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XRCC1 Polymorphisms: Effects on Aflatoxin B_1-DNA Adducts and Glycophorin A Variant Frequency

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Abstract

Hereditary genetic defects in DNA repair lead to increased risk of cancer. Polymorphisms in several DNA repair genes have been identified; however, the impact on repair phenotype has not been elucidated. We explored the relationship between polymorphisms in the DNA repair enzyme, XRCC1 (codons 194, 280, and 399), and genotoxic end points measured in two populations: (*a*) placental aflatoxin B_1 DNA (AFB_1-DNA) adducts in a group of Taiwanese maternity subjects ($n = 120$); and (*b*) somatic glycophorin A (GPA) variants in erythrocytes from a group of North Carolina smokers and nonsmokers ($n = 59$). AFB_1-DNA adducts were measured by ELISA, and erythrocyte GPA variant frequency (*NN* and *NØ*) was assessed in MN heterozygotes with a flow cytometric assay. *XRCC1* genotypes were identified by PCR-RFLPs. The *XRCC1 399Gln* allele was significantly associated with higher levels of both AFB_1-DNA adducts and GPA *NN* mutations. Individuals with the *399Gln* allele were at risk for detectable adducts (odds ratio, 2.4; 95% confidence interval, 1.1–5.4; $P = 0.03$). GPA *NN* variant frequency was significantly higher in 399Gln homozygotes (19.6×10^{-6}) than in *Gln/Arg* heterozygotes (11.4×10^{-6}; $P < 0.05$) or *Arg/Arg* homozygotes (10.1×10^{-6}; $P = 0.01$). No significant effects were observed for other *XRCC1* polymorphisms. These results suggest that the Arg399Gln amino acid change may alter the phenotype of the XRCC1 protein, resulting in deficient DNA repair.

Introduction

Hereditary genetic defects in DNA repair lead to a marked increased risk of developing cancer. Although DNA repair deficiency often arises from mutations in genes that result in a loss of the DNA repair protein, DNA polymorphisms may alter the structure of the DNA repair enzyme and modulate cancer susceptibility. Mutations and polymorphisms have been identified in many of the genes coding for DNA repair enzymes such as *XRCC1*[2] (1–3). *XRCC1* was identified by its ability to restore DNA repair activity in the Chinese hamster ovary cell lines, EM-9 and EM-11, which are hypersensitive to ionizing radiation and alkylating agents (4–6). These cells have increased spontaneous and mutagen-induced sister chromatid exchange and have defects in rejoining single-strand breaks after exposure to X-ray (6, 7). Both EM-9 and EM-11 cells contain a mutated *XRCC1* gene and lack XRCC1 protein (8).

Ionizing radiation and alkylating agents cause DNA base damage and strand breaks that elicit BER. The XRCC1 protein complexes with DNA ligase III via a BRCT domain in its COOH terminus and with DNA polymerase β via the XRCC1 NH_2 terminus domain to repair gaps left during BER (9). PARP detects DNA strand breaks induced by ionizing radiation and is believed to participate in BER (10). XRCC1 negatively regulates PARP by binding to it via the XRCC1 central domain (amino acids 301–402; Ref. 11). This central region also includes a BRCT domain and shares homology to the yeast *rad4/cut5* DNA repair gene (11, 12). Functional importance of this region is also suggested by the determination that the DNA repair-deficient EM-11 cell line contains a cysteine-to-tyrosine mutation at codon 390 (8).

Shen *et al.* (1) identified three coding polymorphisms in the *XRCC1* gene at codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln). These polymorphisms code for nonconservative amino acid changes (including the Arg399Gln change in the PARP binding domain), which suggests potential functional relevance, but their impact on phenotype is unknown. We tested whether *XRCC1* polymorphisms were associated with higher levels of genotoxic damage and found that the *399 Gln* allele was significantly associated with higher levels of AFB_1-DNA adducts and GPA somatic mutations.

