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Modulation of nucleotide excision repair in human lymphocytes by genetic and dietary factors

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Gene–environment interactions determine inter-individual variations in nucleotide excision repair (NER) capacity. Oxidative stress was previously found to inhibit NER, thus supplementation with dietary antioxidants could prevent this inhibition, especially in genetically susceptible subjects. To study the effects of genetic polymorphisms in NER-related genes and dietary intake of antioxidants on an individual's NER capacity, lymphocytes of 168 subjects were isolated before and after a 4-week blueberry and apple juice intervention. Twelve genetic polymorphisms in NER genes *XPA*, *XPC*, *ERCC1*, *ERCC2*, *ERCC5*, *ERCC6* and *RAD23B* were assessed by multiplex PCR with single base extension. Based on specific genotype combinations, a subset of individuals (*n* 36) was selected for phenotypical assessment of NER capacity, which was significantly affected by the total sum of low-activity alleles ($P=0{\cdot}027$). The single polymorphism *XPA* G23A was the strongest predictor of NER capacity ($P=0{\cdot}002$); carriers of low-activity alleles AA had about three times lower NER capacity than *XPA* GG carriers. NER capacity assessed before and after intervention correlated significantly (R^2 0·69; $P<0{\cdot}001$), indicating that inter-individual differences in NER capacity are maintained over 4 weeks. Although the intervention increased plasma trolox equivalent antioxidant capacity from 791 (SE 6·61) to 805 (SE 7·90) μM ($P=0{\cdot}032$), on average it did not affect NER capacity. Nonetheless, carriers of twelve or more low-activity alleles seemed to benefit from the intervention ($P=0{\cdot}013$). Among these, carriers of the variant allele for *RAD23B* Ala249Val showed improved NER capacity upon intervention ($P=0{\cdot}020$). In conclusion, improved NER capacity upon dietary intervention was detected in individuals carrying multiple low-activity alleles. The *XPA* G23A polymorphism might be a predictor for NER capacity.

Nucleotide excision repair: Single nucleotide polymorphisms: Dietary modulation

Molecular epidemiological studies have reported large inter-individual variations in susceptibility to environmental carcinogens and subsequent cancer risk, which may partly be due to genetically determined variations in nucleotide excision repair (NER) capacity[1–4]. The NER pathway protects the integrity of the genome by recognising and eliminating a broad spectrum of bulky lesions such as UV-induced pyrimidine dimers, aromatic DNA adducts, and cross-links. Moreover, several NER-related enzymes have been shown to play a role in the cellular protection against certain types of oxidative DNA damage (including thymine glycols, 8-oxoguanine and cyclodeoxyadenosine), most likely by acting as a cofactor in base excision repair (BER)[5–7]. Thus NER is a versatile DNA repair system, involving the joint action of a variety of enzymes such as XPC–RAD23B, CSB, XPA, XPF–ERCC1, and others[8,9]. It is clear that NER plays a crucial role in cancer prevention, because defects in this pathway lead to several severe human disorders, such as xeroderma pigmentosum[10]. Furthermore, several studies suggest that genetic polymorphisms in various NER genes may have a profound impact on the phenotypical activity of this repair pathway[11–13]. In addition, various genetic polymorphisms in DNA-repair genes have been shown to modulate the levels of bulky DNA adducts[11,14,15] or chromosomal damage[16–18]. So, a number of studies identified associations between polymorphisms in DNA-repair genes with the amount of DNA damage and the capacity to repair these damages.

Next to the effect of single nucleotide polymorphisms (SNP) on DNA-repair activity, also other factors, such as diet and specific dietary compounds, are thought to modulate DNA-repair capacities. Although there is sufficient evidence for chemopreventive effects of certain dietary compounds[19], only a few studies have reported that dietary compounds influence DNA-repair processes (for a review,

Abbreviations: BER, base excision repair; BPDE, benzo[a]pyrene-diolepoxide; ERCC1, excision repair cross-complementing group 1; NER, nucleotide excision repair; RAD23B, RAD23 homologue B (one of two human homologues of *Saccharomyces cerevisiae* Rad23); SBE, single base extension; SNP, single nucleotide polymorphism; TEAC, trolox equivalent antioxidant capacity; TI, tail intensity; TM, tail moment; XPA, xeroderma pigmentosis, complementation group A; XPC, xeroderma pigmentosis, complementation group C.

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see Tyson & Mathers[20]). Several of these studies investigated the dietary modulation of BER or the repair of oxidative lesions. For example, a 3-week intervention with one, two or three kiwi fruits resulted in a significant increase of the BER capacity, as measured by a modified comet assay[21]. In fact, to the best of our knowledge, there are only two studies that investigated the effect of dietary factors on NER capacity in human subjects. Wei *et al.* observed an association between low dietary folate intake and reduced NER capacity[22], while Tyson *et al.* reported no detectable effect of micronutrient supplementation on NER capacity[23]. Therefore, there is an increasing need to study the impact of diet on NER capacity.

We previously showed that especially oxidative stress can inhibit NER capacity[24,25]. Thus, enhanced dietary intake of antioxidants may represent an opportunity for improving NER by reducing oxidative stress. Therefore, we studied the dietary modulation of DNA repair by using samples from a 4-week intervention study with healthy volunteers, consuming 1 litre of blueberry and apple juice per d[26]. This intervention was found to be efficient in enhancing antioxidant defence and reducing the levels of *ex vivo*-induced oxidative DNA damage[26,27]. In the present study, we hypothesised that NER capacity is determined by polymorphisms in DNA-repair genes and that diet may modulate an individual's NER capacity. Therefore, our aims were to (i) investigate the effect of the blueberry and apple juice intervention on the NER capacity; (ii) determine the effect of genetic polymorphisms in NER genes on the phenotypic NER capacity; (iii) identify possible gene–diet interactions.

Materials and methods

Study population

The study population consisted of 168 healthy volunteers, 114 female and fifty-four male, aged 18–45 years (for more details, see references[26,27]). Volunteers were recruited through advertisement in local newspapers and were included if they were non-smokers, did not use any medication (except for oral contraceptives) or any vitamin supplementation. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Maastricht University and the Academic Hospital Maastricht. Written informed consent was obtained from all subjects.

Dietary intervention and study design

The design of the present intervention as well as the efficacy of the washout period was based on a pilot study, described previously[26,27]. Briefly, in a paired design, each subject acted as his or her own control. A 5 d washout period was followed by an intervention period of 4 weeks with a custom-made blueberry and apple juice mixture, produced specifically for the present study by Riedel Drinks (now Friesland Foods, Ede, The Netherlands), of which subjects consumed 1 litre/d. This blueberry and apple juice mixture was about 1·85 times more concentrated than regular fruit juices of Riedel Drinks, consisting of 135 % blueberry juice and 50 % apple juice. As a consequence, it contained high levels of antioxidants, predominantly in the form of flavonoids. Supplementation with this blueberry and apple juice for 4 weeks was reported to be effective, as the intervention significantly increased total plasma antioxidant capacity (trolox equivalent antioxidant capacity (TEAC); $P<0{\cdot}001$) and reduced the levels of *ex vivo*-induced oxidative DNA damage by 20 % ($P=0{\cdot}006$)[26]. The impact of seasonal variation in dietary habits or increased sensitivity was overcome by year-round random sampling.

Collection of samples

After the 5 d washout period and a second time after the 4-week intervention period, blood samples were obtained between 08.00 and 09.00 hours by venepuncture. Volunteers were allowed to have breakfast before sampling, but no juice. Venous blood samples were obtained into one 10 ml EDTA vacuum tube for plasma analyses and into two 10 ml vacuum lithium heparin tubes (venoject II; Terumo-Europe, Leuren, Belgium) for isolation of lymphocytes. The EDTA tubes were centrifuged for 10 min at 265 ***g*** at 4°C to separate plasma for the analysis of the total plasma antioxidant capacity (TEAC) as described previously[26]. All plasma samples were kept at −80°C until analysis. Lymphocytes were isolated using a standard density gradient centrifugation method[28], sampled and stored as cell pellets at −20°C. One sample was used to isolate DNA for genotyping purposes, using standard phenol extraction procedures. Another lymphocyte sample was used to prepare protein extracts to phenotypically assess NER capacity.

