Functional Characterization of Polymorphisms in DNA Repair Genes Using Cytogenetic Challenge Assays

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A major barrier to understanding the role of polymorphic DNA repair genes for environmental cancer is that the functions of variant genotypes are largely unknown. Using our cytogenetic challenge assays, we conducted an investigation to address the deficiency. Using X-rays or ultraviolet (UV) light, we irradiated blood lymphocytes from 80 nonsmoking donors to challenge the cells to repair the induced DNA damage, and we analyzed expression of chromosome aberrations (CA) specific to the inducing agents. We have genotyped polymorphic DNA repair genes preferentially involved with base excision repair (BER) and nucleotide excision repair (NER) activities (*XRCC1, XRCC3, APE1, XPD*) corresponding to the repair of X-ray– and UV light–induced DNA damage, respectively. We expected that defects in specific DNA repair pathways due to polymorphisms would cause corresponding increases of specific CA. From our data, *XRCC1* 399Gln and *XRCC3* 241Met were associated with significant increases in chromosome deletions compared with the corresponding homozygous wild types (18.27 ± 1.1 vs 14.79 ± 1.2 and 18.22 ± 0.99 vs 14.20 ± 1.39, respectively); *XPD* 312Asn and *XPD* 751Gln were associated with significant increases in chromatid breaks compared with wild types (16.09 ± 1.36 vs 11.41 ± 0.98 and 16.87 ± 1.27 vs 10.54 ± 0.87, respectively), $p < 0.05$. The data indicate that *XRCC1* 399Gln and *XRCC3* 241Met are significantly defective in BER, and the *XPD* 312Asn and *XPD* 751Gln are significantly defective in NER. In addition, the variant genotypes interact significantly, with limited overlap of the two different repair pathways. *Key words:* challenge assay, chromosome aberrations, DNA damage, DNA repair, DNA repair gene polymorphisms, genetic susceptibility. *Environ Health Perspect* 111:1843–1850 (2003). doi:10.1289/txg.6632 available via *http://dx.doi.org/* [Online 6 October 2003]

On a daily basis, endogenous and exogenous agents induce cellular DNA damage. If not repaired, the damage can interfere with important cellular functions and can cause serious health problems such as cancer. Therefore, a variety of DNA repair processes such as the base excision repair (BER), nucleotide excision repair (NER), and mismatch and double-strand break repairs have evolved to perform the critical repair functions (Dybdahl et al. 1999; Friedberg 2003). The BER pathway is involved in the repair of DNA damage caused by a variety of internal and external factors including ionizing radiation, alkylating agents, and oxidation. XRCC1 and Ape1 enzymes play important roles in the BER pathway. The Ape1 protein incises the phosphodiester backbone of DNA immediately 5′ to the baseless lesion, leaving a strand break with a normal 3′-hydroxyl group and a nonconventional 5′-abasic terminus (Wilson and Barsky 2001). XRCC1 acts as a scaffold for other DNA repair proteins such as DNA polymerase β and DNA ligase II (reviewed by Caldecott 2003). The XRCC3 protein functions in the homologous DNA double-strand break repair pathway and directly interacts with and stabilizes Rad51 (Bishop et al. 1998). NER is the major pathway in humans for the removal of cyclopyrimidine dimers and 6–4 photoproducts produced by ultraviolet (UV) light and a wide variety of bulky lesions formed by chemical agents (Friedberg et al. 1995). XPD proteins are involved in the NER pathway. They stabilize the transcription factor complex TFIIH and have 5→3′ DNA helicase activity (Lehmann 2001).

Mutations that affect the function of DNA repair enzymes are rare in the human population because they can cause serious health consequences. However, with the advent of the human genome program, variations in DNA sequences of repair genes were discovered recently (Shen et al. 1998). The surprise was that the frequencies of the variant gene alleles, based on single nucleotide polymorphisms, reached the polymorphism level in the population. In addition some of the polymorphisms may not be innocuous variations because the alterations can be predicted to cause the substitution of amino acids in the repair enzymes, presumably altering their repair functions (Mohrenweiser and Jones 1998; Shen et al. 1998). However, the functions of the variant genotypes have not yet been well characterized.

The discovery of DNA repair gene polymorphism has stimulated tremendous interest in research to determine if the variant genotypes are associated with cancer. Significant associations with lung cancer, head and neck cancer, and bladder cancer have been reported (Butkiewicz et al. 2001; Spitz et al. 2001; Stern et al. 2002; Sturgis et al. 2002; Tomescu et al. 2001; Zhou et al. 2003). However, a high number of the observations were not consistent with each other (reviewed by Benhamou and Sarasin 2002; Goode et al. 2002; Hu et al. 2002). In addition unexpected observations were reported. For example, inheritance of *XPA* variant alleles was associated with reduced risk for lung cancer (Wu et al. 2003). In association with the variant genotypes for *XRCC1* and *ERCC3*, the risk for lung cancer decreased as the pack-years of smoking increased (Zhou et al. 2003). There have been many proposed explanations to address the discrepancies. The consistent recommendation is that the functional consequences of the polymorphisms need to be characterized.

