anti-p53 antibodies is dependent on the complexing of the mutant protein with heat shock protein 70 (44). Thus, this might explain why most of the subjects in our study reflected the presence of mutant p53 protein but did not reflect the presence of anti-p53 antibody.

Some previous studies have reported that older people experienced a greater risk of p53 overexpression (45, 46). In our study of PVC workers, we also observed that subject age was directly associated with p53 expression. This phenomenon reflects for PVC workers either an increasing susceptibility to damage with age or the accumulation of long-lived damages. A previous study has also indicated that the capacity of DNA repair decreased with age among healthy subjects (47). In this study, high exposure group was significantly older as compared with the low exposure group. The possible effects of residual confounding could not be excluded, although the effect of age has been considered in the analysis. Our study subjects were relatively young with mean age of 42, and those with higher cumulative dose tended to be older. It is not possible to match low exposed workers with age among the available study subjects.

Previous reports have shown that smoking (48) and HBV infection (49) were associated with the presence of *p53* gene mutations. The quantity of cigarettes smoked by study subjects in the current study was relatively small by comparison to those corresponding figures for the participants of other studies (48, 50); thus, it would seem likely that this was the reason that we observed no significant association between cigarette-smoking and p53 expression in our study. The expression of p53 was not associated with HBV infection in our study, although this result was similar to a previous study of Taiwanese patients suffering from hepatocellular carcinoma (51). On the other hand, cigarette smoking and HBV infection are known to induce diverse types of *p53* mutation (4). Thus, p53 alterations elicited by cigarette smoke or HBV infection may not have been completely recognized by ELISA assay in our study.

In summary, our results have revealed that increased VCM exposure is associated with p53 overexpression and metabolic *CYP2E1*, and DNA repair *XRCC1* genes may modulate the mutation of the *p53* gene among VCM-exposed Taiwanese PVC workers. Those VCM-exposed workers, particularly individuals with susceptible *CYP2E1* and *XRCC1*, may need intensive medical screening, particularly for liver cancer. Clearly thus, the role of metabolic- and DNA-repair genes in VCM-related liver cancer requires additional study.

Acknowledgments

We thank Yu-Chen Lei for her technical assistance.

References

1. Jones, R. D., Smith, D. M., and Thomas, P. G. A mortality study of vinyl chloride monomer workers employed in the United Kingdom in 1940–1974. Scand. J. Work Environ. Health, *14:* 153–160, 1988.

2. Wong, O., Whorton, M. D., Foliart, D. E., and Ragland, D. An industry-wide epidemiologic study of vinyl chloride workers, 1942–1982. Am. J. Ind. Med., *20:* 317–334, 1991.

3. IARC. Overall evaluations of Carcinogenicity: An Updating of IARC Monographs, Vols. 1–42. Monographs on the evaluation of carcinogenic risks to humans, pp. 373–376. Lyon, France: IARC, *Suppl. 7:* 1987.

4. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.

5. Bodner, S. M., Minna, J. D., Jensen, S. M., D'Amico, D., Carbone, D., Mitsudomi, T., Fedorko, J., Buchhagen, D. L., Nau, M. M., Gazdar, A. F., and Linnoila, R. I. Expression of mutant p53 proteins in lung cancer correlates with the class of *p53* gene mutation. Oncogene, *7:* 743–749, 1992.

6. Hollstein, M., Marion, M. J., Lehman, T., Welsh, J., Harris, C. C., Martel-Planche, G., Kusters, I., and Montesano, R. *p53* mutations at *A:T* base pairs in angiosarcomas of vinyl chloride-exposed factory workers. Carcinogenesis (Lond.), *15:* 1–3, 1994.

7. Smith, S., Luo, J. C. J., and Brandt-Rauf, P. Mutant p53 protein as a biomarker of chemical carcinogenesis in human. J. Occup. Environ. Med., *38:* 743, 1996.

8. Smith, S. J., Li, Y., Whitley, R., Marion, M. J., Partilo, S., Carney, W. P., and Brandt-Rauf, P. W. Molecular epidemiology of p53 protein mutations in workers exposed to vinyl chloride. Am. J. Epidemiol., *147:* 302–308, 1998.

9. Luo, J. C., Liu, H. T., Cheng, T. J., Du, C. L., and Wang, J. D. Plasma p53 protein and anti-p53 antibody expression in vinyl chloride monomer workers in Taiwan. J. Occup. Environ. Med., *41:* 521–526, 1999.

10. Labrecque, S., Naor, N., Thomson, D., and Matlashewski, G. Analysis of the anti-p53 antibody response in cancer patients. Cancer Res., *53:* 3468–3471, 1993.

