ERCC2 Genotypes and a Corresponding Haplotype Are Linked with Breast Cancer Risk in a German Population

Christina Justenhoven,[1] Ute Hamann,[2] Beate Pesch,[3] Volker Harth,[3] Sylvia Rabstein,[3] Christian Baisch,[1,2] Caren Vollmert,[4] Thomas Illig,[4] Yon-Dschun Ko,[5] Thomas Brüning,[3] Hiltrud Brauch,[1] and for the Interdisciplinary Study Group on Gene Environment Interactions and Breast Cancer in Germany Network

[1]Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany; [2]Deutsches Krebsforschungszentrum, Heidelberg, Germany; [3]Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin, Ruhr University Bochum, Bochum, Germany; [4]GSF-National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg, Germany; and [5]Department of Internal Medicine, Johanniter Krankenhaus and University of Bonn, Bonn, Germany

Abstract

The polygenic concept of breast cancer susceptibility calls for the identification of genetic variants that contribute to breast cancer risk. Reduced DNA repair proficiencies in women with breast cancer pointed to a possible role of DNA repair enzymes in the risk to develop the disease. The nucleotide excision repair enzyme encoded by the excision repair cross-complementing group 2 gene *ERCC2* (formerly *XPD*) known to cause skin cancer by germ line mutations has multiple regulatory cellular functions, including nucleotide excision repair, basal transcription, cell cycle control, and apoptosis. *ERCC2* polymorphisms *ERCC2*_6540_G>A ($Asp^{312}Asn$) and *ERCC2*_18880_A>C ($Lys^{751}Gln$) within the coding region of this evolutionarily highly conserved gene have been of functional relevance and therefore are potential candidates to confer breast cancer susceptibility. Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, we analyzed genotype frequencies in constitutional DNA of study participants of a German case-control study that included 688 cases of incident breast cancer and 724 population-based, age-matched controls. We identified *ERCC2*_6540_GG ($Asp^{312}Asp$) as an at-risk genotype [odds ratio (OR), 2.06; 95% confidence interval (95% CI), 1.39-3.07]. The *ERCC2*_6540_GG-associated breast cancer risk was even higher in women who were also carriers of the *ERCC2*_18880_CC ($Gln^{751}Gln$) genotype (OR, 3.69; 95% CI, 1.76-7.74). We identified *ERCC2*_6540_G/*ERCC2*_18880_C (Asp^{312}/Gln^{751}) as the most potent risk-conferring haplotype (OR, 3.49; 95% CI, 2.30-5.28). To our knowledge, this is the first study assigning breast cancer risk to both the *ERCC2* genotype encoding $Asp^{312}Asp$ and the haplotype encoding Asp^{312}/Gln^{751}. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2059–64)

Introduction

Breast cancer is viewed as a polygenic disease (1) because known susceptibility genes for hereditary breast cancer cannot explain the high breast cancer incidence in Western countries. Moreover, genetic models showed that susceptibility to breast cancer is likely to be conferred by a large number of loci (1). To explore this polygenic nature, association studies have become popular. In contrast to the previous Mendelian inheritance approach for the identification of single but uncommon predisposing genes, association approaches look for genetic variation across many loci in the population to test their predictive value for defining cancer risk groups. Accordingly, breast cancer risk will be estimated from a combined effect of genetic variations. Critical to this approach are an evidence-based selection of genetic variants to be tested for eligibility as risk factors and the avoidance of major selection bias in the study population subjected to analysis.

Women with breast cancer have been shown to have significantly reduced DNA repair proficiencies (2). This finding calls attention to the intricate network of DNA repair systems that protect the genome from deleterious endogenous and exogenous DNA damage (3). In particular, enzymes of the nucleotide excision repair pathway are known or suspected to be implicated in cancer. They may also participate in other regulatory cellular processes including DNA replication and basal transcription (4), cell cycle progression (5), and apoptosis (6). The DNA helicase encoded by the excision repair cross-complementing group 2 gene *ERCC2* (formerly *XPD*) is one of seven nucleotide excision repair enzymes that cause xeroderma pigmentosum when mutated in the germ line (7). Xeroderma pigmentosum is a rare autosomal recessive disease characterized by an extreme sensitivity to sunlight and a >1,000-fold increased

Received 4/2/04; revised 6/22/04; accepted 7/6/04.

Grant support: Federal Ministry of Education and Research Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0, and 01KW0114, Robert Bosch Foundation of Medical Research (Stuttgart, Germany), Deutsches Krebsforschungszentrum, Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin, and Medizinische Universitäts- und Poliklinik (Bonn, Germany).