Materials and Methods

Subjects. AFB_1-DNA adducts and *XRCC1* genotypes were assessed in 120 placental DNA samples obtained from uncomplicated pregnancies at Taipei Chang Gung Memorial Hospital as described by Hsieh and Hsieh (13). Sixty placentas were collected during high (summer) and low (winter) exposure season. GPA VF was measured in erythrocyte samples obtained from 49 smokers and 10 nonsmokers (17 blacks and 42 whites) heterozygous for the GPA antigen. The subjects were part of a community-based sample comprised of 294 healthy unrelated blacks and whites from Durham and Chapel Hill, North Carolina. This sample population has been used in other genotyping and exposure studies (14, 15). *XRCC1* genotypes were determined using genomic DNA isolated from lymphocytes. One hundred and ten additional whites and 81 additional blacks from the same community sample were included in the genotyping studies to compare *XRCC1* allele frequency in different ethnic groups.

Detection of AFB_1-DNA Adducts. AFB_1-DNA adduct levels were measured by competitive ELISA using monoclonal antibody 6A10 and 50 μg of DNA as described previously (16). The percent of inhibition was calculated by comparison with the nonmodified heat-denatured calf thymus DNA control. DNA samples were quantified relative to an imidazole ring-opened AFB_1-DNA standard, which has a modification level of 4 adducts/10^5 nucleotide. Values below 20% inhibition, corresponding to 0.5 μmol/mol DNA, were considered not detectable. Each sample was measured in triplicate on three different assay dates and had a variability of less than 10%.

Measurement of GPA Variants in Erythrocytes. Blood samples were typed for the M and N antigens using commercial sera (Ortho Diagnostics, Raritan, NJ) to determine MN heterozygous individuals. Fifty-nine individuals were identified and assessed for variants (*NØ* and *NN*) using the BR6 version of the GPA assay as described previously (17, 18). A total of 5×10^6 erythrocytes were analyzed for each sample.

***XRCC1* Genotyping.** *XRCC1* genotypes were detected using a PCR-RFLP technique. A multiplex PCR was used to amplify 491 bp and 615 bp of DNA fragments containing the codon 194 and 399 polymorphisms, respectively. Primers were: (*a*) 26106F gcc ccg tcc cag gta and 26577R agc ccc aag acc ctt

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[2] The abbreviations used are: *XRCC1*, X-ray repair cross-complementing 1; BER, base excision repair; BRCT, BRCA1 COOH terminus; PARP, poly (ADP-ribose) polymerase; AFB_1, aflatoxin B_1; GPA, glycophorin A; OR, odds ratio; CI, confidence interval; VF, variant frequency; LS, least square(s).