We have conducted an investigation to elucidate DNA repair function of certain variant genotypes using our cytogenetic challenge assays (Au 1993; Au et al. 1991; El Zein et al. 1995). The challenge assays have been validated to indicate abnormal DNA repair responses to X rays and to UV light on the basis of studies using the host cell reactivation assay and patients with skin cancer susceptibility (El Zein et al. 1995; Hallberg et al. 1997). Specifically, we have selected two groups of polymorphic DNA repair genes preferentially involved with BER and NER activities that correspond to the repair of X rays and UV light–induced DNA

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damage, respectively. The relationship between variant genotypes and the expression of X rays and UV light–induced chromosome aberrations (CA) in normal human lymphocytes was investigated. Our assumption is that defects in specific DNA repair pathways would lead to a corresponding increase in specific CA. The data indicate that *XRCC1* 399Gln and *XRCC3* 241Met are associated with defects in BER, and *XPD* 312Asn and *XPD* 751Gln with NER.

Materials and Methods

Recruitment of Donors

In this study we recruited volunteers who were healthy and had a presumably stable lifestyle. Therefore, we advertised for healthy males and females who were in the middle age group (35–40 years of age), regardless of ethnicity. Potential volunteers were informed about the objectives of the study, the need to donate a blood sample, and the risk and benefit from participation in the study. Study participants filled out a questionnaire for lifestyle information and medical history and signed consent forms, according to the approved protocol from the University Institutional Review Board. Exclusion criteria were smoking of cigarettes, cigars, and pipes; previous exposure to radiation or hazardous chemicals; on medication; and history of cancer or from cancer families. Each qualified volunteer was asked to donate a single blood sample.

Laboratory Reagents and Cell Cultures

Standard laboratory culture reagents were purchased from Gibco Laboratory (Grand Island, NY) and from Murex Biotech (Dartford, UK). The primers for genotyping, DNA *Taq* polymerase, and restriction enzymes were purchased from Sigma-Aldrich (St. Louis, MO).

Blood specimens were collected into Vacutainer tubes (Sigma-Aldrich) containing sodium heparin as an anticoagulant. The specimens were labeled with a predetermined code and transported to the laboratory as soon as possible after collection. Blood cultures were set up according to the standard procedures in our laboratory (Au et al. 1991). The cultures were normally set up within 2 hr from the time blood samples were drawn from the donors. The culture medium was made up of RMPI 1640 medium that was supplemented with 10% heat-inactivated fetal bovine serum, 2% phytohaemagglutinin, 100 U/mL penicillin, 100 µg/mL streptomycin, and L-glutamine. Cultures were maintained in a water-jacketed CO_2 incubator set up at 37.5°C.

The X-Ray Challenge Assay

The challenge assay used in this study was developed in our laboratory and discussed in detail elsewhere (Au 1993; Au et al. 1991). For this assay, blood cultures were set up by placing 0.5 mL whole blood into culture tubes that each contained 4.5 mL culture medium as described above (Au et al. 1991). A Mark I cesium-137 pneumatic irradiator with a dose rate of approximately 80 cGy/min was used for the irradiation (J.L. Shephard, Glendale, CA). The cells were irradiated in the culture tubes 24 hr after culture initiation and the irradiation dose was 100 cGy. Fifty hours after culture initiation, cells were harvested using the standard Colcemid blocking procedure (Au et al. 1991).

UV-Light Challenge Assay

The UV challenge assay was described in our previous publication (El Zein et al. 1995). Under UV irradiation conditions, target cells need to be irradiated as a monolayer and without overlap from the other cell types. Therefore, lymphocytes were isolated from whole blood using Histopaque 1077 (Sigma-Aldrich) and used to set up the cultures (500,000 cells/mL in 5 mL culture, using the same culture medium described earlier). Twenty-four hours after initiation of the lymphocyte cultures, the culture tubes were centrifuged to pack cells. The supernatant medium was removed and saved for reuse after the irradiation. The packed cells from each culture were resuspended in 2 mL sterile saline and put into a 100-mL Petri dish for irradiation. With the small volume of saline, the cells were therefore spread out into a thin layer with limited overlapping of cells. The source of UV light was a 15-W short-wave tube that produced a peak of intensity of 1,100 $\mu W/cm^2$ of 254 nm at 15 cm distance. The irradiation dose was 4 J/m^2 for 4 sec. The irradiation was performed in a lamina flow hood with the lids of the Petri dishes removed. After the irradiation, cells were resuspended in their own growth medium saved earlier and allowed to grow for an additional 26 hr before harvesting.

Cell Harvesting

Before harvest, cells were blocked with Colcemid (final concentration 0.1 µg/mL) for 1.5 hr. After removal of the culture medium, cells were treated with hypotonic solution (0.075 M KCl) and fixed with Carnoy's fixative (methanol and glacial acetic acid at 3:1 ratio). Cytological preparations were made, coded, and stained with 10% Giemsa solution for 15 min. The stained slides were air dried, and a cover slip was then put onto each slide. Under the microscope, metaphase cells were located and analyzed for the presence of CA (Au et al. 1991). Fifty metaphase cells were analyzed for every exposure condition and the data are expressed as percentages. One individual did all analyses using coded slides.

