Double-strand break DNA repair genotype predictive of later mortality and cancer incidence in a cohort of non-smokers

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1. Introduction

It is plausible that DNA repair genes may influence the risk of disease and/or modulate clinical course and survival, and thus influence mortality. The relationship between common DNA repair gene variants and disease risk has been studied extensively within case–control studies on single diseases (and recently within genome-wide association studies), while the relationship with mortality has been investigated rarely and only for specific outcomes or in relation to specific cancer therapies [1–6]. None of the studies that considered mortality in relation to variants in common genes was prospective in design. Retrospective (case–control) studies can be affected by selection bias and can usually investigate a single or a limited number of diseases or causes of death.

We describe the first prospective study that has investigated total and cause-specific mortality (for all causes), and cancer incidence, in relation to common polymorphisms in a series of DNA repair genes. The advantages of such a design are: (a) lack of selection bias when comparing allele frequencies; (b) the opportunity to investigate all causes of death and incident cancer cases. Concerning point (b), at least for some pathways, like DNA repair, it is likely that they are involved in several types of disease, since they have a key role in protecting DNA from damage. In particular, environmental carcinogens, such as polycyclic aromatic hydrocarbons, aromatic amines or *N*-nitroso compounds, predominantly form DNA adducts but also generate inter-strand cross-links and reactive oxygen species (which induce base damage, abasic sites, and single and double-strand breaks). Unrepaired damage can result in apoptosis or may lead to unregulated cell growth and cancer at several sites. Therefore, if DNA repair is impaired because of functionally relevant genetic variants, both the risk of disease and of poor survival can be affected. For example, after damage to DNA, checkpoints can be activated to arrest the cell cycle, transcription can be upregulated to compensate for the damage or the cell can apoptose [7]. Alternatively, the damage can be repaired at the DNA level enabling the cell to replicate as planned. Complex pathways involving numerous molecules have evolved to perform such repair. Because of the importance of maintaining genomic integrity in the general and specialized functions of cells as well as in the prevention of carcinogenesis, genes encoding for DNA repair molecules have been proposed as candidate cancer-susceptibility genes [8,9].

At least four pathways of DNA repair operate on specific types of damaged DNA, and each pathway involves numerous molecules [10]. The nucleotide excision repair (NER) pathway repairs bulky lesions such as pyrimidine dimers, other photoproducts, larger chemical adducts and cross-links. The base excision repair (BER) operates on small lesions such as oxidized or reduced bases, fragmented or non-bulky adducts, or those produced by methylating agents. Double-Strand Breaks (DSBs) can be produced by replication errors and by exogenous agents such as ionizing radiation; they lead to chromosomal breakage and rearrangement—events that may result in apoptosis or tumorigenesis. Mismatch repair (MMR) is an additional category of DNA repair system that corrects replication errors (base–base or insertion deletion mismatches) caused by the DNA polymerase errors. Finally, alkylated bases are also

Abbreviations: NER, nucleotide excision repair; BER, base excision repair; DSBR, double-strand break repair; MMR, mismatch repair; DRR, direct reversal repair; EPIC, European prospective investigation into cancer and nutrition; SNP, single nucleotide polymorphism; XRCC, X-ray repair cross-complementing.

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directly removed by the suicide enzyme O6-methylguanine-DNA methyltransferase (MGMT), a mechanism known as direct reversal repair (DRR). Studies to date indicate that variation in DNA repair genes in each of the pathways (including polymorphisms analysed in the present study) may influence cancer susceptibility (http://www.epistat.org/); however, results across studies are not consistent, and other potentially important polymorphisms in these and other genes have not yet been explored.

In our prospective study of total mortality and cancer incidence we considered 22 common variants in 16 genes involved in DNA repair pathways and explored whether polymorphisms in these genes were important predictors of mortality or increased risk of cancer in a healthy population of non-smokers. Variants were chosen on the basis of a literature review as those potentially associated with increased cancer risk, in particular variants in genes involved in BER.

2. Materials and methods

2.1. Selection of subjects and collection of specimens

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a multi-center European cohort study in which more than 500,000 healthy volunteers were recruited in 10 European countries (France, Denmark, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, United Kingdom) corresponding to 23 recruitment centers. The cohort includes subjects of both genders, mostly in the age range 35–74 at recruitment. Recruitment took place between 1993 and 1998. Detailed dietary and lifestyle histories collected mainly through self-administered questionnaires, plus a 24-h dietary recall through person-to-person interview (in a 7% sample), anthropometric measurements and a 30–40 ml blood sample are available. All questionnaire information is available in a computerized format. Signed informed consent forms were collected from all participants (except a sub-group of the Oxford cohort who gave consent on postal questionnaires).

The present sub-cohort is formed by the controls of a nested-case–control study within EPIC, Gen-Air, with the aim of studying the relationship between select cancer types and air pollution or environmental tobacco smoke (ETS). Only non-smokers or ex-smokers for at least 10 years were eligible in the Gen-Air sub-study. Details are given elsewhere [11,12]. Gen-Air has been approved by the Ethical Committee of the International Agency for Research on Cancer, and by the local Ethical Committees of the 23 centres.

