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A distinct *ERCC1* haplotype is associated with mRNA expression levels in prostate cancer patients

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Both genetic variants and messenger RNA (mRNA) expression of DNA repair and tumor suppressor genes have been investigated as molecular markers for therapy outcome. However, the phenotypic impact of genetic variants often remained unclear, thus the rationale of their use in risk prediction may be limited. We therefore analyzed genetic variants together with anthropometric and lifestyle factors to see how these affect mRNA levels of *ERCC1*, *MDM2* and *TP53* in primary blood lymphocytes. mRNA expression was measured in 376 prostate cancer patients by quantitative real-time polymerase chain reaction after reverse transcription, and *ERCC1* rs11615 T>C, *ERCC1* rs3212986 C>A, *MDM2* rs2279744 T>G and *TP53* rs17878362 (p53PIN3) polymorphisms were determined. Considerable interindividual differences in mRNA expression were found (coefficients of variation: *ERCC1*, 45%; *MDM2*, 43% and *TP53*, 35%). *ERCC1* expression was positively correlated with plasma levels of β-carotene ($P = 0.03$) and negatively correlated with canthaxanthin ($P = 0.02$) and lutein ($P = 0.02$). Overall, the polymorphisms affected mRNA expression only weakly. Carriers of a distinct *ERCC1* haplotype (CC) showed, however, significantly lower expression values than non-carriers ($P = 0.001$). Applying logistic regression, we found that CC haplotype carriers had a 1.69-fold increased odds ratio (95% confidence interval: 1.06–2.71) for reduced *ERCC1* mRNA levels. This low *ERCC1* expression might be associated with reduced DNA repair and better therapy response. In summary, the association we have found between *ERCC1* genotype and mRNA expression supports recent clinical observations that genetic variation in *ERCC1* can affect treatment outcome and prognosis. Our study further revealed a modulating effect by nutritional factors.

Introduction

There is an urgent need for molecular markers suitable to allow prediction of therapy outcome and to individualize therapeutic protocols (1). Ideal markers should be readily detectable in surrogate tissues such as peripheral blood lymphocytes with high precision and appropriate technical effort (2). As functional characterization of cellular protein markers is often complex and difficult, many studies are currently concentrating on DNA genotype and RNA expression analyses (3,4). Measuring steady-state messenger RNA (mRNA) levels in circulating lymphocytes should be a good choice as this determines not only efficient gene transcription but also correct processing and maintenance of mRNA. Regulation of mRNA expression is, however, tissue specific and can be affected by genetic and endogenous factors such as age (5), gender (6) and potentially body mass index (BMI). Exogenous factors like exposure to tobacco smoke (7) or nutrition-dependent serum antioxidant levels (8) have also been shown to influence mRNA expression. To obtain reliable results, these modifying factors have to be taken into consideration together with high technical standards in isolation and processing of RNA throughout the experimental protocol (9).

Abbreviations: BMI, body mass index; CC carriers, CC haplotype carriers; cDNA, complementary DNA; mRNA, messenger RNA; PCR, polymerase chain reaction; RT, reverse transcription.

In contrast, genetic polymorphisms are inherited genetic variations present in all cells (germinative or somatic and healthy or impaired) of an individual and can easily be determined in DNA from all available tissues. Especially, polymorphisms affecting gene expression might be used instead of costly and time-consuming measurements of mRNA levels. It is, however, difficult to assess the impact of a single-nucleotide polymorphism on a highly complex cellular process such as mRNA expression when using molecular biology methods in cell lines that carry a certain genotype. Association studies evaluating the correlation between genetic variants and mRNA expression in peripheral blood lymphocytes of an ample number of individuals should provide a better rationale for the use of genetic markers.

Here, we analyzed the correlation between genotype and mRNA levels of three DNA damage response or repair genes which were suggested to affect therapy outcome, *TP53*, *MDM2* and *ERCC1*. We selected these genes not only for their possible clinical relevance but also because of preliminary data which indicate that the expression levels of these genes might be modulated by specific polymorphisms (see below). Thus, our study in lymphocytes of 376 prostate cancer patients should reveal whether there is a correlation between these two parameters that may support their use in clinical settings.

For the nucleotide excision repair gene *ERCC1*, there is evidence that *ERCC1* variants affect platinum treatment-induced toxicity (10). Two polymorphisms were reported to be correlated with therapy outcome and mRNA levels. The *ERCC1* rs3212986 C>A (also reported as C8092A) is located in the 3′ untranslated region of the gene and was suggested to influence mRNA stability (11). The *ERCC1* rs11615 T>C polymorphism (Asn118Asn) was correlated with a better survival of lung cancer patients following cisplatin chemotherapy (12–14). In addition, high levels of intratumor mRNA have been associated with platinum resistance in various tumors (13). Cell line experiments revealed that the rs11615 T>C polymorphism was associated with a 50% reduced codon usage and a decreased and delayed induction of *ERCC1* mRNA expression after cisplatin treatment (15).

The *MDM2* gene encodes a protein that is interacting with and negatively regulating the p53 protein. A polymorphism in its promoter region, *MDM2* rs2279744 T>G (also reported as SNP309), was found to be associated with poor prognosis for gastric carcinoma (16). The *MDM2* variant enhanced the affinity of the transcription factor Sp1 to the *MDM2* promoter region by extending the length of the promoter-binding site (17). This resulted in an increased *MDM2* mRNA expression level as shown in a comparison of four tumor cell lines carrying either the homozygous wild-type or variant allele.

The *TP53* PIN3 polymorphism (rs17878362), a 16 bp repeat in intron 3 of the tumor suppressor gene *TP53*, was associated with the risk of developing acute side effects during radiotherapy (18). In addition, this polymorphism was shown to be associated with reduced constitutive *TP53* mRNA expression levels in lymphoblastoid cell lines. A reduced stability of the 16 bp insertion-carrying pre-mRNA was suggested as possible mechanism (19).