DNA Isolation and Genotyping

Genomic DNA was extracted from isolated lymphocytes by a standard nonorganic procedure (Miller et al. 1988). The extracted DNA was used for characterization of the following polymorphic DNA repair genes. These polymorphic genes were chosen because they reportedly are associated with several environmental cancers (Butkiewicz et al. 2001; Misra et al. 2003; Smith et al. 2003; Sturgis et al. 2002; Tomescu et al. 2001; Zhou et al. 2003). Polymerase chain reaction (PCR), followed by restriction fragment length polymorphism, was used for genotyping. All genotype assays were performed twice, and the repeat analyses were conducted independent of each other. Only concordant findings from these analyses were accepted.

For determination of polymorphism *XRCC1* Arg194Trp, 100 ng genomic DNA was amplified in a total volume of 50 µL containing 0.2 µM of the following primer pairs: forward, 5′-GCCCCGTCCCAGGTA-3′, reverse, 5′-AGC CCC AAG ACCC TTT-3′, 1× PCR buffer (150 mM Tris-HCl, pH 0.8, 500 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate (dNTP), and 1 U *Taq* polymerase. The PCR amplification condition consisted of initial denaturation step at 95°C for 2 min, followed by 40 cycles of 94°C for 15 sec, 57°C for 45 sec, 72°C for 45 sec, and final extension step at 72°C for 5 min. The PCR products (490 bp) were digested overnight with the restriction enzyme *PvuII.* The restricted products of *XRCC1* codon 194 Arg/Arg, Arg/Trp, and Trp/Trp genotypes had band sizes of 490, 490/294/196, and 294/196 bp, respectively (Hu et al. 2001).

For the *XRCC1* Arg399Gln genotyping, 100 ng genomic DNA was amplified in a total reaction volume of 50 µL containing 0.2 µM of each of the forward primer, 5′-CAAGTACAGCCAGGTCC-TAG-3′, reverse primer, 5′-CCTTCC-CTCA TCTGGAGTAC-3′, 1× PCR buffer (150 mM Tris-HCl, pH 0.8, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, and 1 U *Taq* polymerase. The PCR amplification condition consisted of initial denaturation step at 95°C for 2 min, followed by 40 cycles of 94°C for 15 sec,

55°C for 30 sec, 72°C for 45 sec, and final extension step at 72°C for 5 min. The 248-bp PCR products were digested with NciI (Promega, Madison, WI); the Arg allele was cut into 89 and 159 bp fragments (Gln allele not digested) (Matullo et al. 2001).

Polymorphism in exon 10 of *XPD*, which contains G/A, *Asp312Asn*, was determined using the method described by Spitz et al. (2001). The oligonucleotide primers 5′-CTGTTGGTGGGTGCCCGTATCT-GTTGGTCT-3 (bases 22872–22901 of *XPD*) and 5′-TAATATCGGGGCTCAC-CCTGCAGCACTTCCT (bases 23592–23616 of *XPD*) were used. PCR was performed in 50-µL reaction mixtures containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 3% dimethyl sulfoxide, 0.2 µM primers, 100 ng template DNA, and 1.5 units *Taq* polymerase in 1× PCR buffer [10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, and 0.1% Triton X-100]. After an initial denaturation at 94°C for 4 min, the DNA was amplified by 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and then by a final extension step of 5 min at 72°C. The PCR product was digested with *Sty*I for 8 hr at 37°C. The digestion products were then resolved on a 3% agarose gel containing ethidium bromide. The homozygous wild-type (Asp/Asp) was identified by two DNA bands (507 and 244 bp), the homozygous mutant type (Asn/Asn) produced three bands (474, 244, and 33 bp), and heterozygotes (Asp/Asn) displayed all four bands (507, 474, 244, and 33 bp).

The *XPD* Lys751Gln polymorphism, a transversion A→C in exon 23 (position 35931), was determined using the primers (forward) 5′-CTGCTCAGCCTGGAG-CAGCTAGA ATCAGAGGACGCTG-3′ and (reverse) 5′-AAGACCTTCTAGCAC-CACCG-3′. The PCR condition consisted of initial denaturation step at 95°C for 2 min, followed by 40 cycles of 94°C for 15 sec, 67°C for 30 sec, 72°C for 45 sec, and final extension step at 72°C for 5 min. The 161-bp PCR product was digested with PstI (Promega); the Gln allele was cut into 41- and 120-bp fragments (Lys allele not digested).

The *XRCC3* Thr241Met polymorphism was determined using the primers (forward) 5′-GCCTGGTGGTCATC-GACTC-3′ and (reverse) 5′-ACAGG-GCTCTGGAAGGCACTGCTCAGCTC ACGCAC̲C-3′ (underlined base modifies primer sequence introducing a cut site in the presence of the Met allele). The PCR condition consisted of initial denaturation step at 95°C for 2 min, followed by 40 cycles of 94°C for 15 sec, 60°C for 30 sec, 72°C for 45 sec, and final extension step at 72°C for 5 min. The 136-bp PCR product was digested with NcoI (Promega); the Met allele was cut into 39- and 97-bp fragments (Thr allele not digested) (Matullo et al. 2001).