Follow-up for cancer incidence and mortality was based on population cancer and mortality registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden, United Kingdom) as well as other methods such as health insurance records, pathology registries and active contact of study subjects or next of kin (France, Germany, Greece), and is virtually complete. In all centres, cancer diagnosis required confirmation through comprehensive pathology review. A detailed protocol entitled "Guidelines for Collection of End-point Data in the EPIC study" for the collection and standardization of clinical and pathological data for each cancer site has been prepared by a special EPIC working group.

Briefly, all the original Genair controls with blood available were 1564, but those with DNA were 1094, which became 1088 after failures in genotyping. These were linked to the EPIC database (containing information on incident cancers) to identify all causes of mortality and incident cancer cases. All recorded health outcomes were coded according to the WHO International Classification of Diseases (ICD10) (http://www.who.int/classifications/icd/en/).

2.2. Genes and laboratory analyses

Single Nucleotide Polymorphisms (SNP) in DNA repair genes were investigated including Base Excision Repair (BER), Nucleotide Excision Repair (NER), Double-Strand Break Repair (DSBR), Direct Reversal Repair (DRR) and apoptosis pathways. DNA was isolated from 200 to 300 μl of buffy coat which was isolated, purified and stored (straws in liquid nitrogen) as previously described [12], and distributed to laboratories at the ISI Foundation in Torino (Dr. G. Matullo) and Cambridge University (Dr. A. Dunning), for genotyping. The TaqMan Nuclease Assay (Applied Biosystems) was used to genotype all the polymorphisms except *hOGG1*-Ser326Cys which was genotyped by the primer extension technique on a Denaturing High Performance Liquid Chromatography (DHPLC) instrument (Varian Inc., Walnut Creek, CA, USA). The Cambridge laboratory used plain TaqMan probes, whereas the Torino laboratory used fluorogenic MGB (minor groove binder) probes; allele specific probes were labelled in both laboratories with Fam and Vic fluorophors.

Methodological validation was performed at the ISI Foundation laboratory, including comparison between Direct Sequencing and Denaturing High Performance Liquid Chromostography (DHPLC), PCR-RFLP and TaqMan assay. At least 10% of genotyping were also randomly repeated for each polymorphism. Concordance was in the range of 99–100% for all comparisons. More detailed methods are described extensively elsewhere [11,12].

2.3. Statistical analysis

The association between SNP and mortality or cancer incidence was analyzed using Cox Proportional Hazard regression models, with age as the time variable and length of follow-up as a covariate. With more than 1000 subjects and overall about 100 deaths we estimated we had 80% power to detect statistically significant ($p = 0.05$) relative risks greater than 1.85 for a genotype with frequency 0.25, and greater than 2.25 for a genotype with frequency 0.10. Due to potential confounding by several demographic and behavioral factors, hazard ratios (HRs) and their 95% confidence intervals were stratified by study centre and adjusted for gender, smoking history (former and never smokers), highest school level attained (as a social class indicator) and age. We computed a score based on the number of variant alleles in different genes belonging to the same DNA repair pathway. The sum of the variant alleles was categorized on the basis of the tertile distribution of the number of minor alleles in the control group. At each locus, the minor allele was treated as the "adverse" allele and the total number of such alleles tallied for each individual. Interaction between two specific DNA repair polymorphisms was assessed by summing the number of at-risk alleles. Dose–response effects for incremental addition of adverse alleles were assessed using a continuous variable for at risk alleles. All analyses were conducted using STATA 8.2 software (StataCorp, TX 77845, USA). Potential confounding/effect modification by dietary and other exposures were explored in multivariate models.

The problem of the inflation of type I error, due to the large number of variants tested, was dealt with by computing, for each significant HR, the false positive report probability (FPRP) as described by Wacholder et al. [13]. This method is based on a Bayesian approach and has been originally applied in the analysis of genetic polymorphisms. To obtain the actual FPRP the investigator must assign to each exposure the 'prior probability' that it is truly associated with the disease of interest. This 'prior probability' is at least in part arbitrary and is based on previous knowledge derived from earlier epidemiological studies and biological plausibility. An FPRP value of 0.2 or less is usually considered satisfactory (for details in computing FPRP see Wacholder et al. [13]).

3. Results

Overall 1088 subjects were available for analysis, 568 men and 520 women (mean age at recruitment 61 years). Median follow-up was 78 months (inter-quartile range 59–93 months); 95 subjects had died. Table 1 shows the hazard ratios for each gene within four pathways for DNA repair. None of the examined genotypes was clearly associated with total mortality, except variants for two DSBR genes, XRCC3 18067 C/T (rs#861539) and XRCC2 31479 G/A (rs#3218536), with statistically significant hazard ratios of around 2 (Table 1). These associations were consistent across countries (results not shown). A statistically significant association was found between total mortality in the second tertile of the distribution of combined BER alleles and a non-significant association in the highest tertile, with no apparent trend (p for trend = 0.37) (Table 2). We also found no evidence of trends associated with the cumulative effect of NER or DSBR gene variants.

We explored whether the excess risk associated with the XRCC3 18067 C > T (Thr241Met) and XRCC2 31479 G > A (Arg188His) polymorphisms could be explained by cancer and/or non-cancer outcomes. Table 3 shows the results separately for all cancer deaths, all non-cancer deaths and all incident cancer cases. We also investigated the most common cancers – including lung, breast, prostate and colorectal – and all circulatory disease deaths (as we noted an excess proportion of circulatory deaths compared to controls among the XRCC3 T/T and C/T genotypes). We found an association between incident prostate cancer and XRCC2 G/A genotype (adjusted hazard ratio of 5.15 (95% C.I. 1.48–17.84), and between all circulatory deaths and XRCC3 C/T genotype (adjusted hazard ratio 2.22 (95% C.I 1.00–5.05).