11. Trivers, G. E., Cawley, H. L., DeBenedetti, V. M., Hollstein, M., Marion, M. J., Bennett, W. P., Hoover, M. L., Prives, C. C., Tamburro, C. C., and Harris, C. C. Anti-p53 antibodies in sera of workers occupationally exposed to vinyl chloride. J. Natl. Cancer Inst., *87:* 1400–1407, 1995.

12. el Ghissassi, F., Barbin, A., and Bartsch, H. Metabolic activation of vinyl chloride by rat liver microsomes: low-dose kinetics and involvement of cytochrome P450 2E1. Biochem. Pharmacol., *55:* 1445–1452, 1998.

13. Guengerich, F. P. Roles of the vinyl chloride oxidation products 1-chlorooxirane and 2-chloroacetaldehyde in the *in vitro* formation of etheno adducts of nucleic acid bases. Chem. Res. Toxicol., *5:* 2–5, 1992.

14. Whysner, J., Conaway, C. C., Verna, L., and Williams, G. M. Vinyl chloride mechanistic data and risk assessment: DNA reactivity and cross-species quantitative risk extrapolation. Pharmacol. Ther., *71:* 7–28, 1996.

15. Wong, R. H., Wang, J. D., Hsieh, L. L., Du, C. L., and Cheng, T. J. Effects on sister chromatid exchange frequency of aldehyde dehydrogenase 2 genotype and smoking in vinyl chloride workers. Mutat. Res., *420:* 99–107, 1998.

16. Huang, C. Y., Huang, K. L., Cheng, T. J., Wang, J. D., and Hsieh, L. L. The *GSTT1* and *CYP2E1* genotypes are possible factors causing vinyl chloride induced abnormal liver function. Arch. Toxicol., *71:* 482–488, 1997.

17. Zdzienicka, M. Z., van der Schans, G. P., Natarajan, A. T., Thompson, L. H., Neuteboom, I., and Simons, J. W. A Chinese hamster ovary cell mutant (EM-C11) with sensitivity to simple alkylating agents and a very high level of sister chromatid exchanges. Mutagenesis, *7:* 265–269, 1992.

18. Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A., and Carrano, A. V. Molecular cloning of the human *XRCC1* gene, which corrects defective DNA strand break repair and sister chromatid exchange. Mol. Cell. Biol., *10:* 6160–6171, 1990.

19. Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. Involvement of *XRCC1* and DNA ligase III gene products in DNA base excision repair. J. Biol. Chem., *272:* 23970–23975, 1997.

20. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol. Cell. Biol., *8:* 3563–3571, 1998.

21. Dosanjh, M. K., Chenna, A., Kim, E., Fraenkel-Conrat, H., Samson, L., and Singer, B. All four known cyclic adducts formed in DNA by the vinyl chloride metabolite chloroacetaldehyde are released by a human DNA glycosylase. Proc. Natl. Acad. Sci. USA, *91:* 1024–1028, 1994.

22. Du, C. L., Chan, C. C., and Wang, J. D. Development of a job exposure matrix model for polyvinyl chloride workers in Taiwan. Inst. Occu. Safety Health J., *9:* 151–166, 2001 (in Chinese).

23. Du, C. L., Chan, C. C., and Wang, J. D. Comparison of personal and area sampling strategies in assessing workers' exposure to vinyl chloride monomer. Bull. Environ. Contam. Toxicol., *56:* 534–542, 1996.

24. International Programme on Chemical Safety. Environmental health criteria 215: Vinyl chloride, pp. 313. Geneva, WHO: International Programme on Chemical Safety, 1999.

25. Gannon, J. V., Greaves, R., Iggo, R., and Lane, D. P. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. EMBO J., *9:* 1595–1602, 1990.

26. Hayashi, S., Watanabe, J., and Kawajiri, K. Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J. Biochem., *110:* 559–565, 1991.

27. Harada, S., and Zhang, S. New strategy for detection of *ALDH2* mutant. Alcohol Alcohol., *28*(Suppl. 1A)*:* 11–13, 1993.

28. Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., and Taylor, J. B. Human glutathione S-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism. Biochem. J., *300(Pt. 1):* 271–276, 1994.

29. Duell, E. J., Wiencke, J. K., Cheng, T. J., Varkonyi, A., Zuo, Z. F., Ashok, T. D., Mark, E. J., Wain, J. C., Christiani, D. C., and Kelsey, K. T. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis (Lond.), *21:* 965–971, 2000.

30. Crabb, D. W., Edenberg, H. J., Bosron, W. F., and Li, T. K. Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive *ALDH2(2)* allele is dominant. J. Clin. Investig., *83:* 314–316, 1989.

31. Pandya, G. A., and Moriya, M. 1. N6-ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. Biochemistry, *35:* 11487–11492, 1996.

32. Vogelstein, B., and Kinzler, K. W. p53 function and dysfunction. Cell, *70:* 523–526, 1992.

33. Stephen, C. W. and Lane, D. P. Mutant conformation of p53. Precise epitope mapping using a filamentous phage epitope library. J. Mol. Biol., *225:* 577–583, 1992.

34. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res., *59:* 2557–2561, 1999.

35. Yu, M. W., Gladek-Yarborough, A., Chiamprasert, S., Santella, R. M., Liaw, Y. F., and Chen, C. J. Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. Gastroenterology, *109:* 1266–1273, 1995.

36. Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X. L., Jerome, R. E., Li, T. K., Wang, S. P., Lin, Y. T., Lu, R. B., and Yin, S. J. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. Am. J. Hum. Genet., *48:* 677–681, 1991.

37. Chen, W. J., Loh, E. W., Hsu, Y. P., Chen, C. C., Yu, J. M., and Cheng. A. T., Alcohol-metabolising genes and alcoholism among Taiwanese Han men: independent effect of *ADH2*. *ADH3* and *ALDH2*. Br. J. Psychiatry, *168:* 762–767, 1996.

38. Nelson, H. H., Wiencke, J. K., Christiani, D. C., Cheng, T. J., Zuo, Z. F., Schwartz, B. S., Lee, B. K., Spitz, M. R., Wang, M., Xu, X., and Kelsey, K. T. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase θ. Carcinogenesis (Lond.), *16:* 1243–1245, 1995.

39. Chen, C. J., Yu, M. W., Liaw, Y. F., Wang, L. W., Chiamprasert, S., Matin, F., Hirvonen, A., Bell, D. A., and Santella, R. M. Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. Am. J. Hum. Genet., *59:* 128–134, 1996.

40. Ketterer, B. Protective role of glutathione and glutathione transferase in mutagenesis and carcinogenesis. Mutat. Res., *202:* 343–361, 1988.

41. Filser, J. G., and Bolt, H. M. Pharmacokinetics of halogenated ethylenes in rats. Arch. Toxicol., *42:* 123–136, 1979.

42. Ivanetich, K. M., Aronson, I., and Katz, I. D. The interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 *in vitro*. Biochem. Biophys. Res. Commun., *74:* 1411–1418, 1977.

43. Their, R., Taylor, J. B., Pemble, S. E., Humphreys, W. G., Persmark, M., Ketterer, B., and Guengerich, F. P. Expression of mammalian glutathione S-transferase 5–5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to dihalomethanes. Proc. Natl. Acad. Sci. USA, *90:* 8576–8580, 1993.

44. Davidoff, A. M., Iglehart, J. D., and Marks, J. R. Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. Proc. Natl. Acad. Sci. USA, *89:* 3439–3942, 1992.

45. Vogl, F. D., Stickeler, E., Weyermann, M., Kohler, T., Grill, H. J., Negri, G., Kreienberg, R., and Runnebaum, I. B. p53 autoantibodies in patients with primary ovarian cancer are associated with higher age, advanced stage and a higher proportion of p53-positive tumor cells. Oncology, *57:* 324–329, 1999.

46. Servomaa, K., Kiuru, A., Kosma, V. M., Hirvikoski, P., and Rytomaa, T. *p53* and *K-ras* gene mutations in carcinoma of the rectum among Finnish women. Mol. Pathol., *53:* 24–30, 2000.

47. D'Errico, M., Calcagnile, A., Iavarone, I., Sera, F., Baliva, G., Chinni, L. M., Corona, R., Pasquini, P., and Dogliotti, E. Factors that influence the DNA repair capacity of normal and skin cancer-affected individuals. Cancer Epidemiol. Biomark. Prev., *8:* 553–559, 1999.

48. Kersting, M., Friedl, C., Kraus, A., Behn, M., Pankow, W., and Schuermann, M. Differential frequencies of p16 (INK4a) promoter hypermethylation, *p53* mutation, and *K-ras* mutation in exfoliative material mark the development of lung cancer in symptomatic chronic smokers. J. Clin. Oncol., *18:* 3221–3229, 2000.

49. Bressac, B., Kew, M., Wands, J., and Ozturk, M. Selective *G* to *T* mutations of *p53* gene in hepatocellular carcinoma from southern Africa. Nature (Lond.), *350:* 429–431, 1991.

50. Ahrendt, S. A., Chow, J. T., Yang, S. C., Wu, L., Zhang, M. J., Jen, J., and Sidransky, D. Alcohol consumption and cigarette smoking increase the frequency of *p53* mutations in non-small cell lung cancer. Cancer Res., *60:* 3155–3159, 2000.

51. Sheu, J. C., Huang, G. T., Lee, P. H., Chung, J. C., Chou, H. C., Lai, M. Y., Wang, J. T., Lee, H. S., Shih, L. N., Yang, P. M., Wang, T. H., and Chen, D. S. Mutation of *p53* gene in hepatocellular carcinoma in Taiwan. Cancer Res., *52:* 6098–6100, 1992.