Selection of polymorphisms for genotyping

In the present study, twelve SNP in NER genes (Table 1) were selected on the basis of (a) their association with cancer development, or (b) their expected influence on DNA repair based on literature review. DNA sequences and allele frequencies were obtained from the Cancer SNP 500 database (http://snp500cancer.nci.nih.gov). Of the twelve SNP analysed here, two have been described before by Wilms *et al.*[26]. In order to genotype the remaining ten SNP (Table 2) we further developed the multiplex PCR method. The development and validation of the adapted multiplex PCR for the new set of ten SNP in various NER genes was based on an approach as described before[29]. The adapted procedure is defined in the following paragraphs.

PCR primer design and multiplex PCR amplification

Primer 3 software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi) and Netprimer software (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html) were used to design PCR primers (for more detailed information, see Knaapen *et al.*[29]). Primers were obtained from Qiagen. First, the isolated DNA containing SNP was amplified in one eightplex and one duplex PCR reaction. For the eightplex PCR, a 10 μl reaction mixture was prepared containing PCR buffer, 10 mM-deoxynucleotide triphosphates, 50 mM-$MgCl_2$, Platinum® Taq Polymerase (5 U/μl; Invitrogen, Carlsbad, CA, USA) and template DNA (40 ng/μl). The final primer concentrations were 2·0 μM (for *XPC*-03, *ERCC6*-01, *ERCC1*-05, *ERCC1*-06 and *ERCC1*-30), 0·8 μM (for *XPA*-02), 1·6 μM

Table 1. Overview of single nucleotide polymorphisms (SNP) included in the present study and their expected effects on DNA-repair capacity

SNP*	Amino acid change	Changed base	Function within NER	Expected effect of variant allele on NER capacity	References	Activity alleles (0/1/2)†
XPA-02	5′-UTR	G → A	Second step of damage recognition (GGR and TCR); binds, stabilises open complex; confirms damage; recruits RPA, ERCC1	Less efficient NER capacity	10,42,48,49	GG/GA/AA
XPC-01	Lys939Gln	A → C	Initial damage recognition; binds lesion in complex with RAD23B (GGR); causes local unwinding; recruits other NER proteins	Reduced repair, higher adduct levels	2,9,48,52,53	AA/AC/CC
XPC-03	Ala499Val	C → T	Initial damage recognition; binds lesion in complex with RAD23B (GGR); causes local unwinding; recruits other NER proteins	Increased repair, lower adduct levels	9,54	TT/CT/CC
ERCC1-05	Asn118Asn	T → C	Forms a heterodimeric protein complex with XPF (also known as ERCC4); endonuclease – 5′ incision (GGR and TCR)	Higher NER capacity	38,42,44	CC/TC/TT
ERCC1-06	Intron 3	G → C	Forms a heterodimeric protein complex with XPF (also known as ERCC4); endonuclease – 5′ incision (GGR and TCR)	More efficient repair	55	CC/GC/GG
ERCC1-30	Gln504Lys	G → T	Forms a heterodimeric protein complex with XPF (also known as ERCC4); endonuclease – 5′ incision (GGR and TCR)	Lower NER capacity	38,42,43	GG/GT/TT
ERCC2-02	Asp312Asn	G → A	Also known as XPD protein; 5′ to 3′ helicase (GGR and TCR); part of the general transcription complex TFIIH; late DNA unwinding	Lower NER capacity, higher adduct levels	38,42,52,56,58	GG/GA/AA
ERCC2-03	Lys751Gln	A → C	Also known as XPD protein; 5′ to 3′ helicase (GGR and TCR); part of the general transcription complex TFIIH; late DNA unwinding	Lower NER capacity, higher adduct levels	12,38,42,43,52,53,56–58	AA/AC/CC
ERCC2-06	Arg156Arg	C → A	Also known as XPD protein; 5′ to 3′ helicase (GGR and TCR); part of the general transcription complex TFIIH; late DNA unwinding	Less efficient NER capacity	57,59	CC/CA/AA
ERCC5-01	His46His	C → T	Also known as XPG protein (GGR and TCR); endonuclease – 3′ incision; stabilises full open complex	More efficient NER capacity	42,44	TT/CT/CC
ERCC6-01	Met1097Val	A → G	Also known as CSB protein (TCR); recruits repair proteins such as TFIIH; displaces stalled transcription complex so that NER proteins can enter	Less efficient NER capacity	40,60	AA/AG/GG
RAD23B-04	Ala249Val	C → T	Initial damage recognition; binds lesion in complex with XPC (GGR); causes local unwinding; recruits other NER proteins	Less efficient NER capacity, higher adduct levels	2,3	CC/CT/TT

NER, nucleotide excision repair; *XPA*, xeroderma pigmentosis, complementation group A; UTR, untranslated region; GGR, global genome repair; TCR, transcription-coupled repair; RPA, replication protein 1; *ERCC1*, excision repair cross-complementing group 1; *XPC*, xeroderma pigmentosis, complementation group C; *RAD23B*, RAD23 homologue B (one of two human homologues of *Saccharomyces cerevisiae* Rad23); XPF, xeroderma pigmentosis, complementation group F; ERCC4, excision repair cross-complementing group 4; *ERCC2*, excision repair cross-complementing group 2; XPD, xeroderma pigmentosis, complementation group D; TFIIH, transcription factor IIH; *ERCC5*, excision repair cross-complementing group 5; XPG, xeroderma pigmentosis, complementation group G; *ERCC6*, excision repair cross-complementing group 6; CSB, Cockayne syndrome B.

* According to the Cancer SNP 500 database (http://snp500cancer.nci.nih.gov)[42,54–62].

† SNP were coded as low-activity alleles, judged on prior knowledge from published literature and their expected modulating effect on the NER capacity: 0 = homozygous for high-activity allele; 1 = heterozygous, carrying one high- and one low-activity allele; 2 = homozygous for low-activity allele.

Table 2. Overview of single nucleotide polymorphisms (SNP) included in the modified multiplex PCR, together with their PCR and single base extension (SBE) primers

SNP*	dbSNP ID*	PCR primers	Product (bp)	SBE primers†	Length (bp)
Run in an eightplex PCR					
XPA-02	rs1800975	Forward 5′-GCAGGCGCTCTCACTCAGAA-3′ Reverse 5′-TGCCGCTTCCGCTCGATA-3′	230	5′-AACTCGGCCGCCGCCATCTC-3′	20
XPC-01	rs2228001	Forward 5′-GCCTCAAAACCGAGAAGATG-3′ Reverse 5′-AGGTGTGGGGCCTGTAGTG-3′	178	5′-AACTGACTATACTACGGCTTCCCACCTGTTCCCATTTGAG-3′	40
XPC-03	rs2228000	Forward 5′-GGTCCAAGAGTGCCTCCAG-3′ Reverse 5′-TTCTGCCTTCTCACCATCG-3′	132	5′-AACTGACTAAACTGCTTGAAGAGCTTGAGGATGCC-3′	35
ERCC6-01	rs2228526	Forward 5′-AATCTGAGGCTAAAGGAGCTGA-3′ Reverse 5′-TCTTCTCCAAGCCTATCATTGC-3′	119	5′-AACACACTAAACCAAGCCTATCATTGCTAGTTACATTACTACTCA-3′	45
ERCC1-05	rs11615	Forward 5′-TCCCTATTGATGGCTTCTGC-3′ Reverse 5′-CTCTGGCCCAGCACATAGTC-3′	124	5′-AACTGACTAAACTAGCTGTTACGTCACGAATCGCCAAATTCCCAGGGCAC-3′	50
ERCC1-06	rs3212948	Forward 5′-GAGGAGGGAGACGGAGAAGT-3′ Reverse 5′-CACTGCTGTCGAATGAATGAA-3′	149	5′-AACTGTCTATACTACGTGTTCGCAACAGATAGTGGCTGGAACTCAGACCTCCTT-3′	54
ERCC1-30	rs3212986	Forward 5′-GGGCACCTTCAGCTTTCTTT-3′ Reverse 5′-AATTCAGAGTCTGGGGAGGAG-3′	124	5′-AACTGACTAAACTAGGTGACTCGTCGTGAAAGTCTCTACACAGGCTGCTGCTGCTGCT-3′	58
RAD23B-04	rs1805329	Forward 5′-GAGAAAGTCAGGCTGTGGTTG-3′ Reverse 5′-GATTCCGCTTTACCTCCAGA-3′	137	5′-AACTGACTAAACTAGGTGCCATGTCGTGAATGTCTGACACAGCCACTGCTGAAGACTGAGGA-3′	62
Run in a duplex PCR					
ERCC2-02	rs1799793	Forward 5′-CCGCAGGATCAAAGAGACAGA-3′ Reverse 5′-AAGCCCAGGAAATGCTCG-3′	265	5′-AACTGACTGGCTCACCCTGCAGCACTTCGT-3′	30
ERCC5-01	rs1047768	Forward 5′-TCTCCCAGATATTAGCATTTGGTT-3′ Reverse 5′-TTTGCAGAGCCGATGAAAC-3′	116	5′-AAGATGAGGATTTTCTATTGAGTTCCC-3′	27

XPA, xeroderma pigmentosis, complementation group A; *XPC*, xeroderma pigmentosis, complementation group C; *ERCC6*, excision repair cross-complementing group 6; *ERCC1*, excision repair cross-complementing group 1; *RAD23B*, RAD23 homologue B (one of two human homologues of *Saccharomyces cerevisiae* Rad23); *ERCC2*, excision repair cross-complementing group 2; *ERCC5*, excision repair cross-complementing group 5.