Table 4 shows the absolute numbers of outcomes (deaths or incidents cases) by combinations of genotypes for XRCC3 and XRCC2, and the corresponding crude and adjusted hazard ratios with 95% confidence intervals. For combinations involving greater than or equal to two adverse alleles, the adjusted risk of all cause mortality rose to 3.62 (95% C.I. 1.60–8.20) with a significant dose–response relationship for each additional polymorphic allele ($p = 0.002$). With combinations of three or more adverse alleles, the adjusted hazard ratio for all cause mortality was 17.29 (95% C.I. 8.13–36.74) and cancer mortality 25.27 (7.55–74.50) (dose–response < 0.001), though numbers were small (15 deaths/24 controls vs. reference group 41/590 for all cause mortality; 7/32 vs. reference 11/620 for cancer mortality).

For all incident cancers and all non-cancer deaths, the hazard ratios were elevated above unity but the results did not reach statistical significance and there was no evidence of a dose–response relationship ($p = 0.10$ and 0.16, respectively). With combinations of three or more adverse alleles, the adjusted hazard ratio for all incident cancers was 5.28 (95% C.I. 2.17–12.85; 8 cases/31 controls vs. reference group 51/580) and all non-cancer deaths 14.24 (95% C.I. 5.25–38.66; 8/24 vs. 32/590); dose–response relationships were also observed for these categories ($p = 0.02$ and 0.06, respectively). We also explored potential confounding/effect modification by dietary and other exposures in multivariate models, but no clear pattern was identified (with the limitation of small numbers to study interactions).

Table 5 shows the results of the false positive report probabilities (FPRPs) from the adjusted Cox Proportional Hazard regression models for the main associations of interest: XRCC3 codon 241 C/T (total, cancer and non-cancer mortality), XRCC2 codon 188 G/A (total mortality, all incident cancer) and XRCC3 codon 241* XRCC2 codon 188 allele with >2 allele combinations (total and cancer mortality) were likely to be true positive associations, using a stringent FPRP cut point of 0.2 at the pre-defined prior probability level and assuming a HR of 1.5. However, considering an FPRP cut point of 0.5, and/or assuming a HR of 2.0, there were substantially more likely associations (Table 5).

4. Discussion

In the first prospective study on gene variants and total mortality, we found that variants in two genes involved in Double-Strand Break DNA repair, XRCC3 18067 C/T (rs#861539) and XRCC2 31479 G/A (rs#3218536), were independently and jointly, associated with a significant increase in total mortality after 78 months of follow-up, among a healthy population of non-smokers. Most but not all of the excess appeared to be attributable to cancer outcomes. We can rule out the excess being explained by bias, since the follow-up of this prospective cohort was completed after the analysis of gene variants, and follow-up was nearly 100% complete for both mortality and cancer incidence. Some causes of death were occasionally missing, but it is unlikely that missing data were associated with DNA repair variants. The exclusion of current and recent smokers also makes the interpretation of the findings easier because smoking-related causes of death were largely absent from this population. After applying a method for the estimation of the number of false positive results, XRCC3 codon 241 C/T (total, cancer and non-cancer mortality), XRCC2 codon 188 G/A (total mortality, all incident cancer) and XRCC3 codon 241* XRCC2 codon 188 allele with >2 allele combinations (total and cancer mortality) are likely to be true positive associations. Assuming a HR of 2.0 and/or a prior probability of 0.5 (suggested by Wacholder et al. [13]) substantially more associations could be considered likely positive findings.

We found a strong and consistent association between mortality and two DNA repair polymorphisms XRCC3-Thr241Met and XRCC2Arg188His. Conversely, in spite of good biological reasons, we did not find a relationship between other DNA repair polymorphisms (including combined effects of minor alleles in specific DNA repair pathways) and mortality (whether cancer or non-cancer). However, we noted a reversal in mortality trend for the XRCC3 IVS6-14 variant allele in strong linkage disequilibrium with the XRCC3-Thr241Met common allele which also appeared to highlight the important functional role of the XRCC3-Thr241Met polymorphism.

XRCC3 and XRCC2 are core components of the Rad51-related protein family and are thought to mediate homologous pairing and strand exchange during the repair of double-strand breaks. Two distinct Rad51 paralogues have been identified, one containing XRCC3, Rad51L1 and Rad51L3, the

Table 1 – DNA repair capacity genes.

