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Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells

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Polymorphisms in several DNA repair genes have recently been identified, but little is known about their phenotypic significance. To determine whether variation in DNA repair genes is related to host DNA damage, we studied the association between polymorphisms in *XRCC1* (codon 399) and *ERCC2* (codon 751) and two markers of DNA damage, sister chromatid exchange (SCE) frequencies ($n = 76$) and polyphenol DNA adducts ($n = 61$). SCE frequencies were determined using a modified fluorescence–Giemsa method and polyphenol DNA adducts were determined using a P1-enhanced ^{32}P-post-labeling procedure. *XRCC1* and *ERCC2* genotypes were identified using PCR–RFLP. Mean SCE frequencies among current smokers who were homozygous carriers of the 399Gln allele in *XRCC1* were greater than those in 399Arg/Arg current smokers. We also observed a possible gene-dosage effect for *XRCC1* 399Gln and detectable DNA adducts, and significantly more adducts among older subjects who were carriers of the 399Gln allele than in younger subjects with the 399Arg/Arg genotype. The polymorphism in *ERCC2* was unrelated to SCE frequency or DNA adduct level. Our results suggest that carriers of the polymorphic *XRCC1* 399Gln allele may be at greater risk for tobacco- and age-related DNA damage.

Introduction

Individuals deficient in the repair of DNA damage are known to be hypersensitive to ultraviolet (UV) light and at increased risk of developing neoplasms (1). Polymorphisms in several DNA repair genes have been reported (2), but the role of these variants in generating DNA damage phenotypes in human populations has been less well studied. Recently, Lunn *et al.* (3) reported that there were more placental aflatoxin B1 adducts and glycophorin A variants among carriers of a polymorphism in exon 10 (Arg→Gln at codon 399) of *XRCC1* (X-ray repair cross-complementing group 1) than in 399Arg/Arg carriers of the gene. *XRCC1* is involved in the repair of single-strand breaks following base excision repair (BER) resulting from exposure to endogenously produced active oxygen, ionizing radiation or alkylating agents (4–6). Codon 399 in *XRCC1* is located within a BRCT (*BRCA1* C-terminus) domain which is believed to be a protein–protein interface that interacts with poly(ADP-ribose) polymerase (PARP) (7,8). PARP is a zinc finger-containing enzyme that detects DNA strand breaks and is involved in BER (9). Chinese hamster cell mutants (EM9 and EM-C11), which have high background sister chromatid exchange (SCE) frequencies and defective single-strand break repair following exposure to ionizing radiation or alkylating agents, revert after transfection of human *XRCC1* (5,6). The *ERCC2* (excision repair cross-complementing group 2, also known as XPD) gene codes for a protein involved in transcription-coupled nucleotide excision repair (NER). NER removes and corrects oligonucleotide fragments containing a variety of lesions such as UV-induced lesions, chemical adducts and crosslinks (10,11). Reduction of photosensitivity in xeroderma pigmentosum (XP) and trichothiodystrophy (TTD) cells by cloned *ERCC2* has been reported (11–14) and several polymorphisms in *ERCC2* have recently been identified (2).

Markers of DNA damage such as SCE and DNA or protein adduct levels have been successfully used to measure human exposure to genotoxic substances such as cigarette smoke (15). Markers of genotoxicity such as SCEs have been found to correlate with inherited predisposition to cancer and defective DNA repair in a limited number of cases (16). In a relatively recent study of baseline SCE frequencies among 136 monozygotic and 88 dizygotic twins, the authors concluded that genetic influences play a significant role (~30%) in the observed variation in baseline SCE frequency (17). The glutathione *S*-transferase T1 and M1 polymorphisms (18,19), but not CYP1A1 or CYP2D6 polymorphisms (19), have explained a portion of this variation. Additional genetic factors may contribute to baseline SCE variability in human cells. We have addressed the potential role of genetic variation on SCE frequencies by analyzing the associations between polymorphisms in *XRCC1* (codon 399) and *ERCC2* (codon 751) with variations in lymphocyte SCE frequencies from 76 healthy subjects.