* According to the Cancer SNP 500 database (http://snp500cancer.nci.nih.gov).

† Neutral non-homologous, non-binding tails are underlined.

(for *XPC*-01) and 4·0 μM (for *RAD23B*-04). For the duplex PCR, the final primer concentrations were 2·7 μM (for *XPD*-02) and 12·3 μM (for *ERCC5*-01) (for corresponding rs-numbers and PCR primers, see Table 2). PCR was conducted as follows: denaturation at 94°C for 3 min; thirty cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. After PCR amplification, the products were pooled (5 μl of the eightplex and 3 μl of the duplex PCR product) and incubated (37°C for 45 min) with 4 μl of ExoSAP-IT (Amersham, Roosendaal, The Netherlands) to digest residual deoxynucleotide triphosphates and primers. Enzymes were deactivated at 75°C (15 min).

Multiplex genotyping

Genotyping was performed by single base extension (SBE) using SnaPShot™ as described by the manufacturer (Applied Biosystems, Nieuwerkerk a.d. IJssel, The Netherlands), with some modifications. Briefly, SBE primers were designed using Primer 3 and Netprimer software to bind immediately adjacent 5′ to the specific SNP, with a template-specific part of 20 to 33 bp and a T_m of 66°C to 69°C (Table 2). To facilitate detection of ten polymorphisms in one single run, the length of the extension primers was adjusted to a distinct size by the addition of a non-homologous tail to their 5′ side[29]. To this end, 5·5 μl of the purified PCR product (containing eleven fragments) were mixed with 2·5 μl of SNaPshot reaction mixture, 1 μl of pooled SBE primers and 1 μl of water. The final concentration for all SBE primers was 4 μM, except for *XPA*-02 and *ERCC5*-01 (2 μM). SBE was performed using twenty-five cycles of 96°C for 10 s and 60°C for 30 s. Subsequently, the samples were incubated at 37°C for 1 h with 1 U of shrimp alkaline phosphatase (Amersham, Roosendaal, The Netherlands), followed by enzyme deactivation at 75°C for 15 min. The SBE products were finally analysed by capillary electrophoresis, for which 1 μl of the (fivefold-diluted) SBE product were mixed with 13 μl of deionised formamide and 0·4 μl of Genescan-120 LIZ™ size marker (Applied Biosystems, Foster City, CA, USA). Samples were denatured at 95°C for 5 min and run on an ABI-Prism® 3100 genetic analyser using a 36 cm capillary array and POP-6 polymer. Analyses were performed with Genescan™ software (version. 3.7; Applied Biosystems)[29].

Measurement of nucleotide excision repair capacity

A subpopulation of thirty-six healthy volunteers (twenty-eight female and eight male, aged 18–45 years) was selected for the phenotypical assessment of NER. Since we previously observed that *ERCC1* expression could be a proxy for NER capacity[24], selection of subjects occurred according to their *ERCC1* genotype. More specifically, samples from homozygous wild types and homozygous carriers of the variant allele for the three studied SNP in *ERCC1* were selected for DNA-repair analysis (for the number of subjects selected per *ERCC1* genotype, see Table 3).

To phenotypically assess the NER capacity in human lymphocytes, we applied a modified comet assay recently developed in our laboratory[30]. Basically, this assay measures the ability of a cell or tissue extract to incise substrate DNA containing benzo[a]pyrene-diolepoxide (BPDE)–DNA adducts.

Table 3. Genotype frequencies as observed in the present study populations

	Frequencies			
	Total population		Subpopulation	
SNP	*n*	%	*n*	%
XPA 5′-UTR (G → A)				
GG	80	48	17	47
GA	71	42	14	39
AA	17	10	5	14
XPC Lys939Gln (A → C)				
AA	68	40	20	56
AC	72	43	15	42
CC	28	17	1	3
XPC Ala499Val (C → T)				
CC	89	53	14	39
CT	66	39	18	50
TT	13	8	4	11
ERCC1 Asn118Asn (T → C)*				
TT	67	40	18	50
TC	69	41	0	0
CC	32	19	18	50
ERCC1 intron 3 (G → C)*				
GG	73	43	18	50
GC	67	40	0	0
CC	28	17	18	50
ERCC1 Gln504Lys (G → T)*				
GG	87	52	18	50
GT	67	38	5	14
TT	17	10	13	36
ERCC2 Asp312Asn (G → A)				
GG	70	42	17	47
GA	76	45	10	28
AA	22	13	9	25
ERCC2 Lys751Gln (A → C)				
AA	56	33	17	47
AC	85	51	9	25
CC	27	16	10	28
ERCC2 Arg156Arg (C → A)				
CC	48	28	13	36
CA	85	51	11	31
AA	35	21	12	33
ERCC5 His46His (C → T)				
CC	57	34	15	42
CT	84	50	15	42
TT	27	16	6	17
ERCC6 Met1097Val (A → G)				
AA	109	65	23	64
AG	49	29	9	25
GG	10	6	4	11
RAD23B Ala249Val (C → T)				
CC	101	60	20	56
CT	60	36	12	33
TT	7	4	4	11

SNP, single nucleotide polymorphism; *XPA*, xeroderma pigmentosis, complementation group A; UTR, untranslated region; *XPC*, xeroderma pigmentosis, complementation group C; *ERCC1*, excision repair cross-complementing group 1; *ERCC2*, excision repair cross-complementing group 2; *ERCC5*, excision repair cross-complementing group 5; *ERCC6*, excision repair cross-complementing group 6; *RAD23B*, RAD23 homologue B (one of two human homologues of *Saccharomyces cerevisiae* Rad23).

* A subpopulation was selected for nucleotide excision repair analysis, according to their *ERCC1* genotype.

Thus, this assay reflects an individual's capacity to recognise and incise damaged DNA, which are important first steps in the NER process. The increase in DNA incisions/breaks, leading to increased tail moments (TM) and percentage DNA in the tail (also known as tail intensity; TI), is indicative of the NER capacity of the cell extracts. After subtracting background levels from all data, the final repair capacity was calculated according to Langie *et al.* [30]. Analyses were performed in duplicate and samples of the same subject isolated before and after the intervention were paired for analysis. Nucleoids exposed to 3 μM-BPDE were used as positive controls to correct for inter-assay variations (TM of BPDE-exposed cells ranged between experiments (n 19) from 1·73 (SE 0·97) to 6·64 (SE 0·77)). Percentage DNA in the tail never exceeded 30 %, indicating that the *in vitro* repair assay in our experiments was not near to saturation.

Statistical analysis

Differences in DNA-repair capacities and TEAC before and after the intervention were analysed by paired-samples t tests. To investigate the effect of the total number of low-activity alleles on the NER capacity, genotypic polymorphisms were coded as number of low-activity alleles, judged on prior knowledge from published literature and their expected modulating effect on the NER capacity (Table 1): 0 (homozygous for high-activity allele); 1 (heterozygous, carrying one high- and one low-activity allele); 2 (homozygous for low-activity allele) (similar approach as previously reported by Ketelslegers *et al.* [31]). Subsequently, the total sum of low-activity alleles was computed and related to NER capacity using linear regression analysis. For obtaining sufficient numbers per group ($n \geq 3$) and subsequent optimal statistical analysis, carriers of five, six, seven or eight low-activity alleles were grouped as carriers of less than nine low-activity alleles. For the same reason, carriers of thirteen, fourteen, fifteen or sixteen low-activity alleles were grouped as carriers of more than twelve low-activity alleles. Stepwise multivariate, linear regression was used to assess the impact of sex, age, TEAC and various polymorphisms on the phenotypically assessed NER capacity. The relationship between NER capacity before and after intervention was assessed by linear regression. Statistical analysis was performed using SPSS (version 15.0; SPSS, Inc., Chicago, IL, USA). In each case, mean values with their standard errors are presented and $P<0·05$ was considered statistically significant.