	Base excision repair (BER)			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC1 Arg194Trp				
C/C	864 (87.0)	82 (86.3)	Ref.	Ref.
C/T	127 (12.8)	13 (13.7)	1.01 (0.56–1.82)	0.91 (0.48–1.72)
T/T	2 (0.2)	0 (0.0)	–	–
Total	993 (100)	95 (100)		
XRCC1 Pro206Pro				
A/A	306 (30.8)	33 (34.7)	Ref.	Ref.
A/G	465 (46.9)	41 (43.2)	0.76 (0.48–1.20)	0.67 (0.41–1.09)
G/G	221 (22.3)	21 (22.1)	0.77 (0.44–1.33)	0.76 (0.43–1.35)
Total	992 (100)	95 (100)		
XRCC1 Arg399Gln				
G/G	436 (43.9)	44 (46.3)	Ref.	Ref.
G/A	444 (44.7)	36 (37.9)	0.88 (0.57–1.38)	0.96 (0.60–1.54)
A/A	113 (11.4)	15 (15.8)	1.45 (0.81–2.62)	1.65 (0.89–3.06)
Total	993 (100)	95 (100)		
APEX Asp148Glu				
T/T	280 (28.2)	27 (28.4)	Ref.	Ref.
T/G	475 (47.8)	48 (50.5)	1.09 (0.68–1.75)	1.04 (0.64–1.70)
G/G	238 (23.0)	20 (21.1)	1.04 (0.58–1.86)	1.02 (0.56–1.86)
Total	993 (100)	95 (100)		
hOGG1 Ser326Cys				
C/C	618 (62.3)	52 (54.7)	Ref.	Ref.
C/G	328 (33.0)	41 (43.2)	1.57 (1.04–2.36)	1.51 (0.98–2.34)
G/G	47 (4.7)	2 (2.1)	0.48 (0.17–1.97)	0.60 (0.14–2.52)
Total	993 (100)	95 (100)		
PCNA 3′-UTR				
G/G	790 (79.6)	75 (79.8)	Ref.	Ref.
G/C	191 (19.2)	19 (20.2)	0.97 (0.58–1.60)	0.98 (0.58–1.64)
C/C	12 (1.2)	0 (0.0)	–	–
Total	993 (100)	94 (100)		
	Nucleotide excision repair (NER)			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
ERCC2/XPD Asp312Asn				
G/G	385 (38.8)	32 (33.7)	Ref.	Ref.
G/A	452 (45.5)	50 (52.6)	1.42 (0.91–2.21)	1.28 (0.80–2.04)
A/A	156 (15.7)	13 (13.7)	1.06 (0.55–2.01)	0.88 (0.45–1.74)
Total	993 (100)	95 (100)		
ERCC2/XPD Lys751Gln				
A/A	359 (36.2)	36 (37.9)	Ref.	Ref.
A/C	453 (45.6)	48 (50.5)	1.10 (0.71–1.70)	1.15 (0.73–1.81)
C/C	181 (18.2)	11 (11.6)	0.69 (0.35–1.35)	0.58 (0.28–1.16)
Total	993 (100)	95 (100)		
ERCC1 Asn118Asn				
T/T	364 (36.7)	37 (39.0)	Ref.	Ref.
T/C	461 (46.5)	37 (39.0)	0.82 (0.52–1.29)	0.83 (0.52–1.35)
C/C	167 (16.8)	21 (22.0)	1.21 (0.71–2.07)	1.25 (0.72–2.18)
Total	992 (100)	95 (100)		

Table 1 – (*Continued*)

	Double-strand break repair (DSBR)			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC3 IVS6-14				
A/A	491 (49.5)	61 (64.2)	Ref.	Ref.
A/G	412 (41.6)	32 (33.7)	0.59 (0.38–0.91)	0.58 (0.37–0.91)
G/G	88 (8.9)	2 (2.1)	0.22 (0.05–0.88)	0.25 (0.06–1.03)
Total	991 (100)	95 (100)		
XRCC3 Thr241Met				
C/C	360 (36.3)	20 (21.0)	Ref.	Ref.
C/T	481 (48.4)	60 (63.2)	2.19 (1.32–3.64)	2.25 (1.32–3.83)
T/T	152 (15.3)	15 (15.8)	1.95 (0.99–3.83)	2.04 (1.00–4.13)
Total	993 (100)	95 (100)		
BRCA1 Pro871Leu				
T/T	339 (44.0)	27 (42.9)	Ref.	Ref.
T/C	352 (45.6)	28 (44.4)	1.08 (0.63–1.84)	0.96 (0.54–1.67)
C/C	80 (10.4)	8 (12.7)	1.36 (0.61–2.99)	1.08 (0.44–2.61)
Total	771 (100)	63 (100)		
BRCA2 Asn372His				
A/A	396 (51.3)	33 (52.4)	Ref.	Ref.
A/C	323 (41.8)	24 (38.1)	0.96 (0.57–1.63)	0.96 (0.55–1.66)
C/C	53 (6.9)	6 (9.5)	1.21 (0.50–2.89)	0.98 (0.36–2.70)
Total	772 (100)	63 (100)		
LIG4 Ala3Val				
C/C	685 (88.0)	60 (95.2)	Ref.	Ref.
C/T	63 (8.1)	2 (3.2)	0.43 (0.10–1.78)	0.30 (0.07–1.29)
T/T	30 (3.9)	1 (1.6)	0.53 (0.07–3.82)	0.63 (0.08–4.66)
Total	778 (100)	63 (100)		
LIG45 Thr9Ile				
C/C	517 (69.9)	52 (83.9)	Ref.	Ref.
C/T	200 (27.1)	8 (12.9)	0.48 (0.23–1.01)	0.49 (0.23–1.05)
T/T	22 (3.0)	2 (3.2)	0.59 (0.14–2.45)	0.61 (0.14–2.70)
Total	739 (100)	62 (100)		
NBS1 Glu185Gln				
C/C	368 (47.4)	33 (53.2)	Ref.	Ref.
C/G	325 (41.9)	24 (38.7)	0.90 (0.53–1.52)	0.77 (0.44–1.34)
G/G	83 (10.7)	5 (8.1)	0.69 (0.27–1.78)	0.41 (0.14–1.21)
Total	776 (100)	62 (100)		
RAD51 5′-UTR				
G/G	675 (86.4)	56 (87.5)	Ref.	Ref.
G/C	101 (12.9)	8 (12.5)	0.99 (0.47–2.10)	0.80 (0.36–1.77)
C/C	5 (0.7)	0 (0.0)	–	–
Total	781 (100)	64 (100)		
RAD512 5′-UTR				
G/G	252 (32.4)	18 (28.1)	Ref.	Ref.
G/T	386 (49.7)	41 (64.1)	1.53 (0.88–2.67)	1.38 (0.77–2.46)
T/T	139 (17.9)	5 (7.8)	0.52 (0.19–1.41)	0.43 (0.14–1.30)
Total	777 (100)	64 (100)		
RAD52 3′-UTR				
C/C	251 (32.8)	27 (43.6)	Ref.	Ref.
C/T	374 (48.8)	25 (40.3)	0.62 (0.36–1.07)	0.67 (0.37–1.19)
T/T	141 (18.4)	10 (16.1)	0.71 (0.34–1.46)	0.78 (0.36–1.68)
Total	766 (100)	62 (100)		