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Requests for reprints: Hiltrud Brauch, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, D-70376 Stuttgart, Germany. Phone: 49-711-8101-3705; Fax: 49-711-859-295. E-mail: hiltrud.brauch@ikp-stuttgart.de

risk of skin cancer (8). Based on its multiple cellular functions and on rare *ERCC2* mutations giving rise to genetic disease, *ERCC2* polymorphisms such as *ERCC2*_6540_G>A and *ERCC2*_18880_A>C may operate as cancer susceptibility factors (9). *ERCC2*_6540_G>A (rs1799793) in exon 10 is implicated in an amino acid exchange from aspartic acid to asparagine in position 312. This residue is in the seven-motif helicase domain of the RecQ family of DNA helicases and is evolutionarily highly conserved, a reason why this substitution may be of functional significance (10). *ERCC2*_18880_A>C (rs1052559) in exon 23 is responsible for an amino acid exchange from lysine to glutamine in position 751.

There are conflicting data on the role of *ERCC2* polymorphisms and the risk for cancers including glioma (11), melanoma (12), basal cell carcinoma (13), and bladder (14, 15), lung (4, 10, 16–22), prostate (23), head and neck (24), and breast cancer (9, 25). Supportive evidence for a role of *ERCC2* polymorphisms in breast cancer comes from observations of an association of the genotype encoding Gln/Gln at position 751 and increased polycyclic aromatic hydrocarbon adduct levels in tumor tissue (25). In addition, the genotype encoding Lys/Lys at position 751 was associated with reduced DNA repair capacity in lymphocytes of patients with breast cancer (9).

A recent review of epidemiologic studies of associations between DNA repair polymorphisms and the risk of cancer was critical of many of the study designs (26). Evaluation of 30 studies showed consistent data only for 3 of 29 polymorphisms in three of eight DNA repair genes. Despite suggestive results in some studies, small sample sizes may have contributed to false-positive or false-negative findings. Altogether, it has been recommended that informative and reliable association studies must be large, favorably >500 cases and controls as well as population based, and that well-designed studies of common polymorphisms in DNA repair are needed to clarify their role in cancer (26).

To elucidate the role of *ERCC2* polymorphisms in breast cancer, we did a population-based association study in Germany and provided evidence for a predictive role of *ERCC2* genotypes and haplotypes in breast cancer.

Materials and Methods

The Interdisciplinary Study Group on Gene Environment Interactions and Breast Cancer in Germany Study Population. Between August 2000 and October 2002, incident breast cancer cases and population-based controls were recruited from the greater Bonn region in Germany, an area of >1 million inhabitants. This is part of a wider effort of the Interdisciplinary Study Group on Gene Environment Interactions and Breast Cancer in Germany (GENICA), which is focused on the identification of breast cancer risks. Works are therefore called the GENICA study. There are 688 breast cancer cases with a first-time diagnosis of primary breast cancer that was histologically confirmed within 6 months of enrollment and 724 population-based controls matched in 5-year age classes. Inclusion criteria were as follows: cases and controls were eligible if they were of Caucasian ethnicity, currently residing in the study region, and ages <80 years. Risk factor information was collected via in-person interviews using the core questionnaire of a German population survey (27), which was extended by questions on reproductive and other factors potentially related to breast cancer. The response rate was 88% for cases and 67% for controls. Characteristics of the study population with respect to potential breast cancer risk factors, including age (<50, ≥50 years; age refers to age at diagnosis for cases), menopausal status (premenopausal, postmenopausal), smoking status (never, former, current), breast cancer in mother and sisters (yes, no), parity (0, ≥1), and hormone replacement therapy (HRT; 0, >0 to <10, ≥10 years), are given in Table 1. For the description of the study population, we chose a cutoff of 50 years, which is frequently considered the boundary for premenopausal and postmenopausal status. The reported menopausal status was premenopausal if women reported bleedings in the year of interview. All other women were considered postmenopausal by either natural or surgically induced menopause. The subgroups obtained by age cutoff and reported menopausal status were similar.

All study participants provided a blood sample drawn into heparin tubes (Becton Dickinson, Franklin Lake, NJ). The GENICA study was approved by the Ethics Committee of the University of Bonn; all study participants gave written informed consent.

Isolation of DNA. Genomic DNA was extracted from 20 mL heparin blood samples using the Puregene kit (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. DNA samples were available from 610 (89%) of the 688 enrolled cases and from 651 (90%) of 724 controls.