The earlier study that reported higher aflatoxin DNA adducts in carriers of the *XRCC1* 399Gln allele (3) postulated that this variant affects a BER-dependent pathway for repair of aflatoxin damage. BER and NER are distinctly different pathways in human cells; only the XPG protein has thus far been shown to carry out overlapping functions within both systems (20). XRCC1, through its interaction with DNA ligase III and polymerase β, is thought to be important in BER specifically (4–6). BER characteristically acts on endogenous oxidative DNA damage in which key intermediates include the formation of abasic sites [apurinic/apyrimidinic (AP) sites]. Recently, several benzene-derived DNA adducts have been identified as

Abbreviations: BER, base excision repair; *ERCC2*, excision repair cross-complementing group 2; NER, nucleotide excision repair; PARP, poly(ADP-ribose) polymerase; SCE, sister chromatid exchange; *XRCC1*, X-ray repair cross-complementing group 1.

good substrates for human AP endonuclease (HAP1) (21), making the BER pathway highly relevant to DNA lesions produced by benzene-related phenols and quinones. We previously developed a ^{32}P-post-labeling assay to detect polyphenol DNA adducts, which we postulate are endogenous in nature and arise through oxidative processes (21–24). Because of the potential importance of *XRCC1* in BER-mediated removal of phenolic adducts, we explored the possible associations of polymorphisms in *XRCC1* with polyphenol DNA adducts in mononuclear cells from 61 healthy subjects; the possible involvement of *ERCC2* in these adducts was also investigated.

Materials and methods

Study population

The study population has been previously described (19). Briefly, the study population consisted of control subjects enrolled in a case-control study of lung cancer at Massachusetts General Hospital (December 1992 to April 1994), and were friends or spouses of enrolled cases. Information on smoking history and occupational/environmental exposures was gathered using an interviewer-administered questionnaire. Since there were many more former smokers than current smokers or non-smokers in this population, former smokers were under-sampled for these analyses (19). Eighty-one of 141 control subjects were sampled for SCE analysis in a previous study (19). Of these 81 subjects, five were excluded as a result of technical problems and incomplete questionnaire or genotype information, leaving 76 for this analysis of SCE frequencies. The 61 subjects for the polyphenol DNA adduct analysis were a previous random sample from the same study population. These study subjects were sampled for the measurement of polyphenol DNA adducts and analysis of associations with lung cancer risk. For six subjects, both SCE frequency and DNA adduct data were available.

Sister chromatid exchange assay

The SCE assay has been described previously (19). Briefly, heparinized blood was processed within 24 h and lymphocytes were cultured using 1% phytohemagglutinin P (Burroughs–Wellcome). 5-Bromo-2-deoxyuridine (50 μM final conc.) was added after 24 h of culture to achieve differential chromatid staining. Cultured lymphocytes were fixed to slides and air-dried. Chromosomes were stained using a modified fluorescence–Giemsa staining technique. For each study subject, 50 metaphases were scored to estimate the mean SCE frequency. High frequency SCE means were also scored from the highest five and the highest 10 SCE counts available from the 50 metaphases scored.

Specimen and data collection, DNA isolation and ^{32}P-post-labeling

Blood samples (30 ml heparinized whole blood) were obtained from each subject and applied to Ficoll–Hypaque density gradients to separate mononuclear cells from erythrocytes and granulocytes. Frozen mononuclear cells were homogenized in 0.1 M Tris, 0.1 M NaCl, 50 mM EDTA pH 8.0 and 1% (v/v) SDS on ice and then extracted twice with equal volumes of chloroform–isoamyl alcohol (24:1). The aqueous supernatant was incubated with RNase A and RNase T1 (250 μg/ml, Sigma, St Louis, MO) at 37°C for 60 min followed by digestion with proteinase K (10 μg/ml, Merck) (37°C for 60 min). The digest was extracted twice with chloroform–isoamyl alcohol, after which sodium acetate (4.0 M final concentration) was added to the aqueous supernatant. DNA was precipitated with ethanol at –20°C and dissolved in 0.1× SSC. The quantity of DNA was determined by a fluorimetric method (Hoescht 33258; Hoefer, San Francisco, CA). Four micrograms of purified DNA were enzymatically digested to deoxynucleotide 3′-monophosphates (3′-dNps) with micrococcal nuclease (Worthington Biochemicals, Lakewood, NJ) and spleen phosphodiesterase. Samples were then treated with P1 nuclease. The modified nucleotides were converted into ^{32}P-labeled deoxynucleotide 3′,5′-diphosphates (3′,5′-dpNps) by incubation with 150 μCi [^{32}P]ATP (6000 Ci/mmol; NEN) and 2.5 μl T4 polynucleotide kinase. The total volume of ^{32}P-labeled 3′,5′-dpNps was applied to each 10×10 cm polyethyleneimine (PEI) cellulose plate.