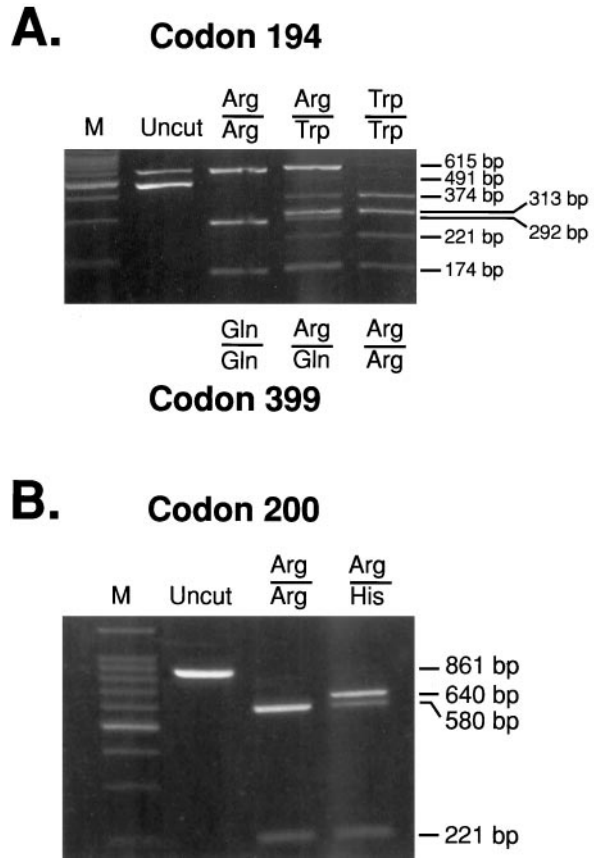

Fig. 1. *A*, 3% Metaphor gel on *Msp*I digestion of the multiplex *XRCC1* PCR products containing codons 194 and 399. *Codon 194* and *399* genotypes are indicated *above* and *below the Lanes*, respectively. *Uncut*, the two undigested products (491-bp and 615-bp, codons 194 and 399, respectively). Representative wild-type homozygotes (*Arg/Arg* and *Arg/Arg*, 194 and 399, respectively), heterozygotes (*Arg/Trp* and *Arg/Gln*, 194 and 399 respectively), and variant homozygotes (*Trp/Trp* and *Gln/Gln*, 194 and 399, respectively) are shown for each polymorphism. *M*, 100-bp molecular weight standard (Promega). *B*, 2% NuSieve gel on *Rsp*I digestion of *XRCC*1 PCR product containing codon 280. *Uncut*, depicts the 861-bp PCR product. Wild-type homozygotes (*Arg/Arg*) and heterozygotes (*Arg/His*) are shown.

tca ct for codon 194; and (*b*) 27776F ttg tgc ttt ctc tgt gtc ca and 28371R tcc tcc agc ctt ttc tga ta for codon 399. A separate PCR using primers 27405F ttg acc ccc agt ggt gct aa and 28247R cgc tgg gac cac ctg tgt t were used to amplify the 861-bp DNA fragment containing the codon 280 polymorphism. PCR conditions for both methods consisted of 50 ng of genomic DNA, 3 mM $MgCl_2$, 200 μM each dNTPS, 0.5 units Taq (Promega, Madison, WI) + TaqStart Antibody (Sigma, St. Louis, MO), and either 0.6 μM (codon 194) or 0.8 μM (codon 280 and 399) each primer in 1× PCR buffer (Promega). PCR program was a 4-min denaturation step at 94°C followed by 30 cycles of 30 s at 94°C and 90 s at 68°C. The *Arg* allele at codon 194 and the *Arg* allele at codon 399 both create *Msp*I sites. The multiplex 491-bp and 615-bp PCR products (codons 194 and 399, respectively) were digested at 37°C for 2 h and resolved on 3% Metaphor agarose gels (FMC Bioproducts, Rockland, ME; see Fig. 1*A*). A 174-bp fragment was present in all of the samples because of an invariant *Msp*I site (in the 491-bp fragment) that served as an internal control for complete digestion. The *Arg*/*Arg*, *Arg*/*Trp*, and *Trp*/*Trp* genotypes for codon 194 resulted in 21-bp and 292-bp; 21-bp, 292-bp, and 313-bp; and 313-bp digestion products, respectively. The *Gln* allele (codon 399) was distinguished from the *Arg* allele as an undigested fragment (615-bp) compared with the 221- and 374-bp digested fragments of the *Arg* allele. *Rsp*I digestion of the 861-bp PCR containing codon 280 was incubated separately at 37°C for 2 h. The digestion fragments—60-bp, 221-bp, 580-bp, and 640-bp—were separated on 2% 3:1 NuSieve agarose gels (FMC Bioproducts; see Fig. 1*B*). The *Arg* allele creates a *Rsp*I site at nucleotide 27466 and results in the 580-bp and 60-bp products that are not recognized by the allele but is contained in the 640-bp fragment. The 221-bp fragment is a result of an invariant *Rsp*I site present in all of the samples.

Statistical Analysis. Allele frequencies were estimated, and differences between the various ethnic groups (blacks, whites, and Taiwanese) were tested by pair-wise comparisons of χ^2 2 × 2 contingency table analysis. The association between *XRCC1* alleles and AFB_1-DNA adducts was evaluated by traditional 2 × *k*-table analysis (OR and 95% CI) and by a logistic regression model controlling for season (adjusted OR and 95% CI). We evaluated the effect of genotype on GPA variants by estimating the LS mean GPA VF (*NN* and *NØ*) for each genotype, using an analysis of covariance model that adjusted for age and smoking status.