For *XRCC3* genotyping,the *XRCC3* Thr241Met polymorphism, a T→C transition in exon 7 (position 18067) was determined using the following primers: sense, 5′-GCCTGGTGGTCATCGACTC-3′; antisense, 5′-ACAGGGCTCTGGAAG-GCACTGCTCAGCTCACGCAC̲C-3′. The PCR conditions consisted of 100 ng genomic DNA, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.5 U *Taq*, and 250 nM of each primer in 1× PCR buffer. The PCR program was as follows: a 3-min denaturation step at 94°C, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by final extension step at 72°C for 5 min. The 136-bp PCR product was digested with *Nco*I restriction enzyme at 37°C for 6 hr; the Met allele was cut into 39- and 97-bp fragments (Thr allele not digested) (Matullo et al. 2001).

For *APE1* genotyping (Hu et al. 2001), the polymorphism in *APE1*, exon 5, T/G, 148 Asp/Glu, was determined using the following primers: forward, 5′-CTGTTTCATTTCTATAGGCTA-3′; reverse, 5′-AGGAACTTGCGAAAG-GCTTC-3′. About 100 ng genomic DNA in a total volume of 50 µL was amplified by PCR. The reaction mixture consisted of PCR buffer (150 mM Tris–HCl, pH 8.0, 500 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, and 1 U *Taq* polymerase. PCR conditions were 95°C for 2 min, followed by 40 cycles of 94°C for 15 sec, 57°C for 45 sec, 72°C for 45 sec, and a final elongation step at 72°C for 5 min. The 64-bp PCR product was digested with the *Bfa*I restriction enzyme at 37°C for 6 hr. The restricted products of *APE1* codon 148 Asp/Asp, Asp/Glu, and Glu/Glu genotypes are represented by band sizes of 164, 164/144/20, and 144/20 bp, respectively.

Statistical Analysis

The genotype and chromosome data were collected by two individuals and entered into a spreadsheet data file without further modification and used for statistical analyses by a third individual. All statistical tests were performed with the software SPSS for Windows (version 10; SPSS Inc., Chicago, IL). CA was expressed as mean ± standard error of the mean (SE). Statistical significance was determined using analysis of variance (ANOVA), followed by Bonferroni's correction for multiple comparisons when the overall *F*-test was significant. CA frequency was further compared using stratified analysis by genotype after adjusting for potential confounders in a general linear model. An alpha error (*p*) of less than 0.05 was used as the criterion of significance. Significant levels (*p*-values) correspond to two-sided tests.

Results

Characteristics of the Study Population

A total of 80 subjects participated in the current study. The use of donated blood specimens for the various assays was determined purely based on the availability of blood from the donors. Whole-blood lymphocyte cultures from 61 donors were used to investigate the effect of DNA repair polymorphisms in X-ray–induced CA. Isolated blood lymphocyte cultures from 49 donors were used to investigate DNA repair polymorphisms in UV-induced CA. As indicated in Table 1, there is no significant difference in age, gender, and distribution of the different genotypes between the entire study population and the two subpopulations. The genotype data were based on two independent and concordant determinations of each genotype. The distribution of the genotypes is not consistent with Hardy-Weinberg equilibrium. This observation is probably due to our limited sample size of a highly selective population. However, our investigation focuses on individual responses based on genotype composition of each individual and is not based on distribution of genotypes in the population. Therefore, the Hardy-Weinberg equilibrium condition does not affect the significance of our investigation.

Effect of X Rays and DNA Repair Gene Polymorphisms on Chromosome Aberrations

Irradiation of the whole-blood cultures from 61 donors with X rays resulted in the induction of different types of CA. Chromatid-type aberrations such as breaks and exchanges and chromosome-type aberrations such as deletions and dicentrics were observed. The frequencies of these aberrations together with aberrant cells indicating the percentage of cells that contained any types of CA are summarized in Table 2. Because X rays preferentially induced chromosome-type aberrations such as deletions and dicentrics (Au et al. 2001), these aberrations were more frequently observed than chromatid-type breaks and exchanges (Table 2). Consequently, the aberrant cells category has high frequency as well. As shown in the table, *XPD* 312 Asn and *XPD* 751Gln are associated with

slightly reduced aberration frequencies in most categories, with a reduction in dicentrics for the combined heterozygous and homozygous variant *XPD* 312Asn and *XPD* 751Gln. The reduction in dicentrics corresponded to the 2-fold increase of another type of translocation, chromatid exchanges (Table 2). Conversely, the *XRCC1*, *XRCC3*, and *APE1* variant genotypes are associated with increased aberrations in most categories. Significant increases were observed for *XRCC1* 194Try in chromatid exchanges, *XRCC1* 399Gln in aberrant cells and deletions, and *XRCC3* 241Met in deletions. Besides the mentioned significant differences, some variations in CA frequencies across the study population were observed, but these are normal observations in population studies. The variations are probably influenced by the presence of other polymorphic genotypes.

Table 1. Selected characteristics of the study population.