Chromatographic conditions

Chromatograms were developed overnight with 0.4 M NaH_2PO_4 (pH 6.8) 16 cm into an attached paper wick, and an additional 5–6 h development in the same direction with 1.0 M lithium formate, 4.5 M urea (pH 3.5) without a wick. For development in the second dimension (D2, at 90° to the first), 0.36 M lithium chloride, 0.22 M Tris–HCl, 3.8 M urea (pH 8.0) were used for ~5–6 h, with 6 cm into a wick. A final chromatographic development, D3 with 1.7 M NaH_2PO_4 (pH 6.0) was carried out for 2–3 h and until the solvent front had migrated 6 cm into the wick.

Table I. Mean SCE frequencies and mean SCE ratios according to *XRCC1* and *ERCC2* genotypes and variables of interest

	n	Mean SCE frequency (95% CI[a])	Adjusted SCE ratio[b] (95% CI)
XRCC1 (codon 399)			
Arg/Arg	38	7.4 (7.0–7.7)	1.0 (referent)
Arg/Gln	29	7.4 (6.9–7.8)	1.0 (0.9–1.1)
Gln/Gln	0	8.0 (6.9–9.1)	1.1 (1.0–1.2)
ERCC2[c] (codon 751)			
Lys/Lys	29	7.5 (7.1–8.0)	1.0 (ref.)
Lys/Gln	36	7.3 (6.9–7.6)	1.0 (0.9–1.0)
Gln/Gln	11	7.8 (7.2–8.4)	1.0 (0.9–1.1)
GST-μ			
present	34	7.1 (6.8–7.5)	1.0 (ref.)
null	42	7.7 (7.3–8.0)	1.1 (1.0–1.1)
Active smoking			
never smoked	22	6.8 (6.4–7.2)	1.0 (ref.)
former smoker	35	7.4 (7.1–7.7)	1.1 (1.0–1.1)
current smoker	19	8.3 (7.7–8.9)	1.2 (1.1–1.3)
			P trend = 0.0006
Pack-years of smoking			
0 (never smoked)	22	6.8 (6.4–7.2)	1.0 (ref.)
<11	15	7.1 (6.8–7.5)	1.0 (0.9–1.1)
11–40	21	7.6 (7.1–8.2)	1.1 (1.0–1.2)
>40	18	8.2 (7.7–8.8)	1.2 (1.1–1.3)
			P trend = 0.0002
Years since quitting smoking			
n/a (never smoked)	22	6.8 (6.4–7.2)	1.0 (ref.)
>20	14	6.7 (6.4–7.1)	1.0 (0.9–1.0)
11–20	11	7.9 (7.5–8.3)	1.1 (1.0–1.3)
1–10	10	7.7 (7.0–8.5)	1.1 (1.0–1.3)
<1 year or is a current smoker	19	8.3 (7.7–8.9)	1.2 (1.1–1.3)
			P trend < 0.0001
Gender			
male	31	7.2 (6.8–7.7)	1.0 (ref.)
female	45	7.6 (7.3–7.9)	1.1 (1.0–1.1)
Age (years)			
<50	18	7.4 (6.5–7.5)	1.0 (ref.)
50–61	20	7.9 (7.4–8.3)	1.0 (1.0–1.1)
62–68	23	7.4 (6.8–8.0)	1.0 (0.9–1.1)
>68	15	7.0 (6.4–7.6)	1.0 (0.9–1.1)
Race			
Caucasian	71	7.4 (7.2–7.7)	1.0 (ref.)
other	5	7.8 (6.5–9.2)	1.0 (0.9–1.2)

[a]CI, confidence interval.
[b]SCE ratio based on multiple linear regression of log-transformed mean SCE frequency adjusted for active smoking, age, gender, GST-μ status and *XRCC1* status.
[c]A separate model was run with *ERCC2* in place of *XRCC1*.