Table 1 – (*Continued*)

	Double-strand break repair (DSBR)			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC2 Arg188His				
G/G	647 (84.2)	47 (75.8)	Ref.	Ref.
G/A	115 (15.0)	15 (24.2)	1.89 (1.05–3.40)	2.12 (1.14–3.97)
A/A	6 (0.8)	0 (0.0)	–	–
Total	768 (100)	62 (100)		
	Direct reversal repair (DRR)			
	Subjects (%)	Deaths (%)	Crude HR	Adjusted HR[a]
MGMT Leu84Phe				
C/C	725 (73.1)	73 (76.8)	Ref.	Ref.
C/T	246 (24.8)	22 (23.2)	0.91 (0.57–1.47)	0.83 (0.50–1.37)
T/T	21 (2.1)	0 (0.0)	–	
Total	992 (100)	95 (100)		
	Cell cycle/apoptosis			
	Subjects (%)	Deaths (%)	Crude HR	Adjusted HR[a]
TP53 Arg72Pro				
G/G	439 (56.7)	39 (63.9)	Ref.	Ref.
G/C	288 (37.2)	20 (32.8)	0.77 (0.45–1.32)	0.67 (0.37–1.20)
C/C	47 (6.1)	2 (3.3)	0.60 (0.14–2.48)	0.47 (0.10–2.18)
Total	774 (100)	61 (100)		

Crude and adjusted hazard ratio of death for any cause with 95% confidence intervals. Gene name, polymorphism and nucleotide substitution.

[a] HR adjusted by sex, age, school level, smoking status and stratified by center.

other containing XRCC2 and Rad51L2 [14,15]. Although the precise roles of XRCC3, XRCC2 and the other Rad51-related proteins are not fully understood, their functions are clearly non-redundant; for example, chromosomal instability and haploinsufficiency have been observed experimentally in cells with knocked out XRCC2 function [16] and XRCC3 deficiency has been shown to decrease repair of double-strand breaks by 25-fold [17]. Mouse embryonic fibroblasts isolated and immortalized from XRCC2-deficient mice were also found to be highly sensitive to agents that induce DNA double-strand

Table 2 – Combined effects of minor alleles in DNA repair pathways.

Tertiles (and number of alleles)	Subjects	Deaths	Crude HR	Adjusted HR[a]
BER DNA repair genes				
1 (0–2)	236 (23.8)	17 (18.1)	Ref.	Ref.
2 (3)	318 (32.1)	37 (39.4)	1.61 (0.91–2.86)	1.95 (1.04–3.64)
3 (4–7)	438 (44.1)	40 (42.5)	1.32 (0.75–2.33)	1.50 (0.81–2.77)
Total	992 (100.0)	94 (100.0)		p trend = 0.37
NER DNA repair genes				
1 (0–1)	327 (33.0)	30 (31.6)	Ref.	Ref.
2 (2)	187 (18.8)	20 (21.0)	1.19 (0.67–2.10)	1.10 (0.61–2.00)
3 (3–6)	478 (48.2)	45 (47.4)	1.10 (0.69–1.75)	1.05 (0.65–1.70)
Total	992 (100.0)	95 (100.0)		p trend = 0.80
DSBR DNA repair genes				
1 (0–6)	182 (26.4)	12 (21.1)	Ref.	Ref.
2 (7–8)	248 (35.9)	19 (33.3)	1.10 (0.53–2.28)	1.11 (0.52–2.36)
3 (9–14)	260 (37.7)	26 (45.6)	1.62 (0.81–3.22)	1.29 (0.61–2.71)
Total	690 (100.0)	57 (100.0)		p trend = 0.49

Crude and adjusted hazard ratio of death for any cause with 95% confidence intervals in relation to tertiles of the distribution of cumulative variant alleles.

[a] HR adjusted by sex, age, school level, smoking status and stratified by center.

Table 3 – XRCC3 18067 C > T (Thr241Met), XRCC2 31479 G > A (Arg188His) allele variants separately in association with (a) total cancer mortality, (b) total non-cancer mortality and (c) all incident cancers.