In summary, the published evidence for a functional impact of these genetic variants is mainly based on cell culture experiments or results from studies with small sample size. Regulation of mRNA expression is, however, complex and differs between cell cultures and primary cells. Systematic evaluations of genotype–phenotype correlations in primary tissues are still missing. To investigate a possible modifying effect of the selected polymorphisms *ERCC1* rs11615 T>C, *ERCC1* rs3212986 C>A, *MDM2* rs2279744 T>G and *TP53* rs17878362

(p53PIN3), expression of these genes was measured by quantitative real-time polymerase chain reaction (PCR) after reverse transcription (RT) in lymphocytes of a cohort of 376 prostate cancer patients and analyzed for possible associations with genotype. Furthermore, *ERCC1* haplotypes as well as potential modifying factors such as age, BMI, serum antioxidant levels and alcohol or tobacco consumption were included in the statistical evaluation.

Materials and methods

Study subjects

A cohort of 405 unselected prostate cancer patients was recruited at the Department of Radiology at the University Hospital in Heidelberg between 2001 and 2005 in a prospective epidemiological study. Only patients receiving radiotherapy were included. The study was approved by the Ethical Committee of the Medical Faculty at the University of Heidelberg (Ref. 206/2000). Patients gave informed written consent to take part in this study. Data on histopathology of the tumor, therapy and radiation-related side effects were collected from patients' hospital records. Demographic and epidemiologic information was gathered using a self-administered patient questionnaire. A detailed epidemiological description of study design and participants will be published elsewhere.

The self-administered questionnaire included detailed information on lifetime smoking habits and alcohol consumption. The participants were asked when they began smoking, the type of product consumed, the amount smoked and the date of cessation or change in their smoking habits. 'Ex-smokers' were persons who reported to have smoked regularly in the past, and 'current smokers' had reported regular smoking at the time of completing the questionnaires. In addition, pack-years were computed as the average number of packs per day times the total number of years of smoking, assuming 20 cigarettes per pack. Patients were stratified into three groups according to the variable pack-years: 0 pack-years, 1–19 pack-years and ≥20 pack-years. Based on their daily alcohol consumption, patients were divided into three groups: 0, 1–18 and ≥19 g/day. Important characteristics of the study cohort are shown in Table I. The mean age was 68 ± 6.7 years. The mean BMI equaled 27 ± 3.7 kg/m^2; 47.4% of the patients were 'never smokers', 43% ex-smokers and 8.6% were current smokers.

Lymphocyte isolation

A venous blood sample of 50 ml was obtained in ethylenediaminetetraacetic acid-layered monovettes from each patient prior to radiotherapy. Within 3 h after blood sampling, peripheral lood lymphocytes were isolated through gradient centrifugation by overlaying whole blood on Lymphoprep™ (Axis-Shield, Oslo, Norway) in Leucosep® tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) and centrifugation at 800*g* for 15 min at room temperature. Lymphocytes were purified in two washing steps with phosphate-buffered saline, and RNA isolation was performed directly afterward. RNA isolated identically from a 500 ml buffy coat of a healthy male subject (received from Heidelberg blood bank) served as a 'calibrator' in RNA quantification (Roche Applied Science, Mannheim, Germany, Technical Note No. LC13/2001) and was used in each experiment.

RNA isolation

Total RNA was isolated by a modified single-step method (20) using Trizol® Reagent (Invitrogen, Karlsruhe, Germany), according to the manufacturer's protocol. Cells were lysed by addition of 1 ml Trizol per 10^7 cells and repetitive pipetting. Aqueous and organic phases were separated by addition of chloroform and subsequent centrifugation. After transfer of the aqueous phase, RNA was recovered by precipitation with isopropanol and centrifugation. After washing with 75% ethanol, the pellets were dried and dissolved in RNase-free H_2O. RNA integrity and concentration were determined using an Agilent 2100 Bioanalyzer with the RNA 6000 Nano LabChip® Kit (Agilent Technologies, Santa Clara, CA, USA).

Synthesis of complementary DNA and analysis of gene expression

Two micrograms of total RNA was reverse transcribed using the Superscript II system (Invitrogen), 0.5 mM deoxynucleotide triphosphates MBI Fermentas, St Leon-Roth, Germany), 40 U RNasin (Promega, Mannheim, Germany) and 0.5 µg oligo-dT primers (Applied Biosystems, Cheshire, UK). Reactions were incubated at 42°C for 50 min followed by incubation at 70°C for 15 min.

Expression levels of the target and reference genes were analyzed on a LightCycler 1.0 (Roche Diagnostics, Mannheim, Germany). A typical 10 µl amplification mixture contained 2 µl of complementary DNA (cDNA) sample, 3 mM $MgCl_2$, 5 µM primers and LightCycler FastStart Master SYBR Green I Mix containing buffer and *Taq* DNA polymerase (Roche Diagnostics). The calibrator sample was measured as a standardized cDNA in each real-time PCR experiment to adjust for possible run-to-run variations. For evaluation of experiments, the so-called 'calibrator normalized relative quantification method with efficiency correction' was used [Roche Applied Science, Technical Note No. LC13/2001 (21)]. Oligonucleotides were designed using the Primer 0.5 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). For each amplicon, one primer covered an exon–exon border to avoid possible amplification of residual contaminating genomic DNA (see Table II). *TBP* was used as a reference gene as its expression level in lymphocytes was expected to be similar to that of the genes to be analyzed. The PCR efficiency was measured using triplicates of a cDNA dilution series (1:10; 1:50; 1:100 and 1:1000). PCR conditions were optimized for each gene in order to obtain PCR efficiencies close to the theoretically optimal value of 2.0.

