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DNA repair polymorphisms and cancer risk in non-smokers in a cohort study

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Environmental carcinogens contained in air pollution, such as polycyclic aromatic hydrocarbons, aromatic amines or *N*-nitroso compounds, predominantly form DNA adducts but can also generate interstrand cross-links and reactive oxygen species. If unrepaired, such lesions increase the risk of somatic mutations and cancer. Our study investigated the relationships between 22 polymorphisms (and their haplotypes) in 16 DNA repair genes belonging to different repair pathways in 1094 controls and 567 cancer cases (bladder cancer, 131; lung cancer, 134; oral–pharyngeal cancer, 41; laryngeal cancer, 47; leukaemia, 179; death from emphysema and chronic obstructive pulmonary disease, 84). The design was a case–control study nested within a prospective investigation. Among the many comparisons, few polymorphisms were associated with the diseases at the univariate analysis: *XRCC1*-399 Gln/Gln variant homozygotes [odds ratios (OR) = 2.20, 95% confidence intervals (CI) = 1.16–4.17] and *XRCC3*-241 Met/Met homozygotes (OR = 0.51, 95% CI = 0.27–0.96) and leukaemia. The recessive model in the stepwise multivariate analysis revealed a possible protective effect of *XRCC1*-399Gln/Gln in lung cancer (OR = 0.22, 95% CI = 0.05–0.98), and confirmed an opposite effect (OR = 2.47, 95% CI = 1.02–6.02) in the leukaemia group. Our results also suggest that the *XPD/ERCC1*-GAT haplotype may modulate leukaemia (OR = 1.28, 95% CI = 1.02–1.61), bladder cancer (OR = 1.38, 95% CI = 1.06–1.79) and possibly other cancer risks. Further investigations of the combined effects of polymorphisms within these DNA repair genes, smoking and other risk factors may help to clarify the influence of genetic variation in the carcinogenic process.

Introduction

Environmental carcinogens contained in air pollution, such as polycyclic aromatic hydrocarbons, aromatic amines or *N*-nitroso compounds, predominantly form DNA adducts but also generate interstrand cross-links and reactive oxygen species [which induce base damage, abasic sites, and single and double strand breaks (SSBs and DSBs)]. Unrepaired damage can result in apoptosis or may lead to unregulated cell growth and cancer. If DNA damage is recognized by the cell's machinery, several responses may occur to prevent replication in the presence of genetic errors. At the cellular level, checkpoints can be activated to arrest the cell cycle, transcription can be upregulated to compensate for the damage or the cell can apoptose (1). 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

Abbreviations: AP, apurinic/apyrimidinic; BER, base excision repair; CA, chromosomal aberrations; CI, confidence interval; COPD, chronic obstructive pulmonary disease; DHPLC, denaturing high performance liquid chromatography; DRC, defect repair capacity; DSB, double strand break; EPIC, European Prospective Investigation into Cancer and Nutrition; ETS, environmental tobacco smoke; FPRP, false positive report probability; IARC, International Agency for Research on Cancer; LD, linkage disequilibrium; MDMT, methylguanine-DNA methyltransferase; MMR, Mismatch repair; NER, nucleotide excision repair; OR, odds ratio; PE, primer extension technique; SNP, single nucleotide polymorphism; SSB, single strand break; UADC, upper aero-digestive cancer.

encoding for DNA repair molecules have been proposed as candidate cancer-susceptibility genes (2,3). At least four pathways of DNA repair operate on specific types of damaged DNA, and each pathway involves numerous molecules (4). The nucleotide excision repair (NER) pathway repairs bulky lesions such as pyrimidine dimers, other photo-products, 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. 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. At least two pathways for DSB repair exist: homologous recombination and non-homologous end-joining. 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 directly removed by the suicide enzyme methylguanine-DNA methyltransferase (*MGMT*).

Studies to date indicate that variation in DNA repair genes in each of the pathways may influence cancer susceptibility, including polymorphisms analysed in the present study (5,6; http://www.perseus.isi.it/huge); however, results are not consistent, and other potentially important polymorphisms in these and other genes have not been explored yet. Hundreds of polymorphisms in DNA repair genes have been identified; however, the impact on repair phenotype and cancer susceptibility remains uncertain for many of these genes (5–8). Among the polymorphisms analysed, few show an evidence of association with phenotype changes (8). Of these, XRCC1-Arg399Gln, has been analysed in a sister chromatid exchange assay, in three different studies and the results are difficult to interpret, but, positive associations were observed in cigarette smokers compared with non-smokers (9). However, the Gln399 homozygote genotype was significantly associated with increased levels of bulky-DNA adducts in leucocytes of non-smokers (10). Using healthy non-smokers, the Gln allele was found to be defective in the repair of X-ray- but not UV-light-induced chromosomal aberrations (CA) (11). The glycophorin A mutation assay indicated that the Gln allele influenced the production of the NN mutation but not the NO mutation (12). However, the observations were not confirmed in a second study with a much larger sample size (13). The *XRCC3*-241Met allele was also found to be significantly associated with increased bulky-DNA adducts in non-smokers (10). This is consistent with a study which indicated that the variant homozygote was deficient in the repair of X-ray- but not UV-light-induced chromosomal aberrations (11). However, *in vitro* experiments performed by Araujo *et al*. (14) have recently suggested that the increased cancer risk associated with the *XRCC3*-241Met allele may not be attributable to an intrinsic homology-directed repair. For the *OGG1*-Ser326Cys polymorphism, a study of the removal of 8-OH deoxyguanosine (8-OHdG) in lymphocyte DNA has been published (15). In this study, patients with the variant Cys allele had significantly higher 8-OHdG levels than those with the wild-type Ser allele. However, this association was not observed in the normal controls. Investigations on the repair of CA indicate that the Asn allele of XPD-Asp312Asn is proficient in the repair of X-ray-induced DNA damage (16), but defective in the repair of UV-light-induced damage (11). The observation was consistent with increased bulky-DNA adduct levels measured in leucocytes of non-smokers (10). For the XPD-Lys751Gln polymorphism, the use of the CA repair assay indicates that the variant Gln allele is defective in the repair of UV-light- but not X-ray-induced DNA damage (11). The XPD-751Gln variant leads to a conformational change of the protein thus altering its interaction with partner proteins within the TFIIH complex (17).