Autoradiography and adduct quantification

Polyphenol DNA adducts were located by autoradiography using Kodak (Rochester, NY) XAR-5 film and a Dupont (Boston, MA) Chronex-Lightning Plus intensifying screen. The films were exposed at –70°C for 3–4 days. The areas of the radioactive spots on the PEI cellulose sheets were measured and the spots were then scraped into liquid scintillation vials containing 5 ml scintillation cocktail (Safety Solve; Research Products, Mt Prospect, IL); radioactivity was determined by liquid scintillation counting. Regions adjacent to the radioactive spots of equal area were scraped, placed into scintillation vials and counted for background determination. Adduct levels were corrected for background counts after adjusting for the area of the TLC sample. The level of modification was calculated as described. For example, assuming that 4 μg of DNA is 1.21×10^4 pmol of 3′-dNp and that the specific activity of the ^{32}P-ATP is 9.36×10^6 c.p.m./pmol, adduct levels were calculated as follows: relative adduct level = c.p.m. in adducts/11.32×10^{10} c.p.m. For each

Table II. Mean SCE frequencies and mean SCE ratios for the joint effects of smoking and DNA repair genes *XRCC1* and *ERCC2*

Smoking status	DNA repair gene[a]	*n*	Mean SCE frequency (95% CI[b])	Adjusted SCE ratio[c] (95% CI)	*P* value[d]
	XRCC1 (codon 399)				
Never smoked	Arg/Arg	14	6.7 (6.2–7.3)	1.0 (ref.)	–
	Arg/Gln + Gln/Gln	8	7.0 (6.1–7.8)	1.0 (0.9–1.2)	0.5
Former smoker	Arg/Arg	16	7.5 (7.1–7.9)	1.1 (1.0–1.2)	0.1
	Arg/Gln	14	7.3 (6.6–8.0)	1.1 (1.0–1.2)	0.2
	Gln/Gln	5	7.2 (6.4–8.0)	1.1 (1.0–1.3)	0.2
Current smoker	Arg/Arg	8	8.1 (7.2–8.9)	1.2 (1.1–1.3)	0.008
	Arg/Gln	8	7.9 (6.9–8.9)	1.2 (1.0–1.3)	0.02
	Gln/Gln	3	9.8 (8.0–12)	1.4 (1.2–1.7)	0.0004
			P value[e] = 0.0007		
	ERCC2 (codon 751)				
Never smoked	Lys/Lys	7	7.1 (6.4–7.8)	1.0 (ref.)	–
	Lys/Gln + Gln/Gln	15	6.7 (6.2–7.3)	1.0 (0.8–1.1)	0.5
Former smoker	Lys/Lys	14	7.3 (6.6–7.9)	1.0 (0.9–1.2)	0.7
	Lys/Gln	15	7.4 (7.0–7.8)	1.0 (0.9–1.2)	0.7
	Gln/Gln	6	7.6 (6.5–8.7)	1.1 (0.9–1.2)	0.5
Current smoker	Lys/Lys	8	8.4 (7.3–9.5)	1.2 (1.0–1.3)	0.05
	Lys/Gln	9	8.1 (7.1–9.2)	1.1 (1.0–1.3)	0.09
	Gln/Gln	2	8.3 (4.7–12)	1.2 (1.0–1.5)	0.09
			P value[e] = 0.002		

[a]Models for *XRCC1* and *ERCC2* were run separately.
[b]CI, confidence interval.
[c]SCE ratios based on multiple linear regression of log-transformed mean SCE frequency adjusted for age, gender and GST-μ.
[d]*P* value based on *t* test of null hypothesis that SCE ratio = 1.0.
[e]*P* value based on ANOVA *f* test.

experiment we ran a positive control sample of catechol- or benzenetriol-treated HL60 DNA. Each sample was run at least twice on different days and the relative adduct levels for all experiments were combined to obtain an average adduct level. All samples were coded and assayed blindly. Mean adduct levels were calculated for each individual and expressed as adducts per 10^{10} nucleotides.

XRCC1 genotyping

DNA was extracted from peripheral lymphocytes by standard methods (Qiagen, Valencia, CA). *XRCC1* genotypes were assayed using a PCR–RFLP assay. An Arg→Gln substitution in exon 10 (codon 399) was amplified to form an undigested fragment of 242 bp using the primer pair 5′-CCCCAAGTA-CAGCCAGGTC-3′ and 5′-TGTCCCGCTCCTCTCAGTAG-3′. Hot-start PCR was performed under the following conditions: denaturation at 94°C for 4 min followed by 35 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C, followed by 10 min at 72°C. *XRCC1* PCR product was digested with *Msp*I at 37°C overnight and resolved on 2% agarose. Arg/Arg genotypes were digested to form 94 and 148 bp fragments. The Arg→Gln change abolishes the *Msp*I restriction site.