The thermal cycling conditions were 1 cycle of 10 min at 95°C, 45 cycles of 15 s denaturation at 95°C, 5 s hybridization at 56°C for *ATLTP6*, *ERCC1* and *MDM2* or 58°C for *TBP* and *TP53* and 10 s elongation at 72°C. Crossing points, representing the PCR cycles at which the PCR products were detectable, were determined for each sample using the second derivative maximum method (LightCycler 3.5.3 software, Roche Diagnostics). Quality control was ensured by a non-template control and the determination of the melting point of each PCR product. Only samples with exact concordance with the melting point of a sequence-proofed amplicon were included. In each LightCycler run, the calibrator sample was measured in triplicate and patient samples were determined in duplicate. Mean crossing point values were used for further calculations. Variability of the real-time PCRs was 8.5%. To determine variation of RT and the PCR together, each RT reaction was spiked with 0.05 ng *ATLTP6* mRNA from *Arabidopsis thaliana* (Stratagene, La Jolla, CA, USA)

Table I. Selected characteristics of the study cohort

Characteristics	Patients[a]		Concentration
	n	%	Median (range)
Age at radiotherapy (years, $n = 405$)			
48–59	39	9.6	
60–64	73	18	
65–69	122	30.1	
70–74	105	25.9	
75+	66	16.3	
Mean ± SD	68 ± 6.7		
BMI (kg/m^2, $n = 404$)			
Normal (18.5–24.9)	121	30.0	
Overweight (25–29.9)	217	53.6	
Adipose (≥30)	66	16.3	
Mean ± SD	27 ± 3.5		
Smoking ($n = 401$)			
Never smokers	192	47.4	
Ex-smokers	174	43.0	
Current smokers	35	8.6	
Tobacco consumption (pack-years[b], $n = 373$)			
0	192	47.4	
1–19	90	22.2	
≥20	91	22.5	
Alcohol consumption (g/day, $n = 397$)			
0	70	17.3	
1–18	150	37.0	
≥19	177	47.7	
Plasma antioxidants (µmol/l, $n = 380$)			
Ascorbic acid	380	93.8	79.83 (12.02–433.57)
α-Carotene	380	93.8	0.10 (0.0–1.25)
β-Carotene	380	93.8	0.30 (0.0–2.45)
Canthaxanthin	380	93.8	0.03 (0.0–0.37)
Cryptoxanthin	380	93.8	0.14 (0.0–1.39)
Lycopene	380	93.8	0.49 (0.0–1.58)
Lutein	380	93.8	0.08 (0.0–0.49)
α-Tocopherol	380	93.8	30.54 (8.32–142.68)
γ-Tocopherol	380	93.8	1.17 (0.0–25.68)

[a]n = number of patients.
[b]Pack-years = (average number of packs per day) × (total number of years of smoking).

Table II. Primer and probes for real-time PCR quantification and genotyping

Gene/SNP[a]	Sequence of primers or probes[b]
Quantification	
ATLTP6	F: 5′-CAGGCTCAAACTTCTGTGGA-3′ R: 5′-GGGAGATCGACACCACATTT-3′
ERCC1	F: 5′-TGTCCAGGTGGATGTGAAAG-3′ R: 5′-AGGATCAATGTGCAGTCGG-3′
MDM2	F: 5′-ATCGAATCCGGATCTTGATG-3′ R: 5′-TCTTGTCCTTCTTCACTAA GGC-3′
TBP	F: 5′-AGCCAAGAGTGAAGAACA GTCC-3′ R: 5′-CCTGGGCATCCTTGAGTTC-3′
TP53	F: 5′-CACCCTTCAGATCCGTGG-3′ R: 5′-CCTGGGCATCCTTGAGTTC-3′
Genotyping	
ERCC1 rs3212986 C>A, chr. 19:50604576 (C8092A)	F: 5′-CCTGAAGCCAGGGCAACT-3′ R: 5′-AGCTGCCAAGGAAACCC-3′ S: 5′-GACAAGAAGCGGAAG**C**AG CAGC-FL-3′ A: 5′-LC RED 640-GCAGCAGCCT GTGTAGTCTGCCCCC-3′
ERCC1 rs11615 T>C, chr. 19:50615493 (Asn118Asn)	F: 5′-TTCCTGAAGTCTGGGGTGG-3′ R: 5′-GGCCCTGTGGTTATCAAGG-3′ S: 5′-CGCAA**C**GTGCCCTGGGAAT-FL-3′ A: 5′-LC RED 640-TGGCGACGTA ATTCCCGACTATGTGCTG-3′
MDM2 rs2279744 T>G, chr. 12:67488847 (SNP309)	F: 5′-GAGGTTTTGTTGGACTGGGG-3′ R: 5′-CCTTTTACTGCAGTTTCGG AAC-3′
TP53 rs17878362, chr. 17:7520369 16 bp insertion (p53PIN3)	F: 5′-CTGAAAACAACGTTCTGGTA-3′ R: 5′-AAGGGGGACTGTAGATGG GTG-3′

SNP, single-nucleotide polymorphism.
[a]SNPs are given with rs number and chromosomal location (chr.). In brackets: names published earlier.
[b]F, forward primer; R, reverse primer; S, sensor probe with the position of polymorphism marked; A, anchor probe; FL, fluorescein; LC RED 640, LightCycler® Red 640.

Recovery of this spike by quantitative real-time PCR served as a control to measure efficiency of the RT step. A coefficient of variation of 17% resulted for RT and quantification determined by RT–PCR.