	(a) Total cancer mortality			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC3 Thr241Met				
C/C	375 (35.5)	5 (13.9)	Ref.	Ref.
C/T	520 (49.2)	25 (69.4)	3.60 (1.37–9.43)	3.29 (1.23–7.82)
T/T	161 (15.3)	6 (16.7)	3.00 (0.91–9.87)	2.84 (0.81–9.90)
Total	1056 (100)	36 (100)		
XRCC2 Arg188His				
G/G	682 (84.1)	12 (63.2)	Ref.	Ref.
G/A	123 (15.2)	8 (36.8)	3.74 (1.52–9.17)	3.17 (1.21–8.30)
A/A	6 (0.7)	0 (0.0)	–	–
Total	811 (100)	20 (100)		
	(b) Total non-cancer mortality			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC3 Thr241Met				
C/C	360 (36.3)	15 (25.4)	Ref.	Ref.
C/T	481 (48.4)	35 (59.3)	1.71 (0.93–3.15)	1.89 (1.00–3.60)
T/T	152 (15.3)	9 (15.3)	1.60 (0.70–3.68)	1.78 (0.75–4.27)
Total	993 (100)	59 (100)		
XRCC2 Arg188His				
G/G	647 (84.2)	33 (78.6)	Ref.	Ref.
G/A	115 (14.0)	9 (21.4)	1.53 (0.77–3.70)	1.72 (0.85–4.08)
A/A	6 (0.8)	0 (0.0)		
Total	768 (100)	42 (100)		
	(c) All incident cancers			
	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
XRCC3 Thr241Met				
C/C	350 (35.5)	30 (21.0)	Ref.	Ref.
C/T	483 (49.0)	58 (63.2)	1.41 (0.91–2.20)	1.34 (0.84–2.13)
T/T	153 (15.5)	14 (15.8)	1.19 (0.63–2.24)	1.16 (0.58–2.31)
Total	986 (100)	102 (100)		
XRCC2 Arg188His				
G/G	640 (84.3)	54 (76.1)	Ref.	Ref.
G/A	113 (14.9)	17 (23.9)	1.89 (1.09–3.29)	1.92 (1.06–3.48)
A/A	6 (0.8)	0 (0.0)	–	
Total	759 (100)	71 (100)		

Crude and adjusted hazard ratio for all cancer deaths with 95% confidence intervals. Gene name, polymorphism and nucleotide substitution.

[a] HR adjusted by sex, age, school level, smoking status and stratified by centre.

breakage, including cancer therapy drugs such as temozolomide, fotemustine and cisplatin [18]. SNPs in DNA repair genes are also known to affect patient responses to anticancer treatments. For example, pancreatic adenocarcinoma patients receiving gemcitabine and radiotherapy treatment, and having at least an XRCC2 31479 A variant allele, were more resistant to treatment and had poorer survival compared to those with the G/G genotype [2]. In another study, the XRCC3 T/T variant genotype was associated with a two-fold decrease in breast cancer survival among patients receiving high dose chemotherapy treatment [5]. In both studies, increasing incremental combination of variant genotypes significantly decreased survival. These findings, together with our own, appear to be contrary to the traditional view in which DNA repair deficiency is thought to improve survival in patients already diagnosed with cancer and treated with DNA-damaging agents [19,20]. This latter paradigm does not appear to be universal though. Mismatch repair (MMR) deficient human tumour cells were tolerant to the presence of high numbers of modified bases in their DNA (which persisted and were mutagenic) but which were not toxic to the cells [21]. The effect of specific gene polymorphisms on the repair efficiency of cytotoxic-induced DNA double-strand breaks is also likely to involve a complex array of factors including inactivation or activation of tumour suppressor genes and oncogenes, cell cycle regulation, cell signalling as well as DNA repair

Table 4 – Combination of gene variants XRCC3 18067 C > T (Thr241Met) and XRCC2 31479 G > A (Arg188His) and association with (a) total mortality, (b) total cancer mortality, (c) total circulatory disease mortality and (d) all incident cancers.

Allele combinations	Subjects	Deaths (%)	Crude HR	Adjusted HR[a]
(a) Total mortality				
0	239 (31.1)	10 (16.1)	Ref.	Ref.
1	351 (45.7)	31 (50.0)	2.38 (1.38–4.11)	2.53 (1.17–5.46)
≥2	178 (23.2)	21 (33.9)	2.10 (1.11–3.98)	3.62 (1.60–8.20)
Total	768 (100)	62 (100)		p trend = 0.002
(b) Total cancer mortality				
0–1	620 (76.7)	11 (50.0)	Ref	Ref.
2	156 (19.3)	4 (18.2)	1.38 (0.44–4.33)	1.26 (0.37–4.28)
≥3	32 (4.0)	7 (31.8)	15.86 (6.00–40.79)	25.27 (7.55–74.50)
Total	808 (100)	22 (100)		p trend < 0.001
(c) Total non-cancer mortality				
0	239 (31.1)	9 (21.4)	Ref.	Ref.
1	351 (45.7)	23 (54.8)	1.87 (1.01–3.49)	1.92 (0.81–4.52)
≥2	178 (23.2)	10 (23.8)	1.31 (0.59–2.90)	1.98 (0.75–5.24)
Total	768 (100)	42 (100)		p trend = 0.16
(d) All incident cancer				
0	231 (30.4)	18 (25.3)	Ref.	Ref.
1	349 (46.0)	33 (46.5)	1.51 (0.94–2.25)	1.30 (0.70–2.42)
≥2	179 (23.6)	20 (28.2)	1.54 (0.87–2.70)	1.75 (0.87–3.55)
Total	759 (100)	71 (100)		p trend = 0.10