Results

Single nucleotide polymorphisms and genotype frequencies

In Table 1, all analysed SNP, amino acid and base changes related to the polymorphism and the expected effect of the variant allele on the NER capacity are listed. Furthermore, the frequencies of the wild-type, heterozygous and variant alleles as observed in the present study population (n 168) are represented in Table 3. Complete genotypes were obtained from all samples and frequencies were in Hardy–Weinberg equilibrium. For validation purposes seventeen of the 168 samples (10 %) were genotyped twice (eight samples of these seventeen were even genotyped in triplicate) and no differences were found.

Effects of dietary intervention on the nucleotide excision repair capacity

The 4-week intervention with blueberry and apple juice was reported to be effective in the total study population (n 168)[26]. The mean TEAC value was significantly ($P<0·001$) increased by the intervention from 781 (SE 3·95) to 800 (SE 4·02) μM. Similar results were found for the selected subpopulation (n 36); mean TEAC values were significantly elevated ($P=0·032$) from 791 (SE 6·61) to 805 (SE 7·90) μM. However, when studying the effect of the dietary intervention on NER capacity, no clear effects of the 4-week blueberry and apple juice intervention on the phenotypically assessed NER capacity and no significant correlations between NER capacity and TEAC values were observed. NER capacity measured as TM before the intervention correlated strongly with the NER capacity detected after the intervention (Fig. 1). Similar results were obtained by using TI as a readout (R^2 0·79; $P<0·001$; slope 0·97).

Effects of genetic factors on the nucleotide excision repair capacity

As a first approach to investigate the influence of the genetic profile on the phenotypic NER capacity, the total sum of putative low-activity alleles was calculated and related to the NER capacity assessed before the dietary intervention. A significant inverse correlation between the amount of low-activity alleles and the NER capacity was observed, when repair capacity was calculated by using TM values (Fig. 2(a)) as well as when TI values were used (Fig. 2(b)).

The impact of all single genetic polymorphisms, as well as age and sex, on NER capacity was assessed by stepwise multivariate linear regression analysis. Sex and age had no effect on

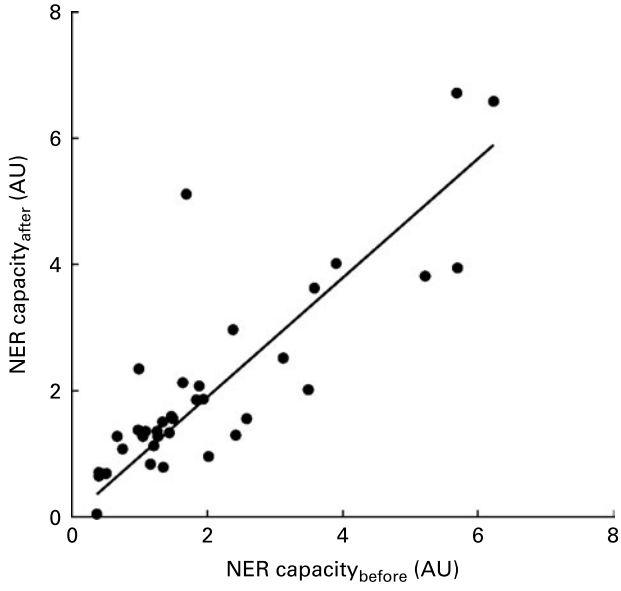

Fig. 1. Correlation between the nucleotide excision repair (NER) capacities measured before and after the blueberry and apple juice intervention (R^2 0·69; $P<0·001$; slope 0·95). Data are presented as the mean of each individual's repair capacity, calculated based on tail moment values. AU, arbitrary units.

NER, while the single genetic polymorphism *XPA* G23A was revealed to be a significant predictor of the NER capacity (Fig. 3(a) and (c)) before the intervention. Individuals that were homozygous for the variant allele of *XPA* G23A (*n* 5) showed a about three times lower NER capacity as compared with those carrying the homozygous wild-type alleles (*n* 17). This association, between NER capacity and the SNP *XPA* G23A, was not affected by the blueberry and apple juice intervention (Fig. 3(b) and (d)).

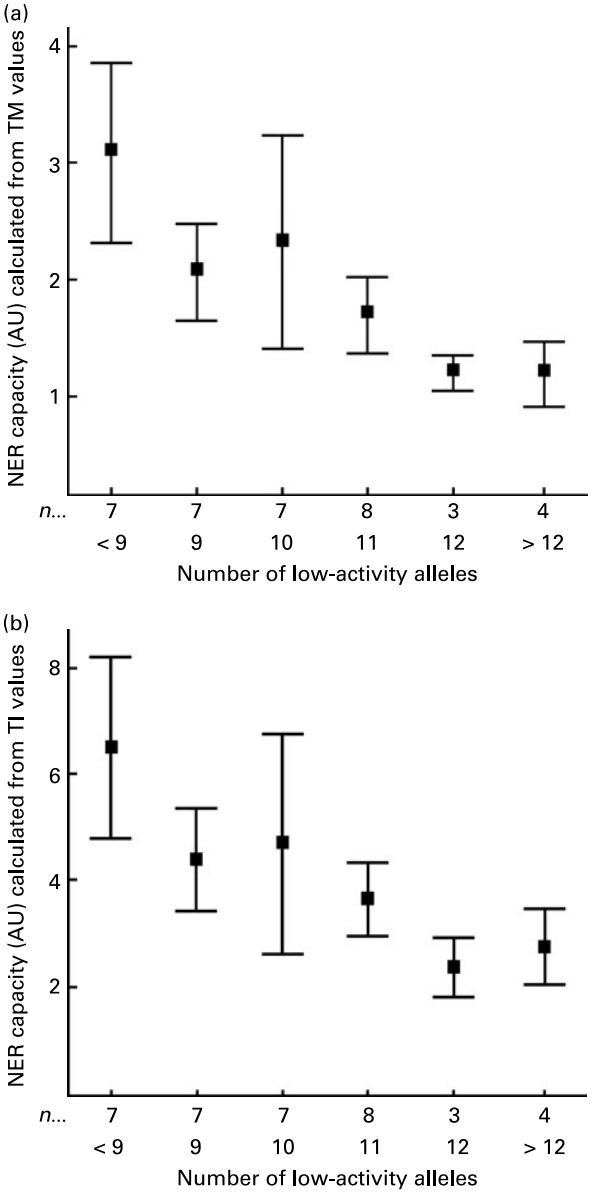

Fig. 2. Relationship between the nucleotide excision repair (NER) capacity and the sum of low-activity alleles in NER-related genes, before intervention. The sum of low-activity alleles was computed for each individual by adding the number of alleles that putatively have adverse effects on NER capacity (Table 1). An increase in this sum of total low-activity alleles was associated with a decreased NER capacity, as calculated (a) from tail moment (TM) values (R^2 0·14; P=0·027) as well as (b) from tail intensity (TI) data (R^2 0·12; P=0·041). Data are means, with standard errors represented by vertical bars. AU, arbitrary units.

Gene–diet interactions

Although the diet did not affect the repair capacity in general, it can be postulated that individuals with a certain genetic background may show an altered NER capacity due to the blueberry and apple juice intervention. To study possible gene–diet interactions regarding changes in NER capacity, differences between the NER capacities measured after and before the intervention were calculated for each individual (ΔNER capacity = NER capacity$_{after}$ − NER capacity$_{before}$). Based on calculations using TM values, improved NER capacity was detected upon dietary intervention in individuals carrying multiple low-activity alleles (Fig. 4(a)); a mean ΔNER capacity of −0·15 (SE 0·13) was observed for carriers of eleven or fewer low-activity alleles (*n* 29), which increased to a mean ΔNER capacity of 0·36 (SE 0·51) and 0·96 (SE 1·64) for carriers of twelve (*n* 3) and more than twelve (*n* 4) low-activity alleles, respectively. Similar results were obtained by using TI as a read-out of the NER capacity (Fig. 4(b)); carriers of eleven or fewer low-activity alleles (*n* 29) showed a mean ΔNER capacity of −0·29 (SE 0·25), which increased to 0·87 (SE 1·04) and 1·63 (SE 1·36) for carriers of twelve (*n* 3) and more than twelve (*n* 4) low-activity alleles, respectively. It should be noted here, however, that the effects of the dietary intervention on the NER phenotype was considerably smaller than the effect of the genotype alone.