ERCC2 genotyping

ERCC2 genotypes were detected using a PCR–RFLP assay. A Lys→Gln in exon 23 (codon 751) was amplified to form an undigested fragment of 184 bp using the primer pair 5′-CCCCCTCTCCCTTTCCTCTG-3′ and 5′-AACCAGGGCCAGGCAAGAC-3′. Hot-start PCR was performed under the following conditions: denaturation at 94°C for 4 min, followed by 30 cycles of 30 s at 94°C, 45 s at 59°C and 30 s at 72°C, followed by 10 min at 72°C. *ERCC2* PCR products were digested with *Mbo*II at 37°C for 1 h and resolved on 4% agarose. Lys/Lys genotypes were digested to form 72 and 112 bp fragments. The Lys→Gln change abolishes the *Mbo*II restriction site.

GST-μ genotyping and smoking history

The GST-μ genotyping assay has been described previously (19). Smoking variables used in analyses were based on active smoking (never smoked, former smoker, current smoker), duration of smoking in years, pack-years of smoking, years since quitting smoking, and number of cigarettes smoked per day (among current smokers).

Statistical analysis

Bivariate analyses were used to screen variables as potential confounders or effect modifiers of mean SCE counts and detectable/non-detectable DNA adducts. Linear regression analyses of mean SCE frequency were performed on log-transformed data. The SCE ratio was calculated by exponentiation of the parameter estimate from a linear regression model of log-transformed mean SCE frequency. The SCE ratio compares the geometric mean SCE frequency in an exposed group with that in a referent (unexposed) group, and can be thought of as the adjusted proportional change in the geometric mean SCE frequency between the two comparison groups. All final linear regression models of mean SCE frequency were evaluated for model fit using regression diagnostics including graphical displays of Cook's influence and Studentized residuals. The final multiple linear regression model of mean SCE was compared with and without one influential observation and found not to differ materially. Interaction was evaluated using joint variables (with a common referent group) for exposure and genotype (additive model), as well as interaction terms and stratification (multiplicative model).

A Wilcoxon rank sum test was used to compare mean polyphenol DNA adduct levels between genotypes. Presented geometric means of DNA adducts were calculated from $\log_e$-transformed data. In bivariate analyses of DNA adducts, Fisher exact tests were used if cell sizes were less than five. In multivariable logistic regression models, DNA adducts were analyzed as a dichotomous outcome variable (detected/undetected). Covariates (potential confounders) were kept in multivariable regression models if the β-coefficient for the exposure changed by >10% in a model without the covariate compared with a model with the covariate. Tests of trend were conducted by ordinal coding of explanatory variables and calculation of the *P* value for the β-coefficient from a logistic or linear regression model. Separate models of SCE frequencies and detectable polyphenol DNA adducts were run for *XRCC1* and *ERCC2*.

Results

Mean SCE frequency among all study subjects did not significantly depart from normality (skewness, 0.27; kurtosis, –0.21; Shapiro–Wilk *W*, 0.99; *P*, 0.6) but natural log transformation gave a slight improvement (skewness, –0.09; kurtosis, –0.42; Shapiro-Wilk *W*, 0.99; *P*, 0.9). Demographic characteristics of the 76 study subjects who participated in the SCE analysis have been described previously (22). Of the 76 study subjects, 71 were white and five were non-white. Arithmetic mean SCE frequencies (with 95% confidence intervals) and adjusted SCE ratios according to DNA repair genotypes and demographic variables of interest are presented in Table 1. All SCE ratios are mutually adjusted for the other variables in the model. Subjects who were homozygous 399Gln carriers in *XRCC1* had slightly higher mean SCE frequencies. Mean SCE frequen-

Table III. Levels of polyphenol DNA adducts according to *XRCC1* and *ERCC2* genotypes and variables of interest