Results and Discussion

Estimated genotype and allele frequencies for *XRCC1* polymorphisms in black, white, and Taiwanese population samples are given in Table 1. All of the distributions were in Hardy-Weinberg equilibrium. The *194Trp* and *280His* alleles were at a low frequency in blacks [0.05 (*194Trp*) and 0.02 (*280His*)] and whites [0.06 (*194Trp*) and 0.03 (*280His*)] but were significantly more prevalent in Taiwanese [0.27 (*194Trp*) and 0.11 (*280His*) $P < 0.001$]. The *399Gln*-allele frequency was significantly different among all of the three populations with the *Gln* allele occurring the highest in whites (0.37), intermediate in Taiwanese (0.26) and lowest in blacks (0.17; Table 1). Shen *et al.* (1) estimated variant allele frequency of 0.25 (*194Trp*), 0.08 (*280His*) and 0.25 (*399Gln*) by sequencing the *XRCC1* gene from 12 unidentified individuals. These frequencies are most similar to that observed in the Taiwanese population (Table 1).

To investigate whether the *XRCC1* polymorphisms were associated with differences in DNA repair, which might be reflected in levels of genotoxic damage, we compared *XRCC1* genotypes with levels of AFB_1-DNA adducts (Tables 2 and 3) and GPA somatic mutations (Table 4). AFB_1-DNA adducts in placental samples obtained from 120 healthy Taiwanese women were measured by competitive ELISA, and the data were previously reported (13). The ELISA assay detects AFB_1-adduct levels greater than 0.5 μmol/μmol DNA. Individuals with genotypes containing the *399Gln* allele were more likely to have detectable AFB_1-DNA adducts (OR, 2.4; 95% CI, 1.1–5.4; $P = 0.03$;

Table 1 *XRCC1 genotype and allele frequency among Taiwanese and North Carolinian (NC) populations of whites (W) and blacks (B)*

	NC community population			
XRCC1 Polymorphisms	Whites (n = 169)	Blacks (n = 98)	Taiwanese (n = 120)	Reported allele frequency (n = 12)[a]
Exon 6, Codon 194				
Arg/Arg	150 (89%)	89 (91%)	67 (56%)	
Arg/Trp	18 (11%)	9 (9%)	42 (35%)	
Try/Trp	1 (0.5%)	0 (0%)	11 (8%)	
Allele frequency (*Trp*)	0.06[b,c,d]	0.05[c,d]	0.27[d]	0.25
Exon 9, Codon 280				
Arg/Arg	159 (94%)	94 (96%)	95 (79%)	
Arg/His	10 (6%)	4 (4%)	23 (20%)	
His/His	0 (0%)	0 (0%)	2 (2%)	
Allele frequency (*His*)	0.03[c,d]	0.02[c,d]	0.11[d]	0.08
Exon 10 Codon 399				
Arg/Arg	65 (38%)	67 (69%)	63 (53%)	
Arg/Gln	83 (49%)	27 (28%)	51 (43%)	
Gln/Gln	21 (13%)	3 (3%)	6 (4%)	
Allele frequency (*Gln*)	0.37[d]	0.17[d]	0.26[d]	0.25

[a] From Shen *et al.* (1).
[b] Allelic distribution for codon 194, 280, and 399 genotypes in Blacks (B), Whites (W), and Taiwanese (T) sample populations were in Hardy Weinberg equilibrium.
[c] Allele frequencies for W *versus* B were not significantly different: codon 194, $P = 0.7$; codon 280, $P = 0.7$.
[d] Allele frequency for the following were significantly different: codon 194, W *versus* T, B *versus* T, $P < 0.0001$; codon 280, W *versus* T, B *versus* T, $P < 0.0005$; codon 399, W *versus* B, $P < 0.0001$; codon 399, W *versus* T, B *versus* T, $P < 0.05$.