Furthermore, this ΔNER capacity as an indicator of the intervention effect was tested by multiple stepwise linear regression analysis against all individual SNP, ΔTEAC, age and sex. No effects of sex, age and ΔTEAC were observed. However, the intervention differentially affected ΔNER capacity in subjects that carried the *RAD23B* Ala249Val polymorphism (Fig. 5); homozygous carriers of the low-activity Val-allele (*n* 4) benefited more from the intervention by a significantly increased NER capacity as compared with subjects homozygous for the wild-type/high-activity Ala-allele (*n* 20). Interestingly, homozygous carriers of the *RAD23B* Val-allele showed about 1·3 times lower NER capacity as their wild-type counterparts (1·49 (SE 0·21) *v.* 1·93 (SE 0·30) and 2·99 (SE 0·51) *v.* 4·05 (SE 0·66), when using TM and TI values, respectively) before the intervention, while after intervention about 1·5 and about 1·3 times higher NER capacity compared with homozygous carriers of the wild-type Ala-allele (2·52 (SE 0·92) *v.* 1·73 (SE 0·23) and 4·88 (SE 1·71) *v.* 3·70 (SE 0·55), when using TM and TI values, respectively) was observed in these subjects. In other words, improved NER capacity upon dietary intervention was detected in individuals carrying low-activity alleles.

Discussion

Until now only a few studies have investigated the relationship between genetic polymorphisms in DNA-repair genes and fruit and vegetable intake, mostly in relation to cancer risk and not directly linked to actual repair capacities[32,33]. The present study is one of the first to report a joint effect of genetic polymorphisms in NER-related genes and dietary intervention on the phenotypically assessed NER capacity. Twelve genetic polymorphisms in NER-related genes were assessed and related to an individual's phenotypic NER capacity. Furthermore, the effect of a 4-week dietary

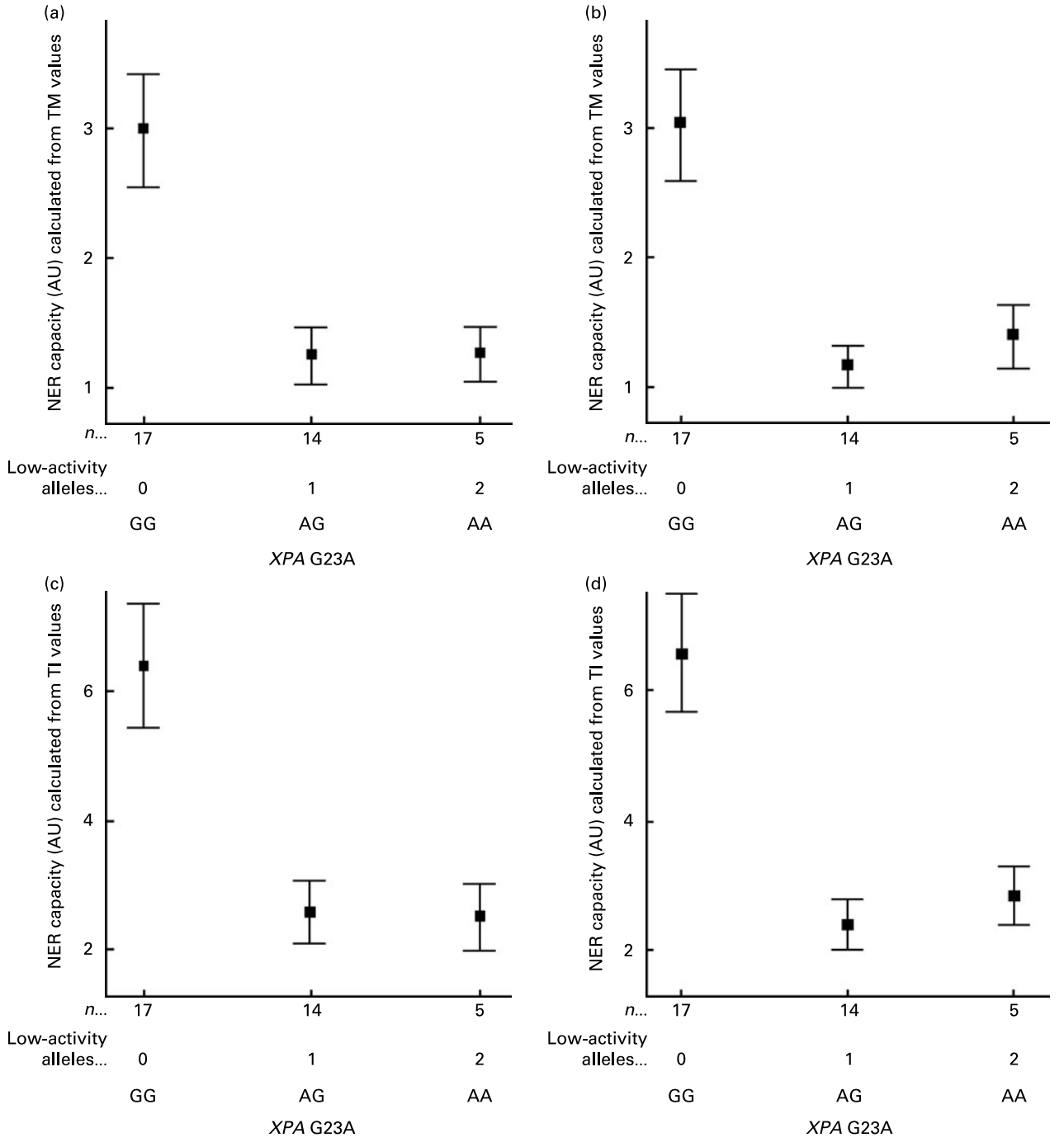

Fig. 3. The effect of the xeroderma pigmentosis, complementation group A (*XPA*) G23A polymorphisms on the phenotypic nucleotide excision repair (NER) capacity. NER capacity calculated from tail moment (TM) values assessed before (a) ($P=0{\cdot}020$) and after (b) ($P<0{\cdot}001$) the blueberry and apple juice intervention, respectively. NER capacity calculated from tail intensity (TI) values assessed before (b) ($P=0{\cdot}020$) and after (d) ($P=0{\cdot}020$) the intervention, respectively. Data are means, with standard errors represented by vertical bars. AU, arbitrary units.

intervention with an antioxidant-rich blueberry and apple juice on the phenotypic NER capacity was evaluated and possible genotype–diet interactions were studied. Although the NER capacity was not affected by the dietary intervention in general, carriers of multiple low-activity alleles seemed to benefit from the intervention. Therefore, the present results support the hypothesis that genetic polymorphisms significantly affect NER capacity, which can be further modulated by diet.

In a previous *in vitro* study[24], we observed that NER capacity was inhibited by oxidative stress. It can thus be postulated that a diet rich in antioxidants may protect NER. However, no overall effects were found in the present intervention study on NER capacity. This is in correspondence with a recent study from Tyson *et al.* in which they reported no detectable effects of micronutrient supplementation on NER capacity[23]. A reasonable explanation for the absence