	DNA adducts not detected (n = 38), n (%)	DNA adducts detected (n = 23)		
		n (%)	Geometric mean[a]	95% CI[b]
XRCC1 (codon 399)				
Arg/Arg	17 (46%)	8 (35%)	2.0	0.8–5.0
Arg/Gln	15 (41%)	10 (43%)	2.9	1.1–7.2
Gln/Gln	5 (13%)	5 (22%)	6.6	0.9–47.5
missing	1	0		
ERCC2 (codon 751)				
Lys/Lys	15 (39%)	12 (52%)	4.4	1.6–11.9
Lys/Gln	17 (45%)	8 (35%)	1.8	0.8–4.1
Gln/Gln	6 (16%)	3 (13%)	2.2	0.4–13.7
GST-μ				
present	19 (50%)	9 (39%)	2.0	0.9–4.4
null	19 (50%)	14 (61%)	3.7	1.6–8.8
Active smoking				
never smoked	7 (19%)	4 (17%)	2.7	0.5–14.2
former smoker	23 (62%)	15 (65%)	2.9	1.4–6.1
current smoker	7 (19%)	4 (17%)	2.8	0.5–14.7
missing	1	0		
Pack-years of smoking				
0 (never smoked)	7 (19%)	4 (17%)	2.7	0.5–14.2
<11	6 (16%)	3 (13%)	2.4	0.4–14.9
11–40	15 (41%)	12 (52%)	3.7	1.5–9.4
>40	9 (24%)	4 (17%)	1.9	0.5–7.1
missing	1	0		
Years since quitting smoking				
n/a (never smoked)	7 (19%)	4 (17%)	2.7	0.5–14.2
>20	7 (19%)	10 (43%)	6.6	2.0–21.3
11–20	7 (19%)	3 (13%)	2.0	0.4–10.5
1–10	9 (24%)	2 (9%)	1.1	0.3–3.8
<1 year or current smoker	7 (19%)	4 (17%)	2.8	0.5–14.7
missing	1	0		
Family history of cancer				
no	15 (43%)	6 (29%)	1.8	0.7–4.8
yes	20 (57%)	15 (71%)	3.4	1.6–7.4
missing	3	2		
Gender				
male	13 (35%)	16 (70%)	5.5	2.3–12.9
female	24 (65%)	7 (30%)	1.6	0.7–3.4
missing	1	0		
Age (years)				
<61	16 (43%)	3 (13%)	1.0	0.4–2.1
61–70	16 (43%)	6 (26%)	2.0	0.7–5.6
>70	5 (14%)	14 (61%)	12.8	4.7–34.8
missing	1	0		

[a]Geometric means were calculated from $\log_e$-transformed polyphenol DNA adduct data.
[b]CI, confidence interval.

cies and ratios did not differ by *ERCC2* genotype. GST-μ null genotype was associated with higher mean SCE levels, as previously reported by Cheng *et al*. (19). Smoking variables (including active smoking, pack-years and years since quitting) were the strongest predictors of higher mean SCE; these variables remained important even after adjustment for gender, age, GST-μ and *XRCC1*. Age, race (not shown) and family history of cancer (not shown) were unrelated to mean SCE frequency. Female gender was weakly associated with higher mean SCE frequency. Analyses of log mean SCE frequencies were also conducted using the mean SCE frequency derived from the five or 10 metaphases with the highest SCE counts (high frequency cells). No differences in the results (parameter estimates and mean SCE ratios) were seen (data not shown). In all analyses of SCE frequencies, unadjusted and adjusted ratios did not differ materially, so only adjusted ratios are presented in the tables.

Table II gives means and adjusted mean SCE ratios for the joint (additive) effects of smoking and polymorphic DNA repair genes *XRCC1* and *ERCC2*. In general, results of interactions based on active smoking (never smoked, former smoker, current smoker) did not differ substantially from those based on other measures of smoking (pack-years, duration and years since quitting) (data not shown). Thus, in the interest of precision, we present only results for interactions with active smoking. Among those who had never smoked, heterozygous and homozygous allele carriers were combined to increase sample size. Mean SCE frequencies and ratios were higher

Table IV. Odds ratios for polyphenol DNA adducts and DNA repair gene polymorphisms