Qiao *et al*. (18) demonstrated that a post-UV defect repair capacity (DRC) for NER can be modulated by genetic polymorphisms of *XPD* in healthy subjects. The combination of XPD variant alleles, XPD-312Asn and XPD-751Gln, was associated with lower DRC of UV-induced DNA damage than homozygous wild-type alleles. However, these effects were not statistically significant, possibly due to the inherent high variation in the host cell reactivation assay. In addition, cigarette smoking may have some confounding effects on the DRC. The *APEX*-148 Glu variant allele has a small but non-significant effect on apurinic/apyrimidinic (AP) endonuclease and DNA binding activities (19). For the other polymorphisms analysed here, insufficient information has been published for any conclusion about their functional consequences to be drawn. Among the many biomarkers described, only two showed consistency with each other. Observations based on the chromosome aberration repair assay for three polymorphisms (*XRCC1*-Arg399Gln, *XRCC3*-Thr241Met and XPD-Lys751Gln) are consistent with those based on the presence of bulky-DNA adducts (10). In this study, smoking habit might interact with genetic differences in DNA repair activity. It becomes clear that investigation of cigarette smokers and cancer patients should be limited to addressing the repair capacities of these individuals and under their respective conditions, not the inherent activities of DNA repair genes. Even fewer molecular epidemiological studies have been published on cancer susceptibility conferred by specific DNA repair gene haplotypes (20–27) and none on the relationship between repair phenotypes and haplotypes, whose allelic combination may be more relevant in determining the possible functional change at the protein level than single low penetrant single nucleotide polymorphisms (SNPs).

In the present study, we analysed 22 biallelic polymorphisms in 16 DNA repair genes (Table I) involved in different repair pathways (BER, NER, DSB and direct repair) in the EPIC cohort, aiming at studying the relationship between some type of cancer (bladder, lung, oral, pharyngeal, laryngeal cancers and leukaemia) or deaths from respiratory diseases [chronic obstructive pulmonary disease (COPD), emphysema], environmental tobacco smoke (ETS) and genetic susceptibility.

Materials and methods

Subjects

EPIC (European Prospective Investigation into Cancer and Nutrition) is a multicentre European study, coordinated by the International Agency for Research on Cancer (IARC) (Lyon), in which >520 000 healthy volunteers were recruited in 10 European countries (France, Denmark, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and UK) corresponding to 23 recruitment centres. The cohort includes subjects of both genders, mostly in the age range 35–74 years 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 10% sample), anthropological measurements and a 30–40 ml blood sample are available. Signed informed consent forms were collected from all participants (except a subgroup of the Oxford cohort who gave consent on postal questionnaires).