Genotyping

Genomic DNA was isolated from whole blood samples using the Qiagen QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All primers and probes are given in Table II. The polymorphisms *ERCC1* rs3212986 C>A (chromosomal locus 19:50604576) and *ERCC1* rs11615 T>C (chromosomal locus 19:50615493) (22) were determined by rapid capillary PCR using sequence-specific probes and melting curve analysis in a LightCycler 2.0 (software version 4.0, Roche Diagnostics). Probes were designed and prepared by Tib Molbiol (Berlin, Germany). Analysis was performed in 10 µl volumes in glass capillaries (Roche Diagnostics) using Qiagen reagents: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 0.1% bovine serum albumin in 2.5% glycerol, 0.5 U *Taq* DNA polymerase, 0.5 µM of each primer, 0.09 µM of each probe for *ERCC1* rs3212986 C>A, 0.045 µM of each probe for *ERCC1* rs11615 T>C and 10 ng of DNA. Reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 59°C (*ERCC1* rs3212986) and 57°C (*ERCC1* rs11615) for 10 s and elongation at 72°C for 12 s. Melting curve analysis was performed with an initial denaturing step at 95°C for 30 s, followed by 45°C for 20 s, slow heating to 80°C with a ramping rate of 0.2°C/s and continuous fluorescence detection. Ten percent of all samples were randomly selected and repeated with this method. In addition, 10% of the samples were analyzed by restriction fragment length polymorphism (23). Genotyping results showed 100% concordance.

The *TP53* rs7520369 (p53PIN3, chromosomal locus 17:7520369) polymorphism, a 16 bp insertion, was determined using the method of Tan *et al.* (18). The *MDM2* rs2279744 T>G polymorphism (chromosomal locus 12:67488847) in the promoter region was determined using a restriction fragment length polymorphism method modified according to Sotamaa *et al.* (24). Analysis was performed in 20 µl volume using Qiagen reagents: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 1.25 U *Taq* DNA polymerase, 0.5 µM of each primer and 20 ng of DNA. Reaction conditions were as follows: initial denaturation at 94°C for 3 min, 40 cycles of denaturation at 94°C for 20 s, annealing at 62.2°C for 20 s, elongation at 72°C for 20 s and a final extension of 3 min at 72°C. The 312 bp PCR products were digested for 16 h in a volume of 10 µl with *MspA1I* (New England Biolabs, Frankfurt, Germany). The visualization on a 3% agarose gel showed three fragments (163, 103 and 46 bp) for the homozygous G-allele carrier and two fragments (209 and 103 bp) for the homozygous T-allele carrier. The PCR product had an additional restriction site resulting in the 103 bp fragment to control for complete digestion. Ten percent of the samples were randomly selected and repeated. A non-template control was ncluded in each amplification set. Genotypes for all polymorphisms were evaluated by two independent observers. Genotyping results for all polymorphisms showed 100% concordance.

Analysis of antioxidant levels

Plasma of each patient was separated from whole blood during lymphocyte isolation and was stored at −80°C. Nine different antioxidants were measured in 380 samples corresponding to 94% of the study population (Table I). High-performance liquid chromatography analyses of tocopherols (α and γ) and carotenoids (α-, β-carotene, canthaxanthin, cryptoxanthin, lycopene and lutein) were done as described by Hess *et al.* (25). Carotenoids have been estimated to be stable for at least 15 years at temperatures below −70°C (26). Ascorbic acid concentrations were determined photometrically at a wavelength of 520 nm (27). Concerning plasma carotenoids and vitamin E, recovery of the standard substances added to plasma samples laid between 94 and 107%, and the coefficient of variation calculated from repeated ($n = 5$) measurements of samples was 2.3%. Mean recovery of ascorbic acid added to plasma samples was 101.6% and the coefficient of variation was 0.7% ($n = 7$).

Statistical analysis

The non-parametric Kruskal–Wallis test was used to determine differences between three categorical values and continuous variables, the non-parametric Wilcoxon test for comparison of two categories. Spearman's rank correlation test was used to analyze for correlation. Each polymorphism was tested for deviation from Hardy–Weinberg equilibrium by comparing the observed and expected genotype frequencies using the chi-square test. Linkage disequilibrium of the two different markers in *ERCC1* (rs3212986 C>A and rs11615 T>C) was estimated using the 'Estimating Haplotypes' program (28), and haplo- and diplotypes were reconstructed using the Phase program, version 2.1 (29). Odds ratios and 95% confidence intervals were computed using multivariate unconditional logistic regression analysis. Estimates were produced by the PHREG procedure of the statistical software package SAS release 9.1 (SAS Institute, Cary, NC).

Results

mRNA expression levels of ERCC1, MDM2 and *TP53*

mRNA levels of *ERCC1*, *MDM2* and *TP53* were determined in lymphocyte samples taken from prostate cancer patients prior to radiotherapy. Complete data for all three genes were obtained in 376 (93%) out of 405 patient samples. Missings were due to insufficient RNA quality (4%) and insufficient PCR product quality (3%).

Median mRNA expression values and ranges are summarized in Table III. Considerable interindividual differences in mRNA levels were observed among study subjects being highest for *MDM2* with up to 37-fold variation, followed by *TP53* with up to 25-fold and *ERCC1* with up to 12-fold variation. Coefficients of variation were calculated from these expression values resulting in 43% for *MDM2*, 35% for *TP53* and 45% for *ERCC1*. This variability clearly exceeded the value observed for experimental variation (17%). Significant correlations of mRNA expression levels were observed among the genes being strongest for *TP53* and *MDM2* (correlation coefficient $r = 0.49$, $P < 0.0001$) and weakest for *MDM2* and *ERCC1* ($r = 0.32$, $P < 0.0001$).