	Undetected adducts (n = 38)	Detected adducts (n = 23)	Unadjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
XRCC1 (codon 399)				
Arg/Arg	17	8	1.0 (ref.)	1.0 (ref.)
Arg/Gln	15	10	1.4 (0.4–4.5)	4.0 (0.8–21.9)
Gln/Gln	5	5	2.1 (0.5–9.5)	6.0 (0.7–52.2)
			P trend[c] = 0.3	P trend = 0.07
ERCC2 (codon 751)				
Lys/Lys	15	12	1.0 (ref.)	1.0 (ref.)
Lys/Gln	17	8	0.6 (0.2–1.8)	0.3 (0.06–1.3)
Gln/Gln	6	3	0.6 (0.1–3.0)	1.2 (0.2–8.2)
			P trend = 0.4	P trend = 0.6
Age (years)/*XRCC1* (joint variable)				
<65, Arg/Arg	11	2	1.0 (ref.)	–
<65, Arg/Gln+Gln/Gln	13	3	1.3 (0.2–9.0)	–
⩾65, Arg/Arg	6	6	5.5 (0.8–35.9)	–
⩾65, Arg/Gln+Gln/Gln	6	12	11.0 (1.8–66.9)	–
			P trend = 0.002	

[a]OR, odds ratio; CI, confidence interval.
[b]Adjusted for age, gender and family history of cancer.
[c]Test for trend in dose-response.

among current smokers who were homozygous carriers of the 399Gln allele in *XRCC1* (mean 9.8, ratio 1.4) than in Arg/Arg current smokers (mean 8.1, ratio 1.2). Mean SCE frequencies and ratios among current smokers did not vary by *ERCC2* genotype. Mean SCE frequencies and ratios by gender, age, race, family history of cancer and GST-μ did not differ by *XRCC1* or *ERCC2* genotypes (data not shown).

To investigate whether the polymorphism in *XRCC1* at codon 399 potentiates the effect of the joint exposure of GST-μ genotype and smoking, we evaluated interaction terms between *XRCC1* genotype and a variable for the joint exposure of active smoking status and GST-μ genotype [six categories: never smoked/GSTM1+ (referent group), never smoked/GSTM1–, former smoker/GSTM1+, former smoker/GSTM1–, current smoker/GSTM1+, current smoker/GSTM1–]. There were too few subjects to separate the three genotypes for *XRCC1*, so heterozygous individuals were combined with those homozygous for the 399Gln allele to increase statistical power. Age- and sex-adjusted mean SCE ratios for combined smoking/GSTM1 groups were only slightly higher among *XRCC1* 399Arg/Gln + Gln/Gln genotypes [1.0 (referent), 1.2, 1.2, 1.2, 1.4, 1.3] than for the Arg/Arg genotype [1.0 (referent), 1.0, 1.1, 1.2, 1.2, 1.2]. We did not observe any differences in mean SCE ratios for combined smoking/GSTM1 stratified by *ERCC2* genotypes. Because of sample size constraints, we were unable to evaluate the joint effects of GST-μ null and DNA repair genotypes and biomarkers of DNA damage.

The distribution of polyphenol DNA adducts was skewed in this sample (skewness, 2.5; kurtosis, 7.6; Shapiro–Wilk W, 0.62; $P < 0.0001$), with only 38% of subjects (n = 23) having detectable DNA adducts. Of the 61 subjects assayed for DNA adducts, 59 were Caucasian and two were non-Caucasian (data not shown). Geometric means of polyphenol DNA adducts and frequency of undetected and detected adducts are presented in Table III. Means of polyphenol DNA adducts showed a weak positive trend with the presence of one or more copies of the *XRCC1* 399Gln allele. Mean adduct levels were slightly higher among GST-μ-null genotypes than among GST-μ-positive genotypes. Smoking (active status, pack-years, years since quitting) was unrelated to mean levels of DNA adducts or to the detection of adducts. Mean adduct levels were slightly higher among those subjects who reported a family history of any cancer. In bivariate analyses of polyphenol DNA adducts as a continuous variable, only greater age (three categories, Wilcoxon rank sum test $P = 0.002$) and male gender (Wilcoxon rank sum test $P = 0.04$) were significantly associated with higher levels of this class of DNA adducts.