Crude and adjusted hazard ratio for all deaths with 95% confidence intervals. Gene name, polymorphism and nucleotide substitution. 0 = [XRCC3 CC and XRCC2 GG], 1 = [XRCC3 CT and XRCC2 GG] or [XRCC3 CC and XRCC2 GA], ≥2 = [XRCC3 CT and XRCC2 GA] or [XRCC3 TT or XRCC2 GG] or [XRCC3 CC or XRCC2 AA] or [XRCC3 TT and XRCC2 GA] or [XRCC3 CT and XRCC2 AA] or [XRCC3TT and XRCC2 AA], ≥3 = [XRCC3 TT and XRCC2 GA] or [XRCC3 CT and XRCC2 AA] or [XRCC3TT and XRCC2 AA].

[a] HR adjusted by sex, age, school level, smoking status and stratified by centre.

[22–24]. In fact, gross genomic instability, with the potent combination of accumulated double-strand breaks and sustained mutations in tumour suppressor genes and oncogenes, is known to be a predominant factor in tumorigenesis and patient prognosis [22,25].

Therefore, it is entirely plausible that decreased DNA repair capacity increases the risk of developing cancer and in turn (independently or in association with the effects of increased resistance to chemotherapy and/or radiotherapy) reduces survival. In support, many studies have shown an etiological association between deficient DNA repair and increased cancer risk [26–29]. XRCC3 deficiency was also found to be significantly associated with increased bulky-DNA adducts in non-smokers [30]. This is consistent with findings which indicated that the XRCC3 T/T variant genotype was deficient in repairing X-ray- but not UV-light-induced chromosomal aberrations [31]. Moreover, observations based on the chromosome aberration repair assay for three polymorphisms (XRCC1-Arg399Gln, XRCC3-Thr241Met and XPD-Lys751Gln) are consistent with studies based on the presence of bulky-DNA adducts [32]. Such studies strongly indicate that it is important for normal tissues to have efficient DNA repair in order to eliminate DNA lesions caused by carcinogens. In the current study, we also found moderate evidence supporting an etiological association of increased cancer risk (incidence) for higher levels of combined XRCC3 and XRCC2 adverse alleles (with significant dose–response effects) and XRCC2 (separately), and increased prostate cancer risk (incidence) with XRCC2 (though a weak non-significant effect was found for XRCC3, separately).

The association between XRCC3 and mortality was still apparent after exclusion of cancer deaths. Various authors have hypothesized that the possible evolution of DNA adducts towards chronic disease may depend on the ability of cells to repair DNA damage [33]. Typically, molecular damage of this type in proliferating cells might evolve towards a neoplastic form. Studies in both humans and experimental animals have shown that there is an accumulation of DNA alterations in cardiac myocytes related to increased age and environmental exposures, for example, tobacco smoke [34–37]. Yet no neoplastic evolution appears to occur since these cells are perennial and fully differentiated and do not proliferate. There is also increasing evidence showing that DNA damage is associated in the progression of atherosclerosis. DNA adducts in vascular smooth muscle cells are predictive of atherosclerosis [38], DNA strand breaks, oxidised pyrimidines and altered purines are significantly higher in patients with coronary artery disease than controls [39], and human plaques show markers of oxidative damage, including DNA strand breaks, expression of 8-oxo-G (an oxidative modification of guanine residues in DNA) and activation of DNA repair enzymes [40].

To conclude, overall, this study is the first to demonstrate prospectively the relationship between variants in double-strand break repair genes, XRCC3 and XRCC2, and total mortality. Significantly increased total and cancer mortality

Table 5 – False positive report probability (FPRP).