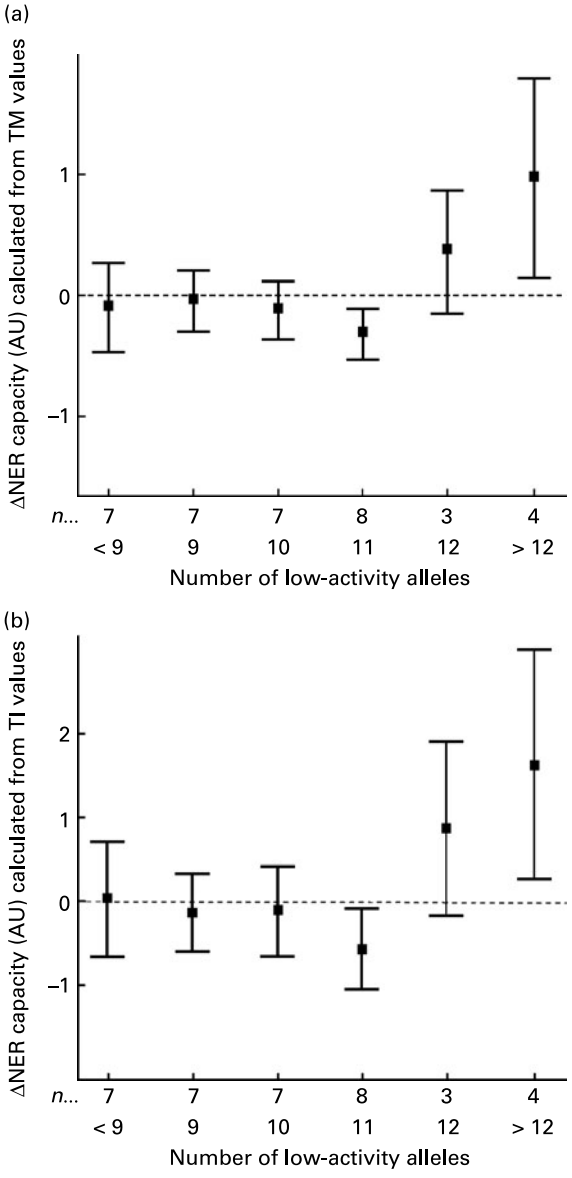

Fig. 4. Relationship between the change in nucleotide excision repair (ΔNER) capacity and the sum of low-activity alleles in NER-related genes. The sum of low-activity alleles was computed for each individual by adding the number of alleles that putatively have adverse effects on NER capacity (Table 1). Improved NER capacity was observed in subjects carrying a total sum of low-activity alleles of twelve or more, when either tail moment (TM) (R^2 0·17; P=0·013) (a) or tail intensity (TI) (R^2 0·14; P=0·023) (b) was used as a read out of the NER capacity. Data are means, with standard errors represented by vertical bars. AU, arbitrary units.

of dietary effects in the present study is that the study population consisted of healthy non-smoking volunteers. Since healthy volunteers encounter relatively low levels of oxidative stress, it may be difficult to detect small additional effects of dietary intake of antioxidants. Future studies on the beneficial effects of diets rich in antioxidants should thus focus on, for instance, subjects suffering from diseases that involve increased oxidative stress. This is supported by observations from previous antioxidant intervention trials with oxidatively stressed subjects (for a review, see Møller & Loft[34]). Additionally, in the present intervention study, inter-individual variations in NER capacity were in the range of about 16-fold, which is similar to variations reported previously in human lymphocytes[23,30,35]. Moreover, a strong correlation was observed between the NER capacity before and after the supplementation period (R^2 0·69, $P<0·001$ and R^2 0·79, $P<0·001$ upon using TM and TI as the read-outs, respectively), indicating that inter-individual variations in NER are maintained over a considerable time, which has been reported before for NER[23] as well as for BER[36]. In contrast, BER seems to be modifiable by the intake of antioxidants even in healthy subjects[21], but data are not consistent[34,37].

Our second aim was to further elucidate the genotype–phenotype relationships with respect to the NER process. Although the majority of genes encoding proteins involved in DNA-repair processes are polymorphic[38], only a limited number of studies have examined the actual phenotypic effects of these genetic polymorphisms. In one of our previous studies, we observed a significant correlation between the *ERCC1* expression and the phenotypic NER capacity *in vitro* [24]. *ERCC1* encodes a subunit of the NER complex, which is required for the incision step of NER[8,9]. However, we did not observe any significant correlation of the studied *ERCC1* polymorphisms with the NER capacity in the present study. Since the functional relevance of SNP in *ERCC1* remains unclear and inconsistent results have been reported[39,40], further investigation into the effect of these polymorphisms on DNA repair is needed.

On the other hand, subjects carrying a high number of putatively low-activity alleles showed lower NER capacity as compared with subjects carrying only a few low-activity alleles. This approach, looking at the combined effect of multiple gene variants rather than investigating the effect of a single nucleotide polymorphism, has been applied before: several studies have reported an association between the number of putatively high-risk alleles in DNA-repair genes and levels of bulky DNA adduct[31,41], while others have observed increased cancer risk with increasing number of putative high-risk alleles[42,43]. All these observations suggest that, at the individual level, studying the combined effect of multiple gene variants may be important in order to define DNA-repair capacity.

Subsequently, to investigate which polymorphisms may have the highest contribution to the inter-individual variations in NER capacity, multivariate linear regression analysis was performed. The common SNP *XPA* G23A seemed to be the most relevant polymorphisms for defining NER capacity. Individuals homozygous for the wild-type allele (GG) exhibited a significantly three times higher NER capacity (approximately) compared with carriers of at least one variant allele. The XPA protein is involved in both global genome repair and the transcription-coupled repair pathway of the NER process, playing an essential role in the assembly of the pre-incision complex[9]. The common G → A single-nucleotide substitution in the 5′ untranslated region of the *XPA* gene is located four nucleotides upstream from the ATG start codon[44]. The functional relevance of this SNP is unknown; however, it has been demonstrated that the 5′ untranslated region may regulate gene expression through post-transcriptional control mechanisms[45,46]. In addition, several studies have shown that individuals with this G → A substitution in the 5′ untranslated

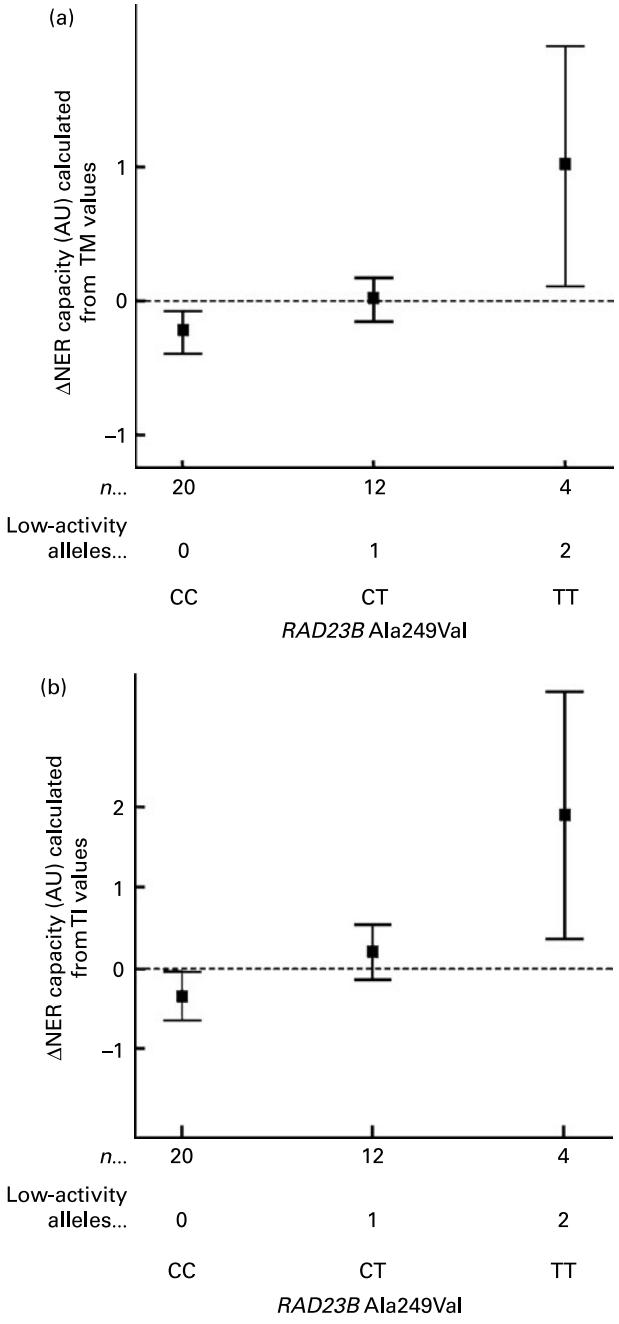

Fig. 5. The influence of the *RAD23B* Ala249Val polymorphism on the effect of the dietary intervention on the change in nucleotide excision repair (ΔNER) capacity. Homozygous carriers of the low-activity Val-allele showed an increased ΔNER capacity as compared with individuals carrying none of the low-activity alleles. (a) ΔNER capacity calculated from tail moment (TM) values ($P=0{\cdot}020$); (b) ΔNER capacity calculated from tail intensity (TI) values ($P=0{\cdot}018$). Data are means, with standard errors represented by vertical bars. AU, arbitrary units.

region of *XPA* have a increased risk of lung cancer[12,47,48]. Furthermore, in agreement with the present results, Wu *et al.* observed a more efficient NER capacity in subjects carrying the *XPA* GG genotype[12]. The association between this *XPA* polymorphism and NER capacity was not affected by the blueberry and apple juice intervention, indicating that the *XPA* G23A SNP might be regarded as a predictor for the NER capacity.