Variable	All subjects, *n* (%)	X rays, *n* (%)	UV, *n* (%)
All subjects	80	61	49
Age, years (mean ± SE)	34.90 ± 0.82	34.51 ± 0.89	34.96 ± 0.99
Gender			
Female	15 (19)	14 (23)	12 (24)
Male	65 (81)	47 (77)	37 (76)
XPD-312			
Asp/Asp	40 (50)	34 (56)	27 (55)
Asp/Asn	34 (43)	23 (38)	18 (37)
Asn/Asn	6 (7)	4 (6)	4 (8)
XPD-751			
Lys/Lys	39 (49)	30 (49)	26 (53)
Lys/Gln	32 (40)	25 (41)	17 (35)
Gln/Gln	09 (11)	06 (10)	06 (12)
XRCC1-194			
Arg/Arg	54 (68)	43 (71)	31 (63)
Arg/Trp	24 (30)	18 (29)	18 (37)
Trp/Trp	2 (2)	0 (0)	0 (0)
XRCC1-399			
Arg/Arg	32 (40)	24 (39)	19 (39)
Arg/Gln	37 (46)	26 (43)	22 (45)
Gln/Gln	11 (14)	11 (18)	08 (16)
XRCC3-241			
Thr/Thr	27 (34)	20 (33)	17 (35)
Thr/Met	48 (60)	37 (61)	29 (59)
Met/Met	5 (6)	4 (6)	3 (6)
APE1-148			
Asp/Asp	42 (53)	31 (51)	25 (51)
Asp/Glu	37 (46)	29 (47)	23 (47)
Glu/Glu	1 (1)	1 (2)	1 (2)

Effect of Ultraviolet Light and DNA Repair Gene Polymorphisms on Chromosome Aberrations

Irradiation of the isolated lymphocyte cultures from 49 donors with UV light resulted in the induction of CA that can be classified as aberrant cells, chromatid breaks, chromatid exchanges, and chromosome deletions (Table 3). Dicentrics, a chromosome-type translocation, were rarely observed; therefore, this type of abnormality was not meaningful for further evaluation. Chromatid-type aberrations such as breaks and exchanges are preferentially induced by UV light (Au et al. 2001); therefore, their frequencies are high and the aberrant cells category also has high frequencies. As shown in the table, *XPD* 312Asn and *XPD* 751Gln are consistently associated with increased CA in every evaluated category (Table 3). Specifically, the variant genotypes are significantly associated with increases in the aberrant cells and chromatid breaks categories. The variant genotypes for *XRCC1*, *XRCC3*, and *APE1* are associated with increased CA in the most observed category (Table 3). However, significant association was observed only in the aberrant cells category for the *XRCC1* 194Trp. As mentioned earlier, normal variations in CA frequencies were observed.

Table 2. Effect of X-ray exposure and DNA repair gene polymorphisms on chromosome aberrations.

	Chromosome aberration type (mean ± SE)[a]				
Genotype (*n*)	Aberrant cells	Chromatid breaks	Chromatid exchanges	Deletions	Dicentrics
XPD-312					
Asp/Asp (34)	27.50 ± 1.20	1.88 ± 0.30	0.65 ± 0.22	16.94 ± 1.10	12.21 ± 0.93
Asp/Asn + Asn/Asn (27)	25.15 ± 1.40	1.56 ± 0.33	1.33 ± 0.68	16.85 ± 1.31	09.85 ± 0.74
XPD-751					
Lys/Lys (30)	27.50 ± 1.27	1.93 ± 0.36	0.67 ± 0.22	17.17 ± 1.20	12.47 ± 1.01
Lys/Gln + Gln/Gln (31)	25.45 ± 1.29	1.55 ± 0.26	1.23 ± 0.60	16.65 ± 1.19	09.90 ± 0.69*
XRCC1-194					
Arg/Arg (43)	26.21 ± 1.11	1.58 ± 0.26	0.51 ± 0.15	16.74 ± 1.03	11.70 ± 0.74
Arg/Trp + Trp/Trp (18)	27.06 ± 1.61	2.11 ± 0.41	2.00 ± 1.01*	17.28 ± 1.47	09.88 ± 1.17
XRCC1-399					
Arg/Arg (24)	24.17 ± 1.33	1.58 ± 0.34	0.92 ± 0.27	14.79 ± 1.20	10.21 ± 1.01
Arg/Gln + Gln/Gln (37)	27.95 ± 1.17*	1.84 ± 0.29	0.97 ± 0.51	18.27 ± 1.10*	11.83 ± 0.80
XRCC3-241					
Thr/Thr (20)	24.45 ± 1.67	2.00 ± 0.44	0.60 ± 0.26	14.20 ± 1.39	10.45 ± 0.95
Thr/Met + Met/Met (41)	27.44 ± 1.06	1.61 ± 0.25	1.12 ± 0.46	18.22 ± 0.99*	11.55 ± 0.82
APE1-148					
Asp/Asp (31)	25.00 ± 1.16	1.61 ± 0.30	0.65 ± 0.23	15.81 ± 1.11	10.61 ± 0.85
Asp/Glu + Glu/Glu (30)	27.97 ± 1.37	1.87 ± 0.33	1.27 ± 0.61	18.03 ± 1.24	11.79 ± 0.94

[a]Expressed as aberrations per 100 cells; aberrant cells contain cells that have any types of aberrations. *$p < 0.05$, ANOVA.