Table 2 *The association between AFB_1–DNA adducts and XRCC1 polymorphisms*

	Nondetectable adducts (n = 51)	Detectable adducts (n = 69)	Crude OR[a]	95% CI; P	Adjusted OR[b]	95% CI; P
Exon 6, codon 194						
Arg/Arg	25	42	1.0[c]		1.0[c]	
Arg/Trp	21	21	0.6	0.3–1.4; 0.27	0.5	0.2–1.2; 0.12
Trp/Trp	5	6	0.7	0.2–3.1; 0.86	0.7	0.2–2.6; 0.61
Arg/Trp + Trp/Trp	26	27	0.6	0.3–1.3; 0.19	0.6	0.3–1.2; 0.14
Exon 9, codon 280						
Arg/Arg	41	54	1.0[c]		1.0[c]	
Arg/His	9	14	1.2	0.4–3.3; 0.91	1.1	0.4–2.9; 0.82
His/His	1	1	0.8	0–61.5; 0.60	0.7	0–12.9; 0.84
Arg/His + His/His	10	15	1.1	0.4–3.1; 1.0	1.1	0.4–2.7; 0.87
Exon 10, codon 399						
Arg/Arg	33	30	1.0[c]		1.0[c]	
Arg/Gln	17	34	2.2	1.0–5.1; 0.07	2.7	1.2–6.1; 0.017
Gln/Gln	1	5	5.5[d]	0.6–131; 0.21	6.4	0.7–60.8; 0.11
Arg/Gln + Gln/Gln	18	39	2.4	1.1–5.4; 0.03	2.9	1.3–6.4; 0.008

[a] Fisher exact OR.
[b] OR adjusted for season, calculated by logistic regression.
[c] Reference group.
[d] Test for trend $\chi^2 = 5.1$; $P = 0.024$.

Table 2). Moreover, a gene-dosage effect was observed (test for trend, $X^2 = 5.12$; $P = 0.024$). That is, individuals homozygous for the *399Gln* allele had a higher risk of having detectable AFB_1-DNA adducts (OR, 5.5; 95% CI, 0.6–131; $P = 0.2$) than heterozygous individuals (OR, 2.2; 95% CI, 1.1–5.1; $P = 0.07$). However, the number of homozygous individuals was very small ($n = 6$), limiting the interpretation of this finding. No statistically significant association was observed between the detection of AFB_1-DNA adducts and the *194Trp* or *280His* alleles; however, individuals carrying a *194Trp* allele were slightly more common in the nondetectable AFB_1-DNA adduct group (OR, 0.6; 95% CI, 0.3–1.3; $P = 0.19$). A larger study may help determine whether this possible difference is real or is due to chance. As reported by Hsieh and Hsieh (13), AFB_1-DNA adducts were observed to be higher during the summer than the winter. The association between DNA damage and the *399Gln* allele was similar for AFB_1-DNA adducts detected in samples collected in the summer (OR, 2.7; (95% CI, 0.7–11.3; $P = 0.16$) and winter (OR, 3.0; 95% CI, 1.0–10.2; $P = 0.04$; data not shown). Adjusting for seasonal variation did not substantially modify the ORs for the association of *XRCC1* genotypes and AFB_1-DNA adducts (Table 2).

We examined the relationship of *XRCC1* genotypes and different levels of AFB_1-DNA adducts (Table 3). Detectable AFB_1-DNA ad-

Table 3 *Codon 399 polymorphisms and AFB_1-DNA adducts stratified by level of adducts*

AFB_1–DNA adduct level	Codon 399 genotype		OR[a]	95% CI; P	OR[b]	95% CI; P
	Arg/Arg	*Arg/Gln + Gln/Gln*				
Nondetectable[c]	33	18	1.0[d]		1.0[d]	
Intermediate[c]	11	23	3.8	1.4–10.7; 0.004	5.2	1.9–14.3; 0.002
High[c]	19	16	1.5	0.6–4.1; 0.38	1.5	0.9–2.4; 0.1
Detectable (intermediate + high)	30	39	2.4	1.1–5.4; 0.03	2.9	1.3–6.4; 0.008

[a] Fisher exact OR.
[b] OR adjusted for season, calculated by logistic regression.
[c] Nondetectable, values are below the limit of detect for the assay; Intermediate, measurable values were less than or equal to the median (2.1 μmol/mol DNA); High, values greater than the median level of adducts.
[d] Reference group.