The effects of different anthropometric and lifestyle factors on mRNA expression were studied. Spearman correlation analyses between age, BMI, alcohol consumption or smoking habits and constitutive mRNA expression showed no statistically significant associations ($P > 0.05$). Similarly, plasma concentrations of ascorbic acid, tocopherols (α and γ) and carotenoids (α-, β-carotene,

canthaxanthin, cryptoxanthin, lycopene and lutein) were not significantly correlated with *MDM2* or *TP53* mRNA expression levels. *ERCC1* mRNA expression displayed a positive correlation with β-carotene levels ($r = 0.11$, $P = 0.03$) and a negative correlation with canthaxanthin ($r = -0.13$, $P = 0.02$) and lutein ($r = -0.12$, $P = 0.02$), respectively.

Table III. mRNA expression levels of *ERCC1*, *MDM2* and *TP53* and correlation among the genes

Variables[a]	Median of mRNA expression (range)	Correlation with *MDM2* mRNA expression	Correlation with *TP53* mRNA expression
		r^b (*P* value)	r^b (*P* value)
ERCC1	0.65 (0.23–2.76)	0.32 (<0.0001)	0.43 (<0.0001)
MDM2	1.19 (0.12–4.44)	—	0.49 (<0.0001)
TP53	0.89 (0.11–2.74)	—	—

[a]Measured in 376 patients.
[b]Spearman's rank correlation coefficients.

Genotyping results

Genotype distributions of the four polymorphisms [*ERCC1* rs11615 T>C, *ERCC1* rs3212986 C>A, *MDM2* rs2279744 T>G and *TP53* rs17878362 (p53PIN3)] were determined for 376 patients (Table IV). The allele frequencies were generally consistent with the published reports on *ERCC1* rs11615 T>C, *ERCC1* rs3212986 C>A (11,23), *MDM2* rs2279744 T>G (30) and *TP53* rs17878362 (p53PIN3) (19). All genotype distributions were in Hardy–Weinberg equilibrium. A high correlation was found between *ERCC1* rs11615 T>C and *ERCC1* rs3212986 C>A polymorphisms indicating linkage disequilibrium ($P = <0.0001$) (23). After reconstruction of diplotypes, association with *ERCC1* expression was analyzed. Eight different diplotypes were observed in this study population (Table V).

Association of mRNA expression and genotype

For both *ERCC1* polymorphisms, rs11615 T>C and rs3212986 C>A, lower *ERCC1* mRNA levels were associated with an increasing

Table IV. Association of genotypes and mRNA expression

Gene	Polymorphism	Variant	Number of patients, $n = 376$ (%)	Frequency of variant allele	mRNA expression, median (range)	Comparison of mRNA expression, *P* value (Kruskal–Wallis)
ERCC1	rs3212986 C>A	CC	212 (56.3)	0.26	0.64 (0.23–2.75)	0.59
		CA	132 (35.2)		0.65 (0.23–1.72)	
		AA	32 (8.5)		0.67 (0.33–2.00)	
ERCC1	rs11615 T>C	TT	138 (36.8)	0.39	0.66 (0.24–2.75)	0.46
		TC	180 (47.8)		0.63 (0.23–2.01)	
		CC	58 (15.4)		0.60 (0.23–2.00)	
MDM2	rs2279744 T>G	TT	153 (40.7)	0.36	1.19 (0.12–3.21)	0.93
		TG	179 (47.6)		1.19 (0.29–4.44)	
		GG	44 (11.7)		1.32 (0.33–2.21)	
TP53	rs17878362 16 bp insertion	A1/A1[a]	269 (71.5)	0.15	0.88 (0.11–2.74)	0.50
		A1/A2[b]	98 (26.1)		0.91 (0.43–1.78)	
		A2/A2	9 (2.4)		0.83 (0.39–1.41)	

[a]A1, without 16 bp insertion.
[b]A2, with 16 bp insertion.

Table V. Association of *ERCC1* mRNA expression and *ERCC1* diplotypes and risk of low expression values according to the diplotype

ERCC1 diplotypes[a]	Number of patients [total = 376, *n* (%)]	Median of mRNA expression (range)	Test for difference *P* value	Number of patients with low mRNA levels[b], *n* (%)	Risk for low mRNA expression		
					Crude odds ratio (95% confidence interval)	Adjusted odds ratio[c] (95% confidence interval)	*P* value
CT:CT	134 (35.6)	0.66 (0.24–2.75)	0.03[d]	67 (50)	Ref.	Ref.	—
CT:AC	107 (28.5)	0.66 (0.31–1.72)		54 (50.5)	0.97 (0.58–1.61)	0.89 (0.51–1.55)	0.70
AC:AC	31 (8.2)	0.66 (0.33–2.00)		16 (51.6)	1.09 (0.50–2.40)	1.86 (0.70–4.91)	0.20
CC:CT	71 (18.9)	0.57 (0.23–2.01)		47 (66.1)	1.78 (0.98–3.21)	1.92 (1.01–3.64)	0.04
CC:AC	20 (5.3)	0.54 (0.23–1.22)		12 (60)	1.54 (0.59–4.02)	1.65 (0.61–4.46)	0.32
CC:CC	7 (1.9)	0.55 (0.27–1.64)		4 (57.1)	Not calculated		
CT:AT	5 (1.3)	0.64 (0.50–1.05)		3 (60)			
AC:AT	1 (0.3)	0.79 (—)		0 (0)			
Non-carrier	278 (73.1)	0.66 (0.24–2.75)	0.001[e]	138 (49.6)	Ref.	Ref.	—
CC carrier	98 (26.1)	0.56 (0.23–2.01)		62 (63.3)	1.69 (1.06–2.71)	1.76 (1.07–2.93)	0.03

[a]Each diplotype consists of a pair of haplotypes that are composed by the respective nucleotide in position of rs3212986 (C or A) followed by the nucleotide in position of rs11615 (T or C).
[b]Dichotomized by median value of the respective reference diplotype.
[c]Due to missing values, the calculation is based on a case number of 348, adjusted for age, alcohol and tobacco consumption and antioxidant plasma levels.
[d]Kruskal–Wallis test.
[e]Wilcoxon test.

number of the respective C allele. This association was, however, not statistically significant (Table IV). Furthermore, there was no correlation of mRNA levels with *MDM2* and *TP53* genotypes.