Specificity of Genotypes on Chromosome Aberrations

By plotting the experimental data from Tables 2 and 3 into graphic formats, we were able to visualize better the genotype-specific effects on CA. The graphic formats also allowed us to hypothesize interactions among the different genotypes. We found that variant gene alleles for BER (*XRCC1* 194Trp, *XRCC1* 399Gln, *XRCC3* 241Met, and *APE1* 148Glu) but not those for NER (*XPD* 312Asn and *XPD* 751Gln) are consistently associated with defective repair of X-ray–induced DNA damage. The most revealing case involves the expression of increased chromosome deletions preferentially induced by X rays (Figure 1). Based on the figure, we hypothesized that the *XRCC* and *APE* variant genotypes would interact with each other but not with *XPD* variant genotypes. The analyses of the interactions support our hypothesis (Table 4). More important, gene dosage effects were documented. Additional interactions of genotypes (in combinations of threes) were not evaluated because the sample size for each group was too small to be meaningful.

Table 3. Effect of UV exposure and DNA repair gene polymorphisms on chromosome aberrations.

Genotype (*n*)	Chromosome aberration type (mean ± SE)[a]			
	Aberrant cells	Chromatid breaks	Chromatid exchanges	Chromosome breaks
XPD-312				
Asp/Asp (27)	09.04 ± 0.68	11.41 ± 0.98	0.44 ± 0.19	0.07 ± 0.07
Asp/Asn + Asn/Asn (22)	12.82 ± 1.02*	16.09 ± 1.36*	1.00 ± 0.37	0.27 ± 0.15
XPD-751				
Lys/Lys (26)	08.46 ± 0.56	10.54 ± 0.87	0.38 ± 0.19	0.15 ± 0.11
Lys/Gln + Gln/Gln (23)	13.30 ± 0.99*	16.87 ± 1.27*	1.04 ± 0.35	0.17 ± 0.12
XRCC1-194				
Arg/Arg (31)	09.60 ± 0.76	12.80 ± 1.18	0.53 ± 0.21	0.13 ± 0.08
Arg/Trp + Trp/Trp (18)	12.53 ± 1.05*	14.63 ± 1.26	0.95 ± 0.39	0.63 ± 0.14
XRCC1-399				
Arg/Arg (19)	10.63 ± 1.05	12.84 ± 1.40	0.53 ± 0.34	0.30 ± 0.16
Arg/Gln + Gln/Gln (30)	10.80 ± 0.83	13.93 ± 1.13	0.80 ± 0.25	0.07 ± 0.07
XRCC3-241				
Thr/Thr (17)	11.06 ± 1.06	13.65 ± 1.67	0.94 ± 0.39	0.35 ± 0.19
Thr/Met + Met/Met (32)	10.56 ± 0.82	13.44 ± 1.02	0.56 ± 0.22	0.06 ± 0.06
APE1-148				
Asp/Asp (25)	10.40 ± 0.89	13.52 ± 1.26	0.64 ± 0.28	0.15 ± 0.11
Asp/Glu + Glu/Glu (24)	11.08 ± 0.95	13.50 ± 1.24	0.75 ± 0.29	0.17 ± 0.12

[a]Expressed as aberrations per 100 cells; aberrant cells contain cells that have any types of aberrations. *$p < 0.05$, ANOVA.

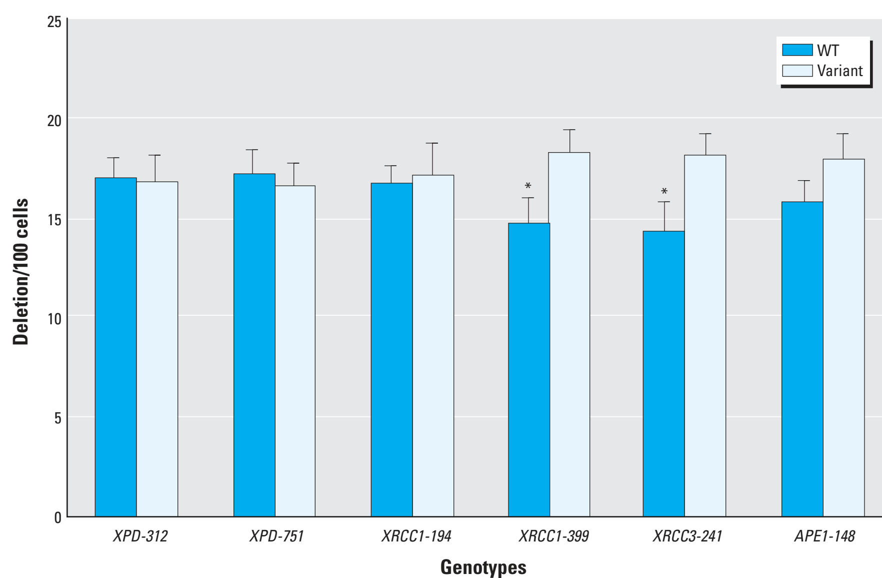

Figure 1. Differential repair of X-ray–induced damage by polymorphic DNA repair genes as indicated by the chromosome deletion frequencies.
*Significant difference between the variant and WT genotypes, $p < 0.05$.