Genotyping. Genotyping was done at loci *ERCC2*_6540_G>A and *ERCC2*_18880 A>C (genomic DNA; Genbank accession no. L47234). Nucleotide positions were determined in genomic DNA sequence starting with the A of the initial ATG as nucleotide 1.

Table 1. Characteristics of study participants

	Cases, *n* (%)	Controls, *n* (%)
Age (y)		
<50	140 (23)	149 (23)
≥50	468 (77)	501 (77)
Menopausal status		
Premenopausal	146 (24)	148 (23)
Postmenopausal	455 (76)	495 (77)
Smoking status		
Never	351 (58)	362 (56)
Former	139 (23)	137 (21)
Current	117 (19)	151 (23)
Breast cancer in mother or sisters		
No	537 (88)	601 (93)
Yes	71 (12)	49 (8)
Parity		
0	123 (20)	120 (19)
≥1	485 (80)	529 (82)
HRT (y)		
Never	295 (49)	327 (51)
>0 to <10	152 (25)	188 (29)
≥10	153 (26)	130 (20)

Table 2. Sequences of primers and masses of extension products of MALDI-TOF MS assays

Single nucleotide polymorphism	Primer	Sequence	Mass (kDa)
*ERCC2*_6540_G>A	PCR primer 1	5′-ACGTTGGATGTGCGAGGAGACGCTATCAGC-3′	
	PCR primer 2	5′-ACGTTGGATGAGTACCGGCGTCTGGTGGAG-3′	
	Extension primer	5′-CTCACCCTGCAGCACTTCGT-3′	5,988.9
	Analyte G	5′-CTCACCCTGCAGCACTTCGTC-3′	6,262.0
	Analyte A	5′-CTCACCCTGCAGCACTTCGTTG-3′	6,606.0
*ERCC2*_18880_A>C	PCR primer 1	5′-ACGTTGGATGAGCAGCTAGAATCAGAGGAG-3′	
	PCR primer 2	5′-ACGTTGGATGCACCAGGAACCGTTTATGGC-3′	
	Extension primer	5′-GAGCAATCTGCTCTATCCTCT-3′	6,332.1
	Analyte A	5′-GAGCAATCTGCTCTATCCTCTT-3′	6,620.3
	Analyte C	5′-GAGCAATCTGCTCTATCCTCTGC-3′	6,934.5

Genotyping of single nucleotide polymorphisms was done using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of allele-specific primer extension products (Mass Array, Sequenom, San Diego, CA). Briefly, 5 ng of genomic DNA were amplified by PCR in a final volume of 6 μL containing locus-specific primers (Table 2) at 167 nmol/L final concentration and 0.1 unit HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). PCR conditions were 95°C for 15 minutes for hot start followed by 44 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension for 1 minute at 72°C and finally followed by incubation at 72°C for 10 minutes. PCR products were treated with shrimp alkaline phosphatase (Amersham, Freiburg, Germany) for 20 minutes at 37°C to remove excess deoxynucleotide triphosphates followed by 10 minutes at 85°C to inactivate shrimp alkaline phosphatase. Base extension (homogenous MassEXTEND assay, Sequenom) reactions in a final volume of 10 μL contained extension primers (Table 2) at a final concentration of 0.54 μmol/L and 0.6 units ThermoSequenase (Amersham). Base extension reaction conditions were 94°C for 2 minutes followed by 40 cycles of 94°C for 5 seconds, 52°C for 5 seconds, and 72°C for 5 seconds. All reactions including PCR amplification, shrimp alkaline phosphatase treatment, and base extension were done in a Tetrad PCR thermal cycler (MJ Research, Waltham, MA). The final base extension products, the fragment lengths of which are given in Table 2, were treated with SpectroCLEAN resin (Sequenom) to remove salts from the reaction buffer. This step was done with a Multimek 96-channel autopipette (Beckman Coulter, Fullerton, CA). For a final volume of 26 μL, 16 μL of resin-water suspension were added into each base extension reaction. Following a quick centrifugation (2,000 rpm for 3 minutes in an Eppendorf centrifuge 5810, Hamburg, Germany), 10 nL of reaction solution were dispensed onto a 384-format SpectroCHIP microarray (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex MALDI-TOF MS was used for data acquisitions from the SpectroCHIP. Genotyping calls were made in real time with Mass Array RT software version 3.0.0.4 (Sequenom).