ERCC1 mRNA expression stratified according to diplotype groups is shown in Table V. Diplotype groups representing <2% of the total study population were excluded from statistical analysis. Significant differences in mRNA expression were found among the remaining five diplotype groups ($P = 0.03$). As CC haplotype carriers (CC carriers) showed decreased mRNA expression in relation to other groups, they were further analyzed and compared with non-CC carriers. The median mRNA expression value of 0.56 in CC carriers was remarkably lower than that in non-carriers (0.66). Using Wilcoxon's test, these differences in mRNA expression were found to be highly significant ($P = 0.001$).

Low *ERCC1* mRNA expression might be associated with reduced cellular DNA repair activity that could lead to increasing genomic instability and better therapy response. We therefore applied a logistic regression model to assess the effect of the diplotypes and haplotypes on the risk for having low mRNA expression (Table V). The risk estimates in this model were adjusted for factors that potentially modify mRNA levels such as antioxidant plasma levels, age, alcohol and tobacco consumption. When carriers of the most frequent diplotype CT:CT were used as reference, an increased odds ratio of 1.92 (95% confidence interval 1.01–3.64) was observed for reduced mRNA expression in CC:CT diplotype carriers. Comparing with non-carriers, CC carriers had an adjusted odds ratio of 1.69 (95% confidence interval: 1.06–2.71) for having low mRNA expression.

Discussion

The aim of this study was to analyze whether genetic variation in DNA damage response or repair genes contributes to mRNA expression measured in peripheral blood lymphocytes. Earlier reports suggested that *ERCC1* rs11615 T>C, *ERCC1* rs3212986 C>A, *MDM2* rs2279744 T>G and *TP53* rs17878362 (p53PIN3) polymorphisms affect mRNA levels in various cell lines (11,15,17,19). In our study with primary lymphocytes of 376 individuals, the observed effects of these polymorphisms, when analyzed separately, were too weak to reach statistical significance. Trends similar to published effects were, however, observed for some polymorphisms. Bond *et al.* (17) observed elevated *MDM2* mRNA expression in cell lines of homozygous GG carriers at *MDM2* rs2279744 T>G. We also found a higher median *MDM2* mRNA level in homozygous GG individuals as compared with T-allele carriers. A decreased mRNA expression was described in cells carrying *TP53* rs17878362 (p53PIN3) (A2/A2) compared with (A1/A1) carrying cells (19). We observed similar differences in mRNA expression with primary lymphocytes carrying these genotypes. No effect of the rs3212986 C>A genotype on *ERCC1* mRNA expression was reported in a small study investigating 67 Korean head and neck cancer patients (31). This observation was confirmed by our results. TT carriers of the *ERCC1* rs11615 T>C polymorphism showed higher *ERCC1* mRNA expression levels in our study, but this is in contrast to results observed in two cell lines carrying this genetic variant (15). Elevated *ERCC1* expression in T-allele carriers was also observed in colorectal tumor tissue (32).

In contrast to the analyses of single polymorphisms, we found a distinct genotype combination of *ERCC1* (CC) that was significantly associated with a decrease in mRNA values. Reconstruction of *ERCC1* haplotypes and subsequent statistical evaluation revealed a distinctly lower mRNA level for CC carriers than for non-carriers ($P = 0.001$). As already one CC allele reduced mRNA expression, it could be argued that the CC haplotype exerts a dominant-negative effect on the regulation of *ERCC1* mRNA expression. This would imply that the genetically polymorphic nature of *ERCC1* can modulate mRNA levels in prostate cancer patients and thus affect therapy outcome. Indeed, different mRNA levels were observed in two study populations when *ERCC1* expression was used as a predictive marker for therapy outcome (13,33,34).

There are more single-nucleotide polymorphisms known in *ERCC1*, *MDM2* and *TP53* than have been analyzed in our study. We can therefore not rule out that other polymorphisms will show stronger correlations with mRNA expression for these genes. We restricted the analysis to selected genetic variants for which measurable genotype–phenotype associations have already been reported, thereby also limiting the number of statistical tests and the risk of false-positive findings.

To avoid important experimental error sources during determination of RNA expression (35–37), we used relative quantification with real-time PCR after RT. This procedure is regarded as the most reliable method for RNA quantification at present (9,38). All experimental steps were optimized by controlling carefully for RNA and cDNA integrity, by applying *TBP* as a reference gene and by normalizing all measurements with a calibrator to adjust for experimental differences during PCRs. These measures increase the probability that the observed interindividual differences in mRNA expression are true differences not affected by high experimental variability. It cannot, however, be excluded that a minor part of these differences is introduced by the normalization step using *TBP* as a reference. *TBP* was selected (i) as it is widely accepted as a reliable reference gene (39–41) and (ii) because it showed an expression level which is comparable with that of the target genes. Validity of our expression data is also supported by the comparability with expression values published in similar studies where *TP53* mRNA expression was determined in lymphocytes of bladder cancer patients (5). The observed interindividual variation for *ERCC1* expression was slightly higher in our prostate cancer patients than it was reported in lung or head and neck cancer patients who were analyzed using conventional multiplex RT–PCR (7,42). To our knowledge, this is the first study to report interindividual variation in *MDM2* expression.

Significant correlations between the mRNA expression levels of *TP53*, *MDM2* and *ERCC1* were observed. The stronger correlation of *TP53* and *MDM2* expression might be explained by the tight functional interaction of these proteins when regulating DNA damage response (43).