As shown in Figure 2, the data indicate that the variant genotypes for NER genes (*XPD* 312Asn and XPD 751Gln) are consistently associated with defective repair of UV light–induced DNA damage, leading to significant increase of chromatid breaks, the aberrations preferentially induced by UV light. Conversely, the BER genes (*XRCC* and *APE*) are not significantly or consistently involved with the repair of this type of damage. Interactive effects of variant genotypes in the repair of UV light–induced DNA damage were further investigated. As shown in Table 4, the only significant interactions were observed with the *XPD* variant genotypes and for chromatid breaks preferentially induced by UV light only. Significant gene–dosage effects were detected. Furthermore, there was no significant interaction between the BER and the NER genes.

Discussion

The results from ongoing investigations into the relationship between polymorphisms in DNA repair genes and susceptibility to environmental cancer have not yet produced consistent results (Benhamou and Sarasin 2002; Goode et al. 2002; Mohrenweiser et al. 2003). However, the inconsistency is most likely due to the complexity of the biological process and of molecular epidemiological investigations. A complementary approach to investigate the role of these polymorphic genes in human disease is to use biomarkers to conduct functional studies. Functional investigations using biomarkers still need to be interpreted carefully on the basis of their experimental design. For example, the use of nonsmoking healthy volunteers would allow us a better chance to elucidate the function of the gene alleles than the use of cigarette smokers or lung cancer patients because the latter conditions could interfere with gene functions. Furthermore, different biomarkers provide different quality of data for understanding the biological mechanisms leading to disease (Bonassi and Au 2002). For example, induction of CA and gene mutations involves the contribution of a spectrum of repair processes, and the data can be used to indicate increased risk for cancer. On the other hand, the presence of DNA adducts is used as a biomarker of exposure, and the extent of DNA repair in modifying the adduct levels is not yet clear.

In our study we observed that *XPD* 312Asn is associated with significant increase in UV light–induced CA (aberrant cells and cells with chromatid breaks, shown in Table 3) but with no increase of

Table 4. Effect of exposure and combined DNA repair gene polymorphisms on chromosome aberrations.

Genotypes						X Rays	UV
XPD-312	*XPD-751*	*XRCC1-194*	*XRCC1-399*	*XRCC3-241*	*APE1-148*	Mean[a] ± SE (*n*)	Mean[a] ± SE (*n*)
							Chromatid breaks
WW	WW						9.96 ± 1.18 (20)
WM or MM	WW						13.79 ± 2.16 (6)
WW	WM or MM						15.57 ± 2.03 (7)
WM or MM	WM or MM						16.93 ± 1.32 (16)*
							$p = 0.002$[b]
						Deletions	
		WW	WW			12.33 ± 1.72 (14)	
		WM or MM	WW			18.14 ± 1.99 (10)	
		WW	WM or MM			18.85 ± 1.17 (29)*	
		WM or MM	WM or MM			16.28 ± 2.24 (8)	
						$p = 0.021$[b]	
						Deletions	
			WW	WW		12.42 ± 2.04 (10)	
			WM or MM	WW		16.00 ± 2.02 (10)	
			WW	WM or MM		16.50 ± 1.70 (14)	
			WM or MM	WM or MM		19.10 ± 1.22 (27)*	
						$p = 0.041$[b]	
						Deletions	
				WW	WW	13.91 ± 2.03 (10)	
				WM or MM	WW	16.65 ± 1.41 (21)	
				WW	WM or MM	14.60 ± 2.02 (10)	
				WM or MM	WM or MM	19.81 ± 1.43 (20)	
						$p = 0.066$[b]	

Abbreviations: MM, mutant homozygous; WM, wild-type/mutant heterozygous; WW, wild-type homozygous.
[a]Mean values are adjusted by age and gender in the general linear model. [b]*p*-Value based on ANOVA test. *$p < 0.05$ compared with WW + WW genotype, based on post-ANOVA testing with Bonferroni's correction for multiple comparisons. Interactions among the other combinations of genotypes were either not significant (see also Figures 1 and 2) or not meaningful because the sample sizes were too small.

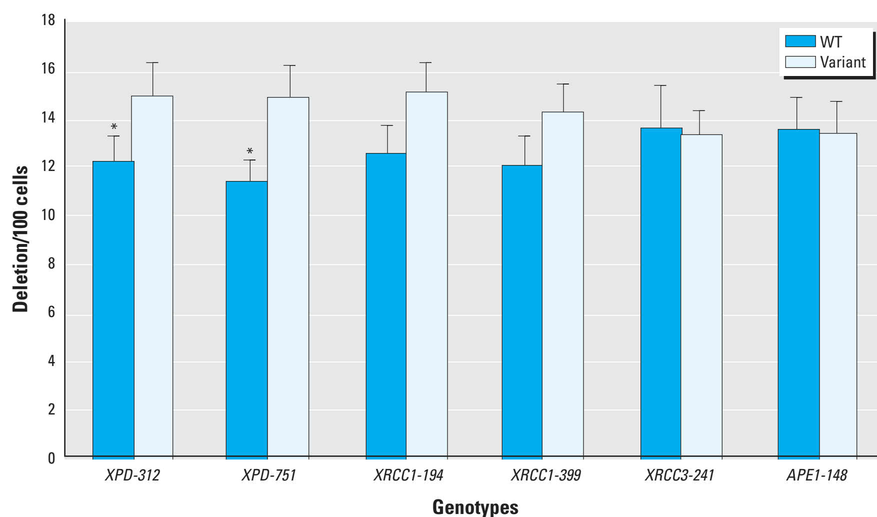

Figure 2. Differential repair of UV light–induced damage by polymorphic DNA repair genes as indicated by the chromatid break frequencies.
*Significant difference between the variant and WT genotypes, $p < 0.05$.