Although we initially did not observe an overall effect of diet on NER capacity, it was still possible that a subgroup may benefit from the intervention due to gene–diet interactions. Indeed, enhanced NER capacity was detected in subjects carrying a sum of twelve or more low-activity alleles. Although we did not observe a general effect of the intervention on the NER capacity in our healthy non-smoking study population, we were able to detect a beneficial effect of the intervention in individuals with an initial low NER capacity. Similar observations were reported by Guarnieri *et al.* detecting beneficial effects of antioxidants only among poorly nourished subjects with low repair activity[49]. As mentioned above, future studies on the beneficial effects of diets rich in antioxidants should thus focus more on specific susceptible subpopulations.

Furthermore, the single polymorphism *RAD23B* Ala249Val seemed to be a predictor for the intervention effect (ΔNER capacity). Subjects homozygous for the variant Val-allele of *RAD23B* had enhanced NER capacity after the intervention, while NER capacity in carriers of the wild-type Ala-allele was unaffected. Moreover, increased ΔNER capacity was observed in subjects that carry two low-activity alleles of *RAD23B* Ala249Val. The protein encoded by *RAD23B* binds XPC, forming a heterodimeric complex[9]. In the global genome repair pathway XPA binds this protein complex, which is essential for the recruitment of all subsequent NER factors in the pre-incision complex. Although the biological function of the *RAD23B* Ala249Val polymorphism is not clear, the variant alleles Val/Val were associated with increased lung cancer risk and higher BPDE sensitivity as compared with the homozygous Ala/Ala wild types[2,3], which is in agreement with the low NER capacity that we detected before the intervention in homozygous carriers of the Val-allele. However, these data need to be interpreted with care, because the group of subjects homozygous for the *RAD23B* Val-allele is small (n 4), and all carried a high number of low-activity alleles. In other words, improved NER capacity upon dietary intervention was especially detected in individuals carrying a high number of low-activity alleles. Nonetheless, our observations suggest that both genetic as well as environmental factors such as diet can modulate an individual's NER capacity, separately or through interaction with each other.

It is not yet clear how this interaction between the genotype and antioxidant intake can be explained. Several studies have suggested that some dietary antioxidants may confer protective properties through mechanisms that are unrelated to their conventional free-radical scavenging abilities, such as up-regulation of antioxidant defence, xenobiotic metabolism or DNA-repair genes[50,51]. For example, quercetin and vitamin C were shown to induce different DNA damage-responsive signalling pathways (for example, p53 and activator protein-1 (AP-1)/NF-κB) that can subsequently enhance the expression of, for instance, NER genes[52,53]. However, results from various *in vivo* intervention studies have been equivocal[50]. Therefore, further and larger studies are needed to clarify possible correlations between an individual's antioxidant capacity and DNA-repair capacity, both in the whole population as well as in several subgroups.

In the present study, we report a joint effect of genetic polymorphisms in NER-related genes and a dietary intervention on

the phenotypically assessed NER capacity. Overall, the present results show that genetic factors have more impact on the NER capacity as compared with the effects of the blueberry and apple juice intervention. Furthermore, the common genetic polymorphism *XPA* G23A might be a predictor for the NER capacity, as it was not affected by the dietary intervention. Still, the present study suggests that the combined effect of multiple gene variants may be more important than the investigation of single nucleotide polymorphism in order to define an individual's DNA-repair capacity. Improved NER capacity upon dietary intervention with blueberry and apple juice was detected in individuals carrying a high number of putative low-activity alleles. In conclusion, studies of genotype–phenotype interactions seem to be helpful in the identification of susceptible subpopulations that may benefit from specific dietary interventions.

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The project was conceived of by J. C. S. K., R. W. L. G. and F. J. vS. The intervention was coordinated by J. C. S. K. and L. C. W. TEAC analysis was performed by L. C. W., while DNA-repair measurements were conducted by S. A. S. L. Modification of the multiplex PCR and genotyping was carried out by S. A. S. L., with help from S. H. Data analysis was carried out by S. A. S. L. and R. W. L. G., and all authors contributed in writing the manuscript.

The authors declare no conflicts of interest.

References

1. Andrew AS, Nelson HH, Kelsey KT, *et al.* (2006) Concordance of multiple analytical approaches demonstrates a complex relationship between DNA repair gene SNPs, smoking and bladder cancer susceptibility. *Carcinogenesis* **27**, 1030–1037.
2. Lin J, Swan GE, Shields PG, *et al.* (2007) Mutagen sensitivity and genetic variants in nucleotide excision repair pathway: genotype–phenotype correlation. *Cancer Epidemiol Biomarkers Prev* **16**, 2065–2071.
3. Shen M, Berndt SI, Rothman N, *et al.* (2005) Polymorphisms in the DNA nucleotide excision repair genes and lung cancer risk in Xuan Wei, China. *Int J Cancer* **116**, 768–773.
4. Lockett KL, Snowhite IV & Hu JJ (2005) Nucleotide-excision repair and prostate cancer risk. *Cancer Lett* **220**, 125–135.
5. Friedberg EC (2001) How nucleotide excision repair protects against cancer. *Nat Rev Cancer* **1**, 22–33.
6. Reardon JT, Bessho T, Kung HC, *et al.* (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. *Proc Natl Acad Sci U S A* **94**, 9463–9468.
7. Kuraoka I, Bender C, Romieu A, *et al.* (2000) Removal of oxygen free-radical-induced 5′,8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells. *Proc Natl Acad Sci U S A* **97**, 3832–3837.
8. Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, *et al.* (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* **73**, 39–85.
9. Sugasawa K (2008) Xeroderma pigmentosum genes: functions inside and outside DNA repair. *Carcinogenesis* **29**, 455–465.
10. Bootsma D, Kraemer KH, Cleaver JE, *et al.* (2002) Nucleotide excision repair syndromes: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. In *The Genetic Basis of Human Cancer*, vol. 2, pp. 211–237 [B Vogelstein and KW Kinzler, editors]. New York: McGraw-Hill.
11. Zhu Y, Yang H, Chen Q, *et al.* (2008) Modulation of DNA damage/DNA repair capacity by XPC polymorphisms. *DNA Repair (Amst)* **7**, 141–148.
12. Wu X, Zhao H, Wei Q, *et al.* (2003) XPA polymorphism associated with reduced lung cancer risk and a modulating effect on nucleotide excision repair capacity. *Carcinogenesis* **24**, 505–509.
13. Dusinska M, Dzupinkova Z, Wsolova L, *et al.* (2006) Possible involvement of XPA in repair of oxidative DNA damage deduced from analysis of damage, repair and genotype in a human population study. *Mutagenesis* **21**, 205–211.
14. Matullo G, Palli D, Peluso M, *et al.* (2001) *XRCC1*, *XRCC3*, *XPD* gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* **22**, 1437–1445.
15. Shen J, Gammon MD, Terry MB, *et al.* (2005) Polymorphisms in *XRCC1* modify the association between polycyclic aromatic hydrocarbon–DNA adducts, cigarette smoking, dietary antioxidants, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **14**, 336–342.
16. Cheng J, Leng S, Dai Y, *et al.* (2007) Association between nucleotide excision repair gene polymorphisms and chromosomal damage in coke-oven workers. *Biomarkers* **12**, 76–86.
17. Mateuca R, Aka PV, De Boeck M, *et al.* (2005) Influence of *hOGG1*, *XRCC1* and *XRCC3* genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. *Toxicol Lett* **156**, 277–288.
18. Mateuca RA, Roelants M, Iarmarcovai G, *et al.* (2008) *hOGG1*(326), *XRCC1*(399) and *XRCC3*(241) polymorphisms influence micronucleus frequencies in human lymphocytes *in vivo*. *Mutagenesis* **23**, 35–41.
19. World Cancer Research Fund & American Institute for Cancer Research (1997) *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. London: World Cancer Research Fund UK.
20. Tyson J & Mathers JC (2007) Dietary and genetic modulation of DNA repair in healthy human adults. *Proc Nutr Soc* **66**, 42–51.
21. Collins AR, Harrington V, Drew J, *et al.* (2003) Nutritional modulation of DNA repair in a human intervention study. *Carcinogenesis* **24**, 511–515.
22. Wei Q, Shen H, Wang LE, *et al.* (2003) Association between low dietary folate intake and suboptimal cellular DNA repair capacity. *Cancer Epidemiol Biomarkers Prev* **12**, 963–969.
23. Tyson J, Caple F, Spiers A, *et al.* (2009) Inter-individual variation in nucleotide excision repair in young adults: effects of age, adiposity, micronutrient supplementation and genotype. *Br J Nutr* **101**, 1316–1323.
24. Langie SA, Knaapen AM, Houben JM, *et al.* (2007) The role of glutathione in the regulation of nucleotide excision repair during oxidative stress. *Toxicol Lett* **168**, 302–309.
25. Gungor N, Godschalk RW, Pachen DM, *et al.* (2007) Activated neutrophils inhibit nucleotide excision repair in human pulmonary epithelial cells: role of myeloperoxidase. *FASEB J* **21**, 2359–2367.