Table 4 *The association of GPA NN and NØ variants and XRCC1 genotypes*

	Mean GPA *NØ* VF (per 10^6 cells)									Mean GPA *NN* VF (per 10^6 cells)								
	All subjects[a]			Smokers[b]			Nonsmokers[b]			All subjects[a]			Smokers[b]			Nonsmokers[b]		
Genotypes	N	LS mean	SE[c]	N	LS mean	SE[c]	N	LS mean	SE[c]	N	LS mean	SE[c]	N	LS mean	SE[c]	N	LS mean	SE[c]
Exon 6, Codon 194																		
Arg/Arg	55	13.6	0.9	45	12.9	0.9	10	16.9	2.9	55	11.6	1.0	45	11.1	1.1	10	13.5	2.7
Arg/Trp	4	13.1	3.5	4	12.9	3.2	0			4	10.4	4.0	4	11.0	3.9	0		
Trp/Trp	0			0			0			0						0		
Exon 9, Codon 290																		
Arg/Arg	56	13.6	0.9	47	12.9	0.9	9	17.1	3.2	56	11.5	1.0	47	11.1	1.1	9	13.3	3.0
Arg/His	3	13	3.9	2	13.3	4.3	1	15.0	9.7	3	11.8	4.5	2	11.1	5.3	1	15.2	9.0
His/His	0			0			0			0			0					
Exon 10, Codon 399																		
Arg/Arg	31	13.3	1.2	26	12.6	1.2	5	18.1	4.8	31	10.1[d]	1.3	26	10.0[d]	1.4	5	12.1	4.4
Arg/Gln	22	13.9	1.4	19	13.3	1.4	3	16.1	6.0	22	11.4[e]	1.5	19	10.6[e]	1.6	3	15.5	5.5
Gln/Gln	6	13.5	2.8	4	12.4	3.1	2	14.9	7.9	6	19.6[d,e]	3.0	4	21.0[d,e]	3.5	2	14.1	7.2

[a] Adjusted for smoking and age.
[b] For smokers or nonsmokers adjusted for age.
[c] Standard error of the mean.
[d] $P < 0.01$, *Gln/Gln versus Arg/Arg*.
[e] $P < 0.05$, *Gln/Gln versus Arg/Gln*.

ducts levels ranged from 0.6 to 6.3 μmol/mol DNA with a median of 2.1 μmol/mol DNA. We classified individuals as nondetectable (≤ 0.5 μmol/mol DNA), intermediate (> 0.5 and ≤ 2.1 μmol/mol DNA), and high (> 2.1 μmol/mol DNA). Individuals with genotypes containing the *399Gln* allele were more likely to have an intermediate level of adducts (OR, 3.8; 95% CI, 1.4–10.7; $P = 0.004$) than a high level of adducts (OR, 1.5; 95% CI, 0.6–6.4; $P = 0.38$). No significant association occurred between *XRCC1* polymorphisms at codon 194 and 280 and adducts (data not shown).

AFB_1 mediates its carcinogenicity mainly through the formation of AFB_1-guanine adducts. These highly unstable adducts can either form more stable ring-opened structures or undergo spontaneous depurination, resulting in apurinic sites and eliciting BER (19–21). Administration of AFB_1 in rats causes single-strand breaks and increases PARP, DNA ligase, and DNA polymerase β enzyme activity (20). These enzymes interact with XRCC1 during BER, suggesting that XRCC1 may be important in the repair of AFB_1-DNA adducts. Thus, the association of AFB_1-DNA adducts and the *399Gln* allele is biologically plausible. Moreover, codon 399 is located in the PARP-binding region of the *XRCC1* gene. The specific functional effect of the Arg399Gln change on XRCC1 binding with PARP remains to be explored.

Although the *399Gln* allele of *XRCC1* was related to the detection of AFB_1-DNA adducts, the effect seems to be greatest at lower adduct levels (Table 3). Possibly, in tissues with higher levels of AFB_1-DNA adducts, the BER pathway may become saturated, which would tend to reduce differences between functional and less functional alleles. A similar phenomena has been observed in rodent exposure studies in which very high levels of AFB_1-induced DNA damage resulted in a decline of PARP activity. (20).