Statistical Analysis. Epidemiologic risk factors, genotype frequencies, and adjusted odds ratios (OR_{adj}) were calculated using SAS/STAT software version 8.02 (28). Risk estimates for the development of breast cancer were calculated as ORs with 95% confidence intervals (95% CI) using logistic regression analysis, conditional on age (<45, 45-50, 50-55, 55-60, 60-65, 65-70, and ≥70 years). In addition, we adjusted the risks for potential breast cancer risk factors such as smoking status (ever, never), history of breast cancer in mother or sisters (no, yes), parity (0, ≥1), and HRT (0, >0 to <10, ≥10 years). To facilitate comparison, OR_{adj} for *ERCC2*_6540_G>A was calculated with the genotype homozygous for the rare A allele as reference based on regular function in apoptosis (10, 29). OR_{adj} for *ERCC2*_18880_A>C was calculated with the genotype homozygous for the frequent A allele as reference. Genotype frequencies were checked for Hardy-Weinberg equilibrium according to Pearson χ^2. For the estimation of risks for combined genotypes, we used the major genotypes *ERCC2*_6540_GG and *ERCC2*_18880_AA as references to obtain stable risk estimates. Furthermore, we individually stratified for potential breast cancer risk factors such as age, menopausal status, and smoking.

Haplotypes were estimated using PHASE (30, 31). Linkage disequilibrium was tested using Arlequin version 2.0 (32).

Results

Study participants of a population-based, case-control study (Table 1) were genotyped for *ERCC2*_6540_G>A and *ERCC2*_18880_A>C polymorphism to investigate a possible association with breast cancer risk. Accuracy and reproducibility of genotyping data were 99.9% based on repeated analysis of ~50% and >10% of randomly selected case and control samples for *ERCC2*_6540_G>A and *ERCC2*_18880_A>C, respectively. Genotype frequencies of cases and controls are given in Table 3. With respect to controls, these frequencies were in Hardy-Weinberg equilibrium.

Comparison of *ERCC2*_6540_G>A ($Asp^{312}Asn$) Genotype Frequencies in Patients and Controls. Our case population showed deviations from the control population. The observed genotype frequencies of cases were not in Hardy-Weinberg equilibrium ($P < 0.001$). A comparison of frequencies between cases and controls identified the GG genotype as a significant breast cancer risk (OR, 2.06; 95% CI, 1.39-3.07; Table 3).

Table 3. Association of *ERCC2* polymorphisms with breast cancer risk

Genotype	Cases, *n* (%)	Controls, *n* (%)	OR_{adj}* (95% CI)
*ERCC2*_6540_G>A ($Asp^{312}Asn$)			
AA	47 (8)	79 (13)	1.00 (reference)
GA	173 (31)	255 (42)	1.15 (0.76-1.74)
GG	347 (61)	276 (45)	2.06 (1.39-3.07)
*ERCC2*_18880_A>C ($Lys^{751}Gln$)			
AA	224 (38)	264 (41)	1.00 (reference)
AC	265 (45)	292 (45)	1.09 (0.85-1.39)
CC	97 (17)	87 (14)	1.32 (0.94-1.86)

*OR_{adj} conditional on age in 5-year groups, adjusted for smoking, history of breast cancer in mother or sisters, HRT, and parity.

Comparison of *ERCC2*_18880_A>C ($Lys^{751}Gln$) Genotype Frequencies in Patients and Controls. Genotype frequencies of cases and controls were in Hardy-Weinberg equilibrium. No statistically significant differences in genotype frequencies were observed between cases and controls (Table 3).

Stratification of *ERCC2*_6540_G>A ($Asp^{312}Asn$) and *ERCC2*_18880_A>C ($Lys^{751}Gln$) Genotypes by Potential Breast Cancer Risk Factors. *ERCC2*_6540_G>A and *ERCC2*_ 18880_A>C genotypes were stratified by age (<50, ≥50 years), menopausal status (premenopausal, postmenopausal), and smoking (never, ever). No intensified breast cancer risk was observed as shown for the *ERCC2*_6540_G>A genotypes (Table 4). Although ORs indicate increased breast cancer risks, these risks were not substantially different from the risk previously attributed to the *ERCC2*_6540_GG genotype (Table 3).

Combined Genotype and Haplotype Frequencies. The observed increased breast cancer risk of *ERCC2*_6540_GG carriers was even higher when women were carriers of the *ERCC2*_18880_CC genotype (OR, 3.69; 95% CI, 1.76-7.74). It was less pronounced in combination with the *ERCC2*_18880_ AC genotype (OR, 2.61; 95% CI, 1.77-3.85; Table 5). When phase was established from genotypes by haplotype analysis, we observed an association of the *ERCC2*_6540_G/*ERCC2*_18880_C haplotype with increased breast cancer risk (OR, 3.49; 95% CI, 2.30-5.28; Table 6).