The measured mRNA expression values could be affected by a number of endogenous and exogenous factors, such as age, tobacco consumption and antioxidant levels in plasma. A decrease in mRNA expression with increasing age has recently been reported for *ERCC1* and *TP53* (5,6). This was not detected in our study, probably because of the absence of younger patients within our study cohort with 90% being older than 60 years. Tobacco consumption had also no significant effect on mRNA expression in our study. This is in contrast to the association found for *ERCC1* levels and smoking in head and neck cancer patients (31) but may be due to the low percentage of current smokers in our cohort.

Antioxidants, e.g. from nutritional sources, are important cellular protective agents against oxidative damage and act in concert with other cellular defense mechanisms such as DNA repair (44). It is conceivable that antioxidants which modulate the intracellular levels of reactive oxidative species might also influence expression of the investigated genes. Our analyses showed no correlation of antioxidant levels in blood plasma with mRNA expression of *TP53* and *MDM2*. In contrast, *ERCC1* expression was positively associated with plasma levels of β-carotene and negatively correlated with canthaxanthin and lutein. There are controversial reports on the effect of carotenoids on mRNA expression of DNA repair genes. An intervention study with fruit, vegetables and antioxidants showed no effect of these components on *ERCC1* expression in healthy subjects (45). Astley *et al.* (46) reported, however, enhanced DNA repair activities when healthy subjects received a carotenoid-enriched diet. Our results show a discernible effect of antioxidants on *ERCC1* mRNA expression in prostate cancer patients. These patients might be inflicted with stronger oxidative stress due to their disease (47–49), making the effects of antioxidants more pronounced in patients compared with healthy subjects (50). Consequently, the observed modifying effect of antioxidants on *ERCC1* expression has to be considered when *ERCC1* mRNA expression is explored as a biomarker for therapy outcome.

We found low *ERCC1* mRNA expression in CC carriers after accounting for modifying factors such as antioxidants. Low *ERCC1* mRNA expression might be used as a risk marker as it has also been found to be related with reduced DNA repair capacity (51). Furthermore, low *ERCC1* expression in patients with ovarian tumors has been reported to be associated with increased clinical chemotherapy sensitivity (33). *ERCC1* expression in peripheral blood lymphocytes was recently found to reflect expression of this gene in head and neck cancer tissue, and it was suggested to be indicative for therapy success (31). The ratio of *ERCC1* mRNA expression in tumor head and neck versus normal tissue was reported to predict the response to neoadjuvant therapy with cisplatin, 5-fluorouracil and radiation (34).

In summary, our results demonstrate considerable interindividual differences in *ERCC1* mRNA expression among 376 prostate cancer patients. These differences can be at least partially attributed to genetic variation but can also be modified by extrinsic and intrinsic factors such as antioxidants. Only weak effects of single polymorphisms on *ERCC1* expression were detected but carriers of *ERCC1* CC haplotype had significantly lower expression values than non-carriers. This low *ERCC1* expression might be associated with reduced DNA repair and better chemotherapy or radiotherapy response. Our study used primary lymphocytes as suitable surrogate cells to assess the impact of molecular markers in clinical/epidemiological settings, this is because target tissue samples are more difficult to obtain and only available by using invasive procedures (2). Published studies that aimed to characterize the functional impact of polymorphisms were mainly performed in immortalized tumor cell lines. However, these cells might have cellular characteristics considerably different from those of primary lymphocytes thus explaining some of the observed inconsistencies. Therefore, our *ex vivo* results in patients contribute to a better understanding of the impact of genetic variants on mRNA expression of DNA repair genes. Once validated in further analyses, such markers should provide valuable tools for the assessment of therapy response.