Unadjusted and adjusted odds ratios for detection of polyphenol DNA adducts and polymorphisms in *XRCC1* and *ERCC2* are presented in Table IV. Detection of polyphenol DNA adducts was positively associated with one or more copies of the *XRCC1* 399Gln allele, but not the *ERCC2* 751Gln allele. Odds ratios for detection of DNA adducts and *XRCC1* genotype became more pronounced after adjustment for the effects of age, gender and family history of cancer. A trend test of odds ratios for detectable DNA adducts and *XRCC1* genotype gave a P value of borderline significance ($P = 0.07$). Because the nature of polyphenol DNA adducts is believed to be endogenous, and because we detected an effect of age on this class of adducts, we evaluated the potential interaction of *XRCC1* genotype and age using an additive model. Unadjusted odds ratios and 95% confidence intervals (CI) for detection of polyphenol DNA adducts and the joint effect of age and *XRCC1* genotype are presented in Table IV. Due to small sample size, age was dichotomized at the sample median (<65 years, ⩾65 years) and *XRCC1* 399Arg/Gln genotypes were combined with 399Gln/Gln genotypes; furthermore, only unadjusted odds ratios are presented. The relationship between age and the *XRCC1* 399Gln allele was more than additive [11.0 > (1.3 + 5.5 – 1.0)]. Joint variables for smoking/*XRCC1* and smoking/GST-μ were not associated with the presence of detectable polyphenol DNA adducts (data not shown).

Discussion

We investigated the relationship between polymorphisms in the BER gene *XRCC1* and the NER gene *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells from healthy

subjects. Our results suggest that *XRCC1* (in addition to cigarette smoking and GST-μ) may contribute to baseline SCE frequency. In a small subset of subjects, we found evidence that mean SCE frequencies among current smokers homozygous for the *XRCC1* 399Gln allele were greater than those in current smokers with the 399Arg/Arg genotype. While these findings are based on small numbers, they are consistent with the results of Lunn *et al*. (3) who found more DNA damage (aflatoxin DNA adducts and somatic glycophorin A variants) among carriers of the same allele (399Gln) in *XRCC1*.

Because oxidative DNA damage is repaired via BER-related processes, we analyzed polyphenol DNA adducts and polymorphisms in *XRCC1* (as well as *ERCC2*). Our results suggest that the *XRCC1* allele 399Gln may be associated with higher DNA adduct levels and the presence of detectable adducts, a result consistent with the proposed role of *XRCC1* in BER of oxidative DNA damage. Lunn *et al.* (3) found a gene dosage effect with the same *XRCC1* allele and (detectable/undetectable) aflatoxin DNA adducts. When, however, the authors evaluated aflatoxin adduct levels, 399Gln allele carriers (homozygous plus heterozygous) were more likely to have intermediate, rather than higher, levels of adducts. In the present study, mean DNA adduct levels among individuals with two copies of the *XRCC1* 399Gln allele (6.6 adducts/10^{10} nucleotides) were higher compared with those in heterozygous (2.9 adducts/10^{10} nucleotides) and Arg/Arg (2.0 adducts/10^{10} nucleotides) individuals, but differences were not statistically significant (Wilcoxon rank sum test $P = 0.17$, comparing Gln/Gln with Arg/Arg individuals). The association of age with DNA adducts and the potential interaction of age with *XRCC1* genotype supports the endogenous nature of these modifications and a possible role for *XRCC1* and BER in clearing DNA adducts at higher endogenous concentrations that may occur with greater age. Together, these findings suggest a role for *XRCC1* in removal of DNA damage in human mononuclear blood cells represented by SCEs and polyphenol DNA adducts.

The mechanism responsible for the association of the *XRCC1* 399Gln allele and higher levels of baseline DNA damage is unknown. However, the 399Gln allele is located within the central BRCT domain of *XRCC1*, which contains a binding site for PARP and is conserved in several proteins involved in DNA damage repair, cell cycle control, and recombination (8,25). In Chinese hamster ovary cells lines with non-conservative amino acid substitutions within the BRCT domain, reduced repair of single-strand breaks and hypersensitivity to ionizing radiation has been observed (26). Similar cell lines deficient in *XRCC1* exhibit elevated spontaneous SCE frequency (5, 6). This is the second report to evaluate DNA damage phenotypes and the 399Gln allele in *XRCC1*. Our results, together with those of Lunn *et al.* (3), suggest that this polymorphism in *XRCC1* may be important in more than one type of DNA damage phenotype, and that further mechanistic studies of this protein are needed. Our results also suggest that the polymorphism in *ERCC2* has no phenotypic effect on SCE frequencies or the presence of polyphenol DNA adducts.

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