When we estimated putative haplotype frequencies from allele frequencies, we found that *ERCC2*_6540_G/*ERCC2*_18880_A and *ERCC2*_6540_A/*ERCC2*_18880_C haplotypes were more frequent than the *ERCC2*_6540_G/*ERCC2*_18880_C and *ERCC2*_6540_A/*ERCC2*_18880_A haplotypes. This was confirmed by linkage disequilibrium analyses that showed linkage disequilibrium between *ERCC2*_6540_G and *ERCC2*_18880_A alleles as well as for the *ERCC2*_6540_A and *ERCC2*_18880_C alleles. This was true for both cases and controls and highly significant ($P < 0.0001$).

Discussion

We identified an increased breast cancer risk for female carriers of the *ERCC2*_6540_GG genotype in a German population. It is important to consider this result in light of the multiple cellular functions of ERCC2. *ERCC2*_6540_GG encodes the frequent enzyme phenotype $Asp^{312}Asp$, and carriers are predicted to have normal DNA repair proficiency. The nucleotide excision repair aspect of ERCC2 function by itself therefore does not provide a rationale for the increased breast cancer risk. Rather, we may consider that ERCC2, which is part of the basal transcription repair complex TFIIH (3, 33), binds p53, a key regulator of apoptosis (34). TFIIH-p53-regulated apoptosis has been linked with polymorphisms of ERCC2 in that cell lines homozygous for Asn at position 312 had more apoptotic cells than cell lines homozygous for Asp or heterozygous cell lines (29). Our patient-based observations are in line with these *in vitro* data, and we may infer that carriers of the *ERCC2*_6540_GG genotype, homozygous for Asp at position 312, may have a lower apoptotic capacity and thus be at increased risk to develop breast cancer. Conversely, the lower breast cancer risk of individuals homozygous for Asn at position 312 may be due to a higher apoptotic response. Our findings of an association between breast cancer risk and this evolutionarily highly conserved *ERCC2* polymorphism (35) with important functional implications may encourage efforts toward

Table 4. *ERCC2*_6540_G>A ($Asp^{312}Asn$) genotypes and potential breast cancer risk factors

	AA			GA			GG		
	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
Age (y)									
<50	15 (12)	19 (13)	1.00* (reference)	29 (23)	65 (46)	0.59* (0.3-1.4)	82 (65)	57 (40)	2.02* (0.9-4.5)
≥50	32 (7)	60 (13)	1.00* (reference)	144 (33)	190 (41)	1.44* (0.9-2.3)	259 (60)	219 (47)	2.17* (1.4-3.5)
Menopausal status									
Premenopausal	15 (12)	17 (12)	1.00* (reference)	34 (27)	65 (46)	0.60* (0.3-1.4)	81 (62)	58 (41)	1.56* (0.7-3.4)
Postmenopausal	32 (8)	62 (13)	1.00* (reference)	137 (32)	185 (40)	1.4* (0.9-2.3)	255 (60)	217 (47)	2.20* (1.4-3.5)
Smoking status									
Never	27 (8)	42 (12)	1.00† (reference)	105 (32)	133 (39)	1.20† (0.7-2.1)	195 (60)	163 (48)	1.77† (1.0-3.0)
Ever	20 (9)	37 (14)	1.00† (reference)	68 (29)	122 (45)	1.01† (0.5-1.9)	146 (62)	113 (42)	2.25† (1.2-4.1)

*OR_{adj} conditional on age in 5-year groups, adjusted for smoking, history of breast cancer in mother or sisters, HRT, and parity.
†OR_{adj} conditional on age in 5-year groups, adjusted for history of breast cancer in mother or sisters, HRT, and parity.

the investigation of apoptotic responses of epithelial cells with different genotypes.