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26. Wilms LC, Boots AW, de Boer VC, *et al.* (2007) Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on *ex vivo* induced lymphocytic DNA damage in human volunteers. *Carcinogenesis* **28**, 1800–1806.
27. Wilms LC, Hollman PC, Boots AW, *et al.* (2005) Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE–DNA adducts in human lymphocytes. *Mutat Res* **582**, 155–162.
28. Boyum A (1976) Isolation of lymphocytes, granulocytes and macrophages. *Scand J Immunol* Suppl. 5, 9–15.
29. Knaapen AM, Ketelslegers HB, Gottschalk RW, *et al.* (2004) Simultaneous genotyping of nine polymorphisms in xenobiotic-metabolizing enzymes by multiplex PCR amplification and single base extension. *Clin Chem* **50**, 1664–1668.
30. Langie SA, Knaapen AM, Brauers KJ, *et al.* (2006) Development and validation of a modified comet assay to phenotypically assess nucleotide excision repair. *Mutagenesis* **21**, 153–158.
31. Ketelslegers HB, Gottschalk RW, Godschalk RW, *et al.* (2006) Interindividual variations in DNA adduct levels assessed by analysis of multiple genetic polymorphisms in smokers. *Cancer Epidemiol Biomarkers Prev* **15**, 624–629.
32. van Gils CH, Bostick RM, Stern MC, *et al.* (2002) Differences in base excision repair capacity may modulate the effect of dietary antioxidant intake on prostate cancer risk: an example of polymorphisms in the XRCC1 gene. *Cancer Epidemiol Biomarkers Prev* **11**, 1279–1284.
33. Misra RR, Ratnasinghe D, Tangrea JA, *et al.* (2003) Polymorphisms in the DNA repair genes *XPD*, *XRCC1*, *XRCC3*, and *APE/ref-1*, and the risk of lung cancer among male smokers in Finland. *Cancer Lett* **191**, 171–178.
34. Møller P & Loft S (2004) Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutat Res* **551**, 79–89.
35. Gaivão I, Piasek A, Brevik A, *et al.* (2007) Comet assay-based methods for measuring DNA repair *in vitro*; estimates of inter- and intra-individual variation. *Cell Biol Toxicol* **25**, 45–52.
36. Collins AR, Dusinska M, Horvathova E, *et al.* (2001) Inter-individual differences in repair of DNA base oxidation, measured *in vitro* with the comet assay. *Mutagenesis* **16**, 297–301.
37. Møller P, Vogel U, Pedersen A, *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.
38. Mohrenweiser HW, Xi T, Vazquez-Matias J, *et al.* (2002) Identification of 127 amino acid substitution variants in screening 37 DNA repair genes in humans. *Cancer Epidemiol Biomarkers Prev* **11**, 1054–1064.
39. Zhou W, Liu G, Park S, *et al.* (2005) Gene–smoking interaction associations for the *ERCC1* polymorphisms in the risk of lung cancer. *Cancer Epidemiol Biomarkers Prev* **14**, 491–496.
40. Zienolddiny S, Campa D, Lind H, *et al.* (2006) Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. *Carcinogenesis* **27**, 560–567.
41. Matullo G, Peluso M, Polidoro S, *et al.* (2003) Combination of DNA repair gene single nucleotide polymorphisms and increased levels of DNA adducts in a population-based study. *Cancer Epidemiol Biomarkers Prev* **12**, 674–677.
42. Gu J, Zhao H, Dinney CP, *et al.* (2005) Nucleotide excision repair gene polymorphisms and recurrence after treatment for superficial bladder cancer. *Clin Cancer Res* **11**, 1408–1415.
43. Crew KD, Gammon MD, Terry MB, *et al.* (2007) Polymorphisms in nucleotide excision repair genes, polycyclic aromatic hydrocarbon–DNA adducts, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **16**, 2033–2041.
44. Butkiewicz D, Rusin M, Harris CC, *et al.* (2000) Identification of four single nucleotide polymorphisms in DNA repair genes: *XPA* and *XPB* (*ERCC3*) in Polish population. *Hum Mutat* **15**, 577–578.
45. Akiri G, Nahari D, Finkelstein Y, *et al.* (1998) Regulation of vascular endothelial growth factor (VEGF) expression is mediated by internal initiation of translation and alternative initiation of transcription. *Oncogene* **17**, 227–236.
46. Larsen LK, Amri EZ, Mandrup S, *et al.* (2002) Genomic organization of the mouse peroxisome proliferator-activated receptor β/δ gene: alternative promoter usage and splicing yield transcripts exhibiting differential translational efficiency. *Biochem J* **366**, 767–775.
47. Vogel U, Overvad K, Wallin H, *et al.* (2005) Combinations of polymorphisms in *XPD*, *XPC* and *XPA* in relation to risk of lung cancer. *Cancer Lett* **222**, 67–74.
48. Butkiewicz D, Popanda O, Risch A, *et al.* (2004) Association between the risk for lung adenocarcinoma and a (−4) G-to-A polymorphism in the *XPA* gene. *Cancer Epidemiol Biomarkers Prev* **13**, 2242–2246.
49. Guarnieri S, Loft S, Riso P, *et al.* (2008) DNA repair phenotype and dietary antioxidant supplementation. *Br J Nutr* **99**, 1018–1024.
50. Cooke MS, Evans MD, Mistry N, *et al.* (2002) Role of dietary antioxidants in the prevention of *in vivo* oxidative DNA damage. *Nutr Res Rev* **15**, 19–42.
51. Silva JP, Gomes AC & Coutinho OP (2008) Oxidative DNA damage protection and repair by polyphenolic compounds in PC12 cells. *Eur J Pharmacol* **601**, 50–60.
52. Lunec J, Holloway KA, Cooke MS, *et al.* (2002) Urinary 8-oxo-2′-deoxyguanosine: redox regulation of DNA repair *in vivo*? *Free Radic Biol Med* **33**, 875–885.
53. Ye R, Goodarzi AA, Kurz EU, *et al.* (2004) The isoflavonoids genistein and quercetin activate different stress signaling pathways as shown by analysis of site-specific phosphorylation of ATM, p53 and histone H2AX. *DNA Repair (Amst)* **3**, 235–244.
54. Qiao Y, Spitz MR, Shen H, *et al.* (2002) Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic *XPC* and *XPD/ERCC2* genotypes. *Carcinogenesis* **23**, 295–299.
55. Vodicka P, Kumar R, Stetina R, *et al.* (2004) Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. *Carcinogenesis* **25**, 757–763.
56. Shen J, Desai M, Agrawal M, *et al.* (2006) Polymorphisms in nucleotide excision repair genes and DNA repair capacity phenotype in sisters discordant for breast cancer. *Cancer Epidemiol Biomarkers Prev* **15**, 1614–1619.
57. Ma H, Xu L, Yuan J, *et al.* (2007) Tagging single nucleotide polymorphisms in excision repair cross-complementing group 1 (*ERCC1*) and risk of primary lung cancer in a Chinese population. *Pharmacogenet Genomics* **17**, 417–423.
58. Spitz MR, Wu X, Wang Y, *et al.* (2001) Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* **61**, 1354–1357.
59. Møller P, Wallin H, Dybdahl M, *et al.* (2000) Psoriasis patients with basal cell carcinoma have more repair-mediated DNA strand-breaks after UVC damage in lymphocytes than psoriasis patients without basal cell carcinoma. *Cancer Lett* **151**, 187–192.
60. Hou SM, Ryk C, Kannio A, *et al.* (2003) Influence of common *XPD* and *XRCC1* variant alleles on p53 mutations in lung tumors. *Environ Mol Mutagen* **41**, 37–42.
61. Vogel U, Olsen A, Wallin H, *et al.* (2005) Effect of polymorphisms in *XPD*, *RAI*, *ASE-1* and *ERCC1* on the risk of basal cell carcinoma among Caucasians after age 50. *Cancer Detect Prev* **29**, 209–214.
62. Chen M, Kamat AM, Huang M, *et al.* (2007) High-order interactions among genetic polymorphisms in nucleotide excision repair pathway genes and smoking in modulating bladder cancer risk. *Carcinogenesis* **28**, 2160–2165.

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