Differences in AFB_1-DNA adducts also reflect differences in exposure. External exposure information was not available for the subjects so that the direct effects of adduct repair cannot be assessed. *In vitro* or *in vivo* studies using a fixed AFB_1 exposure should provide insight into the effect of genotype on the repair of AFB_1-DNA adducts.

We also examined the relationship of *XRCC1* genotypes and somatic mutations characterized by the GPA assay (Table 4). The GPA assay detects two types of allele loss variants *NØ* (allele loss) and *NN* (allele loss and duplication) present in erythrocytes. We measured the VF of both *NØ* and *NN* mutations in smokers and nonsmokers who were heterozygous for GPA. The mean VF (*NØ* and *NN*) was estimated for individuals with the different *XRCC1* genotypes (codons 194, 280, and 399) using a LS regression model adjusting for smoking and age (Table 4). Smoking did not affect VF for either *NØ* ($P = 0.13$) or *NN* ($P = 0.31$). Age was associated with increased *NN* VF ($P = 0.005$) but not *NØ* VF ($P = 0.26$). The LS mean *NN* VF was highest in individuals with two *Gln* alleles (*Gln*/*Gln*, 19.6×10^{-6}), intermediate with one *Gln* allele (*Arg*/*Gln*, 11.4×10^{-6}), and lowest with no *Gln* alleles (*Arg*/*Arg*, 10.1×10^{-6}). Differences in LS mean VF were significant ($P < 0.05$) when compared with the *Gln*/*Gln* genotype. The association between the 399 genotypes and mean GPA VF was greater in smokers than in nonsmokers. *NØ* VF was similar in all of the 399 genotypes. The *194Trp* allele and *280His* allele did not significantly affect either *NØ* or *NN* VF; however, these alleles are rare in the GPA study population (whites and blacks), which limits the interpretation of this negative finding.

GPA VF is a marker of DNA damage and increases after exposure to ionizing radiation, benzene, chemotherapy, and other mutagens (22, 23). Individuals with diseases of DNA repair and metabolisms, such as ataxia telangiectasia and Bloom Syndrome, have significant elevations in GPA VF, thus implicating it as a marker of exposure, damage, and cancer risk (22). *NØ* and *NN* variants arise from independent molecular mechanisms (22). Gene inactivation mechanisms such as point mutations, deletions, and chromosome loss are likely to result in *NØ* variants, whereas mitotic recombination, gene conversion, and chromosome missegregation are more important in generating *NN* variants (23). The relationship of *399Gln* allele to *NN* but not *NØ* variants may result from a greater role of the central domain of *XRCC1* in recombination repair than repair of lesions that cause gene inactivation. Both PARP and XRCC1 participate in DNA strand-break rejoining and homologous recombination (7, 24–26). Cells without the *XRCC1* gene and mice lacking the *PARP* gene exhibit high levels of sister chromatid exchange, which suggests increased recombination activity (7, 26). PARP inhibitors cause an increase in recombination frequency and genomic instability (27), and XRCC1 expression is elevated during male meiosis in the mouse, which implies a role in meiotic recombination (28).

This is the first report to investigate associations between phenotype (measures of genotoxic damage) and three missense polymorphisms—194(Arg to Trp), 280 (Arg to His), and 399 (Arg to Gln)—in the *XRCC1* gene. We find evidence to suggest that the *XRCC1 399Gln* allele is associated with increased levels of DNA damage that may be due to reduced DNA repair function. Individuals with the *Gln* allele were more likely to have higher levels of AFB_1-DNA adducts and GPA *NN* somatic variants. BER is important in the repair of AFB_1-DNA adducts, whereas errors in recombination may generate GPA *NN* variants. *XRCC1* is implicated in both of the repair processes. Moreover, the *Arg399Gln* polymorphism occurs in a region of the *XRCC1* gene that contains biologically important domains (PARP binding and BRCT) and has homology with another DNA repair-related gene (yeast *rad4/cut5* gene). Future studies need to characterize the role of the *XRCC1 399Gln* allele in functional DNA repair assays and to test to see whether it affects the levels of other biomarkers of DNA damage.

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