Due to its functional relevance, we included the *ERCC2*_18880_A>C polymorphism in our studies because amino acid residue 751 is within the interaction domain of ERCC2 and its helicase activator p44 protein inside TFIIH (7, 36). We did not find an association of breast cancer risk at this single locus. However, combined genotype frequencies revealed that the *ERCC2*_6540_GG ($Asp^{312}Asp$)-associated breast cancer risk increased significantly to >3-fold when women were also carriers of the *ERCC2*_18880_CC ($Gln^{751}Gln$) genotype. We identified *ERCC2*_6540_G/*ERCC2*_18880_C (Asp^{312}/Gln^{751}) as the at-risk haplotype. With respect to calculated haplotype frequencies, our data are reminiscent of that by Butkiewicz et al. (10), who suggested linkage disequilibrium for codons 312 and 751. Similarly, our study suggests that carriers of Asp^{312} are most likely to be also carriers of Lys^{751}. Furthermore, our population-based controls revealed similar calculated haplotype frequencies when compared with those observed in segregation analysis of three-generation families by Butkiewicz et al. (10). From this, we may infer that *ERCC2* haplotype frequencies of the German and Polish populations are similar. In addition, we compared the calculated haplotype frequencies and observed an imbalance in allelic combinations. This led us to estimate linkage disequilibrium, which was highly significant. In particular, we observed an overrepresentation of the Asn^{312}/Gln^{751} and the Asp^{312}/Lys^{751} haplotypes and an underrepresentation of the Asp^{312}/Gln^{751} and Asn^{312}/Lys^{751} haplotypes. This imbalance was less pronounced for the Asn^{312}/Gln^{751} haplotype in cases that establishes its role in breast cancer risk.

Although our data agree with the functional role of ERCC2 and its polymorphisms in apoptosis control, it is important to compare our results with those of others to point out consistencies and inconsistencies. *ERCC2* polymorphisms have been subject to many cancer susceptibility studies; direct comparisons between studies, however, are frequently hampered by differences in ethnicity, organ sites, study size, and type of controls. Interestingly, a hospital-based, breast cancer case-control study of women from Korea did not report an association of breast cancer risk with the ERCC2 $Asp^{312}Asn$ polymorphism (37). This discrepancy may be explained by different ethnic background in both studies (i.e., Asian versus Caucasian) and/or differences in type of controls (i.e., hospital-based versus population-based). When these polymorphisms were investigated in lung cancer, conflicting results were also obtained (4, 10, 16-22). This may be explained by variations in study size and smoking exposure, a risk factor considered of higher magnitude than *ERCC2* polymorphism (18). Yet, in line with our study, Butkiewicz et al. (10) showed similar genotype risk and protection assignments. In contrast, the genotype encoding $Asn^{312}Asn$ has been assigned as the at-risk genotype in prostate cancer (23). In addition to the gender difference, the prostate cancer study also differed with respect to our case-control study by using sibs, that is, cases and their brothers, which may explain a variation in risk allele assignment.

Note that our molecular results were adjusted for suspected breast cancer risk factors including smoking status, breast cancer in mother or sisters, parity, and HRT without effect on the significance of results. When we stratified our data by suspected breast cancer risks such as age, menopausal status, and smoking status, we did not observe any intensified effect of the *ERCC2*_6540_GG-associated breast cancer risk. Thus, in our study population, the observed increase in breast cancer risk may be fully attributed to the influence of the polymorphic *ERCC2* gene.

Our study benefits from population-based design as well as from sufficient size and statistical power for the main effects. Our data are highly consistent with the functional interpretations derived from independent *in vitro* studies. Accordingly, the polymorphic ERCC2 acts as an intrinsic part of the organism's defense machinery by modulation of the p53 tumor suppressor function. Our analyses of clinical samples and controls support the concept that imbalances due to ERCC2 $Asp^{312}Asn$ and $Lys^{751}Gln$ polymorphisms may contribute to breast cancer susceptibility by allowing the outgrowth of DNA-damaged breast epithelial cells. The origin of such DNA damage has not been subject to our study and therefore remains elusive. Yet, to our knowledge, this is the first study assigning breast cancer risk to both the *ERCC2* genotype encoding $Asp^{312}Asp$ and the haplotype encoding Asp^{312}/Gln^{751}. In the future, it will be important to clarify the relevance of these *ERCC2* polymorphisms in the prediction of breast cancer risk and prevention of the disease.

Table 5. Combined genotypes of *ERCC2*_6540_G>A ($Asp^{312}Asn$) and *ERCC2*_18880_A>C ($Lys^{751}Gln$)

Genotypes		Cases, *n* (%)	Controls, *n* (%)	OR_{adj}* (95% CI)
*ERCC2*_6540	*ERCC2*_18880			
GG	AA	187 (34)	216 (36)	1.00 (reference)
GG	AC	113 (21)	50 (8)	2.61 (1.77-3.85)
GG	CC	34 (6)	10 (2)	3.69 (1.76-7.74)
GA	AA	23 (4)	34 (6)	0.82 (0.46-1.44)
GA	AC	116 (21)	197 (32)	0.70 (0.52-0.95)
GA	CC	31 (6)	22 (4)	1.62 (0.90-2.91)
AA	AA	3 (1)	5 (1)	0.75 (0.17-3.23)
AA	AC	15 (3)	27 (4)	0.67 (0.35-1.31)
AA	CC	29 (5)	47 (8)	0.70 (0.42-1.16)

*OR_{adj} conditional on age in 5-year groups, adjusted for smoking, history of breast cancer in mother or sisters, HRT, and parity.