CA from X-ray–irradiated cultures. This indicates specific defects in NER. The chromosome data are consistent with the involvement of the same genotype with a nonsignificant increase of transversion *p53* gene mutation in lung cancer patients (Hou et al. 2003) but not with DNA adducts levels in lymphocytes of lung cancer patients (Pastorelli et al. 2002). Similarly, our observation of specific DNA repair defects in *XPD* 751Gln is consistent with the significant increase of DNA adducts among healthy nonsmokers (Matullo et al. 2001) and with increased transversion *p53* mutation in lung cancer patients (Hou et al. 2003) but not with changes in DNA adducts in lymphocytes of lung cancer patients (Pastorelli et al. 2002). Our data also showed that *XPD* 312 Asn and *XPD* 751Gln are associated with slightly reduced aberration frequencies in X-ray–challenged cells, with more reduction in dicentrics for the combined heterozygous and homozygous variant *XPD* 312Asn and *XPD* 751Gln (Table 2). However, this unexpected reduction in dicentrics may be caused by the corresponding 2-fold increase of another type of translocation, chromatid exchanges.

With respect to *XRCC1* 194Trp, we observed an association with increased CA, but the increase is not consistent or indicative of specific DNA repair defect. This is similar to the lack of association with *p53* gene mutation in oral cancer (Hsieh et al. 2003) and the lack of increased DNA adducts (Pastorelli et al. 2002). Nevertheless, the defective DNA repair activities interacted with *XRCC1* 399Gln to cause significant increase in X-ray–induced chromosome deletions (Table 4).

XRCC1 399Gln is associated with significant and specific CA indicative of defects in BER. This observation is consistent with the report that *XRCC1* 399Gln is associated with significant increase of *p53* gene mutation in oral cancer patients (Hsieh et al. 2003), significant increase of glycophorin A mutations in normal smokers and nonsmokers (Lunn et al. 1999), and significant increase of DNA adducts in normal nonsmokers (Matullo et al. 2001).

However, our data are not consistent with the lack of increase of *p53* gene mutations in lung cancer patients (Hou et al. 2003) and the lack of increased DNA adducts in lung cancer patients (Pastorelli et al. 2002).

We showed that *XRCC3* 241Met is associated with BER defects. However, our data are not consistent with the lack of increase of DNA adducts in healthy donors (Matullo et al. 2001).

APE1 148Gln is associated with some repair defects and significant interactions with XRCC3 241Met. However, we have not been able to find any biomarker data in the literature to support or refute our observations.

On the basis of our data, we have observed a significant genotype-specific repair defects to the inducing agents, especially *XRCC1* 399Gln and *XRCC3* 241Met for X-rays, and *XPD* 312Asn and *XPD* 751Gln for UV light. More important, heterozygous and homozygous variant genotypes are consistently associated with higher CA than the wild-type genes. Combinations of variant genotypes also showed an increase in CA compared with the corresponding combined wild-type genes. Our precise observation is supported by a variety of studies in the literature although there are discrepancies. Most of the discrepancies are due to incompatible comparison with different studies; for example, the use of cigarette smokers, cancer patients, and inappropriate biomarkers in the other investigations. We believe that we are able to show the relatively precise response because our experimental conditions are vigorously controlled and the conditions are favorable for the elucidation of the function of the variant genotypes. For example; the use of X rays and UV light to elucidate DNA repair activities is not affected by many confounding factors that can influence similar investigations using chemicals; for example, individual differences in cellular uptake, metabolism, and distribution of chemicals. The existence of polymorphic chemical-metabolizing genes in the population can further complicate the interpretation of results from studies using chemicals. In addition, the use of different types of CA indicative of repair defects from X rays and UV light allows us to elucidate the efficiency in DNA repair more precisely compared with other biomarkers.

Our data also indicate that the functions of the variant genotypes are complementary to each other in the same repair pathway, with limited overlap of genes from the other pathways. This suggests that individuals who have different variant genotypes that act in the same repair pathway may have further elevated risk for environmental disease due to synergistic interactions among the genotypes.

Using carefully selected DNA damage–inducing agents and appropriate biomarkers, we have provided data to indicate that some of the variant genotypes are associated with specific DNA repair defects. Specifically, *XRCC1* 399Gln and *XRCC3* 241Met in BER and *XPD* 312Asn and *XPD* 751Gln for NER are significantly defective. In addition, these genotypes interact significantly with each other without significant overlap of different repair pathways. No genotypes, either singly or in combination, are associated with enhanced repair activities that lead to significantly increased CA under our experimental conditions. The information is helpful in understanding the functions of polymorphic DNA repair genes and their role for susceptibility to environmental cancer.

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