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References

1. Mader,R.M. (2006) Links between biology, prognosis and prediction of response to chemotherapy in colorectal cancer. *Onkologie*, **29**, 334–341.
2. Burczynski,M.E. *et al.* (2005) Transcriptional profiles in peripheral blood mononuclear cells prognostic of clinical outcomes in patients with advanced renal cell carcinoma. *Clin. Cancer Res.*, **11**, 1181–1189.
3. Bartsch,H. *et al.* (2007) Genetic risk profiles for cancer susceptibility and therapy response. *Recent Results Cancer Res.*, **174**, 19–36.
4. Efferth,T. *et al.* (2005) Pharmacogenetics for individualized cancer chemotherapy. *Pharmacol. Ther.*, **107**, 155–176.
5. Gu,J. *et al.* (2005) Roles of tumor suppressor and telomere maintenance genes in cancer and aging–an epidemiological study. *Carcinogenesis*, **26**, 1741–1747.
6. Vogel,U. *et al.* (2006) ERCC1, XPD and RAI mRNA levels in lymphocytes are not associated with lung cancer risk in a prospective study of Danes. *Mutat. Res.*, **593**, 88–96.
7. Cheng,L. *et al.* (2002) Expression of nucleotide excision repair genes and the risk for squamous cell carcinoma of the head and neck. *Cancer*, **94**, 393–397.
8. Chalabi,N. *et al.* (2004) The effects of lycopene on the proliferation of human breast cells and BRCA1 and BRCA2 gene expression. *Eur. J. Cancer*, **40**, 1768–1775.
9. Bustin,S.A. *et al.* (2004) Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. *J. Biomol. Tech.*, **15**, 155–166.
10. Suk,R. *et al.* (2005) Polymorphisms in ERCC1 and grade 3 or 4 toxicity in non-small cell lung cancer patients. *Clin. Cancer Res.*, **11**, 1534–1538.
11. Chen,P. *et al.* (2000) Association of an ERCC1 polymorphism with adult-onset glioma. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 843–847.
12. Isla,D. *et al.* (2004) Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann. Oncol.*, **15**, 1194–1203.
13. Reed,E. (2005) ERCC1 and clinical resistance to platinum-based therapy. *Clin. Cancer Res.*, **11**, 6100–6102.
14. Ryu,J.S. *et al.* (2004) Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer*, **44**, 311–316.
15. Yu,J.J. *et al.* (2000) Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. *Int. J. Oncol.*, **16**, 555–560.
16. Ohmiya,N. *et al.* (2006) MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. *J. Clin. Oncol.*, **24**, 4434–4440.
17. Bond,G.L. *et al.* (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell*, **119**, 591–602.
18. Tan,X.L. *et al.* (2006) Association between TP53 and p21 genetic polymorphisms and acute side effects of radiotherapy in breast cancer patients. *Breast Cancer Res. Treat.*, **97**, 225–262.
19. Gemignani,F. *et al.* (2004) A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. *Oncogene*, **23**, 1954–1956.
20. Chomczynski,P. *et al.* (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159.
21. Pfaffl,M.W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.*, **29**, e45.
22. Yin,J. *et al.* (2002) Rapid genotyping of human ERCC1 exon 4 polymorphism with fluorescence analysis using fluorophore-labeled hybridization probes and a lightcycler. *Biochem. Genet.*, **40**, 143–148.
23. Zhou,W. *et al.* (2004) Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin. Cancer Res.*, **10**, 4939–4943.
24. Sotamaa,K. *et al.* (2005) p53 codon 72 and MDM2 SNP309 polymorphisms and age of colorectal cancer onset in Lynch syndrome. *Clin. Cancer Res.*, **11**, 6840–6844.
25. Hess,D. *et al.* (1991) Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int. J. Vitam. Nutr. Res.*, **61**, 232–238.
26. Comstock,G.W. *et al.* (1993) Reported effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in serum or plasma summarized. *Clin. Chem.*, **39**, 1075–1077.
27. Speitling,A. *et al.* (1992) Methodenhandbuch der Verbundstudie Ernährungserhebung und Risikofaktoren Analytik. Wissenschaftlicher Fachverlag Dr. Fleck, Niederkleen, Germany.
28. Xie,X. *et al.* (1993) Testing linkage disequlibrium between a disease gene and marker loci. *Am. J. Hum. Genet.*, **53** (suppl): 313.
29. Stephens,M. *et al.* (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet.*, **73**, 1162–1169.
30. Pine,S.R. *et al.* (2006) MDM2 SNP309 and SNP354 are not associated with lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 1559–1561.
31. Yang,M. *et al.* (2006) Effects of ERCC1 expression in peripheral blood on the risk of head and neck cancer. *Eur. J. Cancer Prev.*, **15**, 269–273.
32. Park,D.J. *et al.* (2002) ERCC1 polymorphism is associated with differential ERCC1 mRNA levels. *Proc. AACR 2002*, **321**.
33. Reed,E. (1998) Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. *Cancer Treat. Rev.*, **24**, 331–344.
34. Warnecke-Eberz,U. *et al.* (2004) High specificity of quantitative excision repair cross-complementing 1 messenger RNA expression for prediction of minor histopathological response to neoadjuvant radiochemotherapy in esophageal cancer. *Clin. Cancer Res.*, **10**, 3794–3799.

35. Hurteau,G.J. *et al*. (2002) mRNA-specific reverse transcription-polymerase chain reaction from human tissue extracts. *Anal. Biochem.*, **307**, 304–315.
36. Ovstebo,R. *et al*. (2003) PCR-based calibration curves for studies of quantitative gene expression in human monocytes: development and evaluation. *Clin. Chem.*, **49**, 425–432.
37. Stahlberg,A. *et al*. (2004) Properties of the reverse transcription reaction in mRNA quantification. *Clin. Chem.*, **50**, 509–515.
38. Bustin,S.A. *et al*. (2005) Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clin. Sci. (Lond)*, **109**, 365–379.
39. Almeida,A. *et al*. (2004) Gene expression analysis by real-time reverse transcription polymerase chain reaction: influence of tissue handling. *Anal. Biochem.*, **328**, 101–108.
40. Lapillonne,H. *et al*. (2006) High WT1 expression after induction therapy predicts high risk of relapse and death in pediatric acute myeloid leukemia. *J. Clin. Oncol.*, **24**, 1507–1515.
41. Radonic,A. *et al*. (2005) Reference gene selection for quantitative real-time PCR analysis in virus infected cells: SARS corona virus, Yellow fever virus, Human Herpesvirus-6, Camelpox virus and cytomegalovirus infections. *Virol. J.*, **2**, 7.
42. Cheng,L. *et al*. (2000) Reduced expression levels of nucleotide excision repair genes in lung cancer: a case-control analysis. *Carcinogenesis*, **21**, 1527–1530.
43. Harris,S.L. *et al*. (2005) The p53 pathway: positive and negative feedback loops. *Oncogene*, **24**, 2899–2908.
44. Moller,P. *et al*. (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutat. Res.*, **551**, 79–89.
45. Moller,P. *et al*. (2003) No effect of 600 grams fruit and vegetables per day on oxidative DNA damage and repair in healthy nonsmokers. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 1016–1022.
46. Astley,S.B. *et al*. (2004) Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes. *Br. J. Nutr.*, **91**, 63–72.
47. Chen,L. *et al*. (2001) Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J. Natl Cancer Inst.*, **93**, 1872–1879.
48. Dakubo,G.D. *et al*. (2006) Altered metabolism and mitochondrial genome in prostate cancer. *J. Clin. Pathol.*, **59**, 10–16.
49. El Zein,R. *et al*. (2005) Chromosomal instability in peripheral blood lymphocytes and risk of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 748–752.
50. Stacewicz-Sapuntzakis,M. *et al*. (2005) Role of lycopene and tomato products in prostate health. *Biochim. Biophys. Acta*, **1740**, 202–205.
51. Vogel,U. *et al*. (2000) DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation to mRNA levels in primary lymphocytes. *Mutat. Res.*, **461**, 197–210.

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