Table 6. Haplotype frequencies of *ERCC2*_6540_G>A ($Asp^{312}Asn$) and *ERCC2*_18880_A>C ($Lys^{751}Gln$) in breast cancer cases and controls

*ERCC2*_6540	*ERCC2*_18880	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
A	A	55 (5)	85 (7)	1.00 (reference)
A	C	208 (19)	328 (27)	0.98 (0.67-1.44)
G	A	614 (56)	693 (57)	1.37 (0.96-1.10)
G	C	219 (20)	97 (8)	3.49 (2.30-5.28)

Acknowledgments

We thank all women who participated in this study, Werner Schroth for helpful discussion, and the associated members of the GENICA Network Scientific Advisory Board: Hermann

M Bolt, M.D., Ph.D. (Institut für Arbeitsphysiologie an der Universität Dortmund), Michel Eichelbaum, M.D. (Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Stuttgart), and Hans Vetter, M.D. (Medizinische Universitäts-Poliklinik Bonn); clinicians and hospitals: Walther Kuhn, M.D. (Universitätsklinikum Bonn), Uwe-Jochen Goehring, M.D., and Horst Wilms, M.D. (Johanniter-Krankenhaus Bonn), Heinrich Mause, M.D. (Gemeinschaftskrankenhaus St. Elisabeth-St. Petrus Bonn), Volker Pelzer, M.D. (Marien-Hospital Bonn), Michael Kaiser, M.D. (Malteser-Krankenhaus Bonn-Hardtberg), Rolf-Eberhard Herzog, M.D. (Evangelisches Waldkrankenhaus Bad-Godesberg), Sigrid Milz, M.D., and Wolfgang Nohl, M.D. (St. Johannes Krankenhaus Bad-Honnef), Karl-Heinz Schlensker, M.D. (Klinikum Rhein-Sieg), Robert Kampmann, M.D. (St. Josef-Hospital Troisdorf), Bernd Werling, M.D. (Krankenhaus Troisdorf- Sieglar), Norbert Golz, M.D. (Marien-Hospital Euskirchen), and Matthias Winkler, M.D., and Peter-Karl Abramowski, M.D. (Kreiskrankenhaus Mechernich); pathologists: Reinhard Büttner, M.D., and Hans-Peter Fischer, M.D. (Universitätsklinikum Bonn); Magdolna Bollmann, M.D., Reinhard Bollmann, M.D., Dietmar Kindermann, M.D., and Rainer Nikorowitsch, M.D. (Bonn), Jürgen Vogel, M.D., Mathias Feldmann, M.D., and Jürgen Gerlach, M.D. (Siegburg); epidemiologists: Ulrich Ranft (Institut für Umweltmedizinische Forschung, Düsseldorf), Heinz-Erich Wichmann, M.D., Ph.D., Rolf Holle, Ph.D., and Hannelore Nagl (GSF-National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg); interviewers: Mette Besuden, Martin Black, Sandra Gschwendtner, Miriam Knie, Annette Kramer, Robert Priemke, Astrid Prömse, Miriam Rossa, Johanna Scholl, Heiko Schneider, Katja Tamme, Matthias Wenghöfer, Bernd Wiesenhütter, and Melanie Wollenschein; and laboratory technical assistants: Sandra Brod and Silke Schöneborn.