Gene/SNP	OR (95% CI)	*p*-Value	HR = 1.5 power	Prior probability				HR = 2.0 power	Prior probability			
				0.5	0.25	0.1	0.01		0.5	0.25	0.1	0.01
Total mortality												
XRCC3 cod. 241 C/T	2.25 (1.32–3.83)	0.003	0.10	**0.04**	**0.11**	0.27	0.80	0.33	**0.01**	**0.02**	**0.07**	0.46
XRCC3 cod. 241 T/T	2.04 (1.00–4.13)	0.047	0.20	**0.19**	0.42	0.69	0.96	0.48	**0.09**	0.23	0.47	0.91
XRCC2 cod. 188 G/A	2.12 (1.14–3.97)	0.019	0.14	**0.12**	0.29	0.55	0.93	0.43	**0.04**	**0.12**	0.28	0.81
XRCC3 cod. 241*XRCC2 cod. 188												
Allele combinations = 1	2.53 (1.17–5.46)	0.018	0.09	**0.16**	0.37	0.64	0.95	0.28	**0.06**	**0.16**	0.37	0.87
Allele combinations > 1	3.62 (1.60–8.20)	0.002	0.02	**0.10**	0.26	0.52	0.92	0.08	**0.03**	**0.07**	**0.19**	0.72
Total non-cancer mortality												
XRCC3 cod. 241 C/T	1.89 (1.00–3.60)	0.050	0.24	**0.18**	0.40	0.66	0.96	0.57	**0.08**	0.22	0.46	0.90
XRCC3 cod. 241 T/T	1.78 (0.75–4.27)	0.196	0.35	0.36	0.63	0.83	0.98	0.60	0.25	0.49	0.75	0.97
XRCC2 cod. 188 G/A	1.72 (0.85–4.08)	0.218	0.38	0.37	0.63	0.84	0.98	0.63	0.26	0.51	0.76	0.97
XRCC3 cod. 241*XRCC2 cod. 188												
Allele combinations = 1	1.92 (0.81–4.52)	0.135	0.29	0.32	0.59	0.81	0.98	0.54	**0.20**	0.43	0.69	0.96
Allele combinations > 1	1.98 (0.75–5.24)	0.169	0.29	0.37	0.64	0.84	0.98	0.51	0.25	0.50	0.75	0.99
Total cancer mortality												
XRCC3 cod. 241 C/T	3.29 (1.23–7.82)	0.007	0.04	**0.16**	0.36	0.63	0.95	0.13	**0.05**	**0.14**	0.33	0.84
XRCC3 cod. 241 T/T	2.84 (0.81–9.90)	0.101	0.16	0.39	0.66	0.85	0.980.97	0.29	0.26	0.51	0.76	0.97
XRCC2 cod. 188 G/A	3.17 (1.21–8.30)	0.019	0.06	0.23	0.45	0.73	0.99	0.17	**0.10**	0.24	0.49	0.91
XRCC3 cod. 241*XRCC2 cod. 188												
Allele combinations = 2	1.26 (0.37–4.28)	0.711	0.61	0.54	0.78	0.91	0.76	0.77	0.48	0.74	0.89	0.99
Allele combinations > 2	25.27 (7.55–74.5)	<0.001	0.01	**0.03**	**0.09**	0.22	0.97	0.02	**0.00**	**0.00**	**0.02**	**0.18**
All incident cancer												
XRCC3 cod. 241 C/T	1.34 (0.84–2.13)	0.216	0.68	0.24	0.49	0.74	0.99	0.96	**0.18**	0.40	0.67	0.96
XRCC3 cod. 241 T/T	1.16 (0.58–2.31)	0.673	0.77	0.47	0.72	0.89	0.94	0.94	0.42	0.68	0.87	0.99
XRCC2 cod. 188 G/A	1.92 (1.06–3.48)	0.032	0.21	**0.13**	0.31	0.58	0.98	0.55	**0.05**	**0.15**	0.34	0.85
XRCC3 cod. 241*XRCC2 cod. 188												
Allele combinations = 1	1.30 (0.70–2.42)	0.407	0.67	0.38	0.64	0.84	0.97	0.91	0.31	0.57	0.80	0.98
Allele combinations > 1	1.75 (0.87–3.55)	0.121	0.34	0.27	0.52	0.77		0.64	**0.16**	0.36	0.63	0.95

Prior probabilities ranging from 0.5 to 0.01 with estimated statistical power to detect an OR of 1.5 or 2.0 (or 0.67 and 0.5, respectively, if observed OR < 1.0) and α level equal to observed *p*-value. Bold type indicates FPRP value below the 0.2 threshold.

were associated with increasing number of adverse genotypes for XRCC3 and XRCC2. Since both are key functionally discrete components in Rad51-mediated DNA repair [14,15], the association has strong biological plausibility. The results could be mediated via an association of increased cancer risk with higher levels of combined adverse alleles. We were not able to evaluate the possible survival-effect of XRCC3 and XRCC2 on the repair efficiency of cancer therapy-induced DNA double-strand breaks, but this should be explored in future investigations.

The strengths of the study are that it employs a prospective design with 100% follow-up complete for both mortality and cancer incidence and validated methods of data collection and standardization. It is important to confirm our observations in future prospective studies. In particular, further research is required to assess the relationship between DNA damage (using adducts measured prospectively before cancer mortality or onset of cancer), DNA repair genotype and clinical outcome.

Conflict of interest

The Authors declare that they have no conflicts of interest.

Acknowledgements

This paper was made possible by a grant of the European Community (5th Framework Programme) to PV (grant QLK4CT199900927) and a grant of the Compagnia di San Paolo to the ISI Foundation. All authors are independent from funders. Mortality data for The Netherlands were obtained from "Statistics Netherlands".

Also, the work described in the paper was carried out with the financial support of:

- Europe Against Cancer Program of the European Commission (SANCO).
- ISCIII, Red de Centros RCESP, C03/09.
- Deutsche Krebshilfe.
- Deutsches Krebsforschungszentrum.
- German Federal Ministry of Education and Research.
- Danish Cancer Society.
- Health Research Fund (FIS) of the Spanish Ministry of Health.
- Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra.
- Cancer Research U.K.
- Medical Research Council, United Kingdom.
- Stroke Association, United Kingdom.
- British Heart Foundation.
- Department of Health, United Kingdom.
- Food Standards Agency, United Kingdom.
- Wellcome Trust, United Kingdom.
- Greek Ministry of Education and Ministry of Health.
- Italian Association for Research on Cancer (AIRC).
- Italian National Research Council.
- Dutch Ministry of Public Health, Welfare and Sports.
- World Cancer Research Fund.
- Swedish Cancer Society.
- Swedish Scientific Council.
- Regional Government of Skåne, Sweden.
- Norwegian Cancer Society.
- Research Council of Norway.

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