References

1. Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33–6.
2. Helzlsouer KJ, Harris EL, Parshad R, Perry HR, Price FM, Sanford KK. DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl Cancer Inst 1996;88:754–5.
3. Hoeijmakers JH. Human nucleotide excision repair syndromes: molecular clues to unexpected intricacies. Eur J Cancer 1994;30A: 1912–21.
4. Spitz MR, Wu X, Wang Y, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001;61:1354–7.
5. Robles AI, Wang XW, Harris CC. Drug-induced apoptosis is delayed and reduced in XPD lymphoblastoid cell lines: possible role of TFIIH in p53-mediated apoptotic cell death. Oncogene 1999;18:4681–8.
6. Barnes DM, Camplejohn RS. P53, apoptosis, and breast cancer. J Mammary Gland Biol Neoplasia 1996;1:163–75.
7. Coin F, Marinoni JC, Rodolfo C, Fribourg S, Pedrini AM, Egly JM. Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. Nat Genet 1998;20:184–88.
8. Eveno E, Bourre F, Quilliet X, et al. Different removal of ultraviolet photoproducts in genetically related xeroderma pigmentosum and trichothiodystrophy diseases. Cancer Res 1995;55:4325–32.
9. Lunn RM, Helzlsouer KJ, Parshad R, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000;21:551–5.
10. Butkiewicz D, Rusin M, Enewold L, Shields PG, Chorazy M, Harris CC. Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis 2001;22:593–7.
11. Caggana M, Kilgallen J, Conroy JM, et al. Associations between ERCC2 polymorphisms and gliomas. Cancer Epidemiol Biomarkers Prev 2001;10:355–60.
12. Winsey SL, Haldar NA, Marsh HP, et al. A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res 2000;60:5612–6.
13. Vogel U, Hedayati M, Dybdahl M, Grossman L, Nexo BA. Polymorphisms of the DNA repair gene XPD: correlations with risk of basal cell carcinoma revisited. Carcinogenesis 2001;22: 899–904.
14. Matullo G, Guarrera S, Carturan S, et al. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. Int J Cancer 2001;92:562–7.
15. Stern MC, Johnson LR, Bell DA, Taylor JA. XPD codon 751 polymorphism, metabolism genes, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1004–11.
16. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and ^{32}P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001;22:1437–45.
17. Hou SM, Falt S, Angelini S, et al. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. Carcinogenesis 2002;23:599–603.
18. Zhou W, Liu G, Miller DP, et al. Gene-environment interaction for the ERCC2 polymorphisms and cumulative cigarette smoking exposure in lung cancer. Cancer Res 2002;62:1377–81.
19. Zhou W, Liu G, Miller DP, et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2, smoking, and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2003;12:359–65.
20. Park JY, Lee SY, Jeon HS, et al. Lys^{751}Gln polymorphism in the DNA repair gene XPD and risk of primary lung cancer. Lung Cancer 2002;36:15–6.
21. David-Beabes GL, Lunn RM, London SJ. No association between the XPD (Lys^{751}G1n) polymorphism or the XRCC3 (Thr^{241}Met) polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:911–2.
22. Vogel U, Laros I, Jacobsen NR, et al. Two regions in chromosome 19q13.2-3 are associated with risk of lung cancer. Mutat Res 2004;546: 65–74.
23. Rybicki BA, Conti DV, Moreira A, Cicek M, Casey G, Witte JS. DNA repair gene XRCC1 and XPD polymorphisms and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13: 23–9.
24. Sturgis EM, Zheng R, Li L, et al. XPD/ERCC2 polymorphisms and risk of head and neck cancer: a case-control analysis. Carcinogenesis 2000;21:2219–23.
25. Tang D, Cho S, Rundle A, et al. Polymorphisms in the DNA repair enzyme XPD are associated with increased levels of PAH-DNA adducts in a case-control study of breast cancer. Breast Cancer Res Treat 2002;75:159–66.
26. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1513–30.
27. Gieseke B, Nagl H. DIAMON-Rechnergestützte Fragebogenentwicklung und Interviewführung, Dokumentation. GSF Bericht 2001;03/01.
28. Changes and enhancement through release 8.0. SAS/STAT software. Cary (NC): SAS Institute; 2000.
29. Seker H, Butkiewicz D, Bowman ED, et al. Functional significance of XPD polymorphic variants: attenuated apoptosis in human lymphoblastoid cells with the XPD 312 Asp/Asp genotype. Cancer Res 2001; 61:7430–4.
30. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–9.
31. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
32. Schneider S, Roessli D, Excoffier L. ARLEQUIN Ver. 2.0: a software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva; 2000.
33. Hoeijmakers JH, Bootsma D. Molecular genetics of eukaryotic DNA excision repair. Cancer Cells 1990;2:311–20.
34. Wang XW, Vermeulen W, Coursen JD, et al. The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev 1996;10:1219–32.
35. Zhu Y, Spitz MR, Amos CI, Lin J, Schabath MB, Wu X. An evolutionary perspective on single-nucleotide polymorphism screening in molecular cancer epidemiology. Cancer Res 2004;64:2251–7.
36. Benhamou S, Sarasin A. ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis 2002;17:463–9.
37. Kang D. Genetic polymorphisms and cancer susceptibility of breast cancer in Korean women. J Biochem Mol Biol 2003;36:28–34.

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Christina Justenhoven, Ute Hamann, Beate Pesch, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:2059-2064.