Table I. List of DNA repair genes and polymorphisms analysed

Gene name	Repair pathway	Polymorphism	Nucleotide substitution	Current name	rs no.	Chromosomal location
APEX	BER	Asp148Glu	T→G	*APEX* 2197 T/G	3136820	14q11.2–q12
hOGG1	BER	Ser326Cys	C→G	*OGG1* 8055 C/G	1052133	3p26.2
PCNA	BER	3′-UTR	G→C	*PCNA* 6084 G/C	3626	20pter–p12
XRCC1	BER	Arg194Trp	C→T	*XRCC1* 26304 C/T	1799782	19q13.2
XRCC1	BER	Pro206Pro	A→G	*XRCC1* 26651 A/G	915927	19q13.2
XRCC1	BER	Arg399Gln	G→A	*XRCC1* 28152 G/A	25487	19q13.2
ERCC1	NER	Asn118Asn	C→T	*ERCC1* 19007 C/T	3177700	19q13.2–13.3
ERCC2/XPD	NER	Asp312Asn	G→A	XPD 23591 G/A	1799793	19q13.2–13.3
ERCC2/XPD	NER	Lys751Gln	A→C	XPD 35931 A/C	13181	19q13.2–13.3
MGMT	DRR	Leu84Phe	C→T	*MGMT* 127215 C/T	12917	10q26
BRCA1	DSBR	Pro871Leu	C→T	*BRCA1* P871L	799917	17q21
BRCA2	DSBR	Asn372His	A→C	*BRCA2* N372H	144848	13q12.3
LIG4	DSBR	Ala3Val	C→T	*LIG4* A3V	1805389	13q33–q34
LIG4	DSBR	Thr9Ile	C→T	*LIG4* T9I	1805388	13q33–q34
NBS1	DSBR	Glu185Gln	G→C	*NBS1* E185Q	1805794	8q21–q24
RAD51	DSBR	5′UTR	G→C	*RAD51* 135 G/C	1801320	15q15.1
RAD51	DSBR	5′UTR	G→T	*RAD51* 172 G/T	1801321	15q15.1
RAD52	DSBR	3′UTR	C→T	*RAD52* 2259 C/T	11226	12p13–p12.2
XRCC2	DSBR	Arg188His	G→A	*XRCC2* 31479 G/A	3218536	7q36.1
XRCC3	DSBR	IVS6 -14	A→G	*XRCC3* 17893 A/G	1799796	14q32.3
XRCC3	DSBR	Thr241Met	C→T	*XRCC3* 18067 C/T	861539	14q32.3
TP53	CC/Ap	Arg72Pro	G→C	*TP53* R72P	1042522	17p13.1

The follow-up was based on population cancer registries in seven of the participating countries: Denmark, Italy, the Netherlands, Norway, Spain, Sweden and the UK. In France, Germany and Greece a combination of methods was used, including health insurance records, cancer and pathology registries, and active follow-up through study participants and their next-of-kin. Mortality data were also obtained from either the cancer registry or mortality registries at the regional or national level. All the information is centralized both at the national level and at IARC.

Gen-Air is a case–control study nested within the EPIC cohort, aiming at studying the relationship between some types of cancer and air pollution or ETS. Eleven centres (in France, Italy, Denmark, Sweden, the Netherlands, and Potsdam, Germany) collected data on exposure to ETS, investigating about place of exposure and exposure during childhood. In the present study we considered any exposure to ETS (someone regularly smokes at home and/or work). Individual exposure to air pollution was assessed using concentration data from monitoring stations in routine air quality monitoring networks. Individual pollutants (NO_2, O_3, PM10, SO_2) and residence nearby heavy traffic roads were assessed. Cases were subjects with bladder, lung, oral, pharyngeal, or laryngeal cancers or leukaemia, all newly diagnosed after recruitment. Also deaths from respiratory diseases (COPD, emphysema) were identified and included. These diagnoses were chosen because they were suspected of being associated with air pollution or ETS exposure. Only non-smokers or ex-smokers since at least 10 years have been included in Gen-Air. We matched three controls per case for exposure assessment and the analysis of questionnaire data, and two controls per case for laboratory analyses. Matching criteria were gender, age (±5 years), smoking status (never or former smoker), country of recruitment and follow-up time. Matching was introduced to allow strict control of potentially confounding variables, considering that other risk factors may be stronger than ETS or air pollution. In addition, matching was needed for laboratory analyses, to avoid differential sample degradation between cases and controls. Mean follow-up was 89 months (minimum 51, maximum 123).

Gen-Air has been approved by the Ethical Committee of the IARC and by the local Ethical Committees of the 23 centres.

We identified 4051 subjects (1074 cases and 2977 controls) who met the protocol criteria. Of these subjects, 2410 had blood samples (843 cases and 1564 controls). Blood samples of cases and controls for centres which have released an ethical approval was sent to laboratories for investigation. The Malmo center had decided not to allow the use of their blood samples, but it participated in the rest of the project.

The distribution of cases by cancer site or cause of death was as follows: bladder cancer 227, lung cancer 271, oral/pharyngeal cancer 73, laryngeal cancer 58, leukaemias 311, deaths from respiratory diseases 134.

DNA extraction and polymorphisms

DNA was extracted from 200–300 μl of buffy coat in the laboratory of M.P. according to a standard phenol–chloroform protocol (28), except for the Danish samples that were extracted and purified from lymphocytes using a salting out procedure. DNA was available for 1661 subjects, 567 cases and 1094 Controls; cases were distributed as follows: bladder cancer, 124; lung cancer, 116; oral/pharyngeal cancer, 43; laryngeal cancer, 39; leukaemias, 169; deaths from respiratory diseases (COPD), 77. DNA has been sent in dry ice to ISI Foundation, Torino (G.M.) and to Cambridge (A.M.D.) laboratories for the analysis of DNA repair gene polymorphisms. A list of the analysed DNA repair gene SNPs is reported in Table I with all the relevant information.

Genotyping of DNA repair polymorphisms

5′ Nuclease Assay (TaqMan) was used to genotype all the polymorphisms reported in Table I except for *OGG1*-Ser326Cys. Cambridge laboratory used plain TaqMan probes, whereas Torino laboratories used fluorogenic MGB (minor groove binder) probes; allele specific probes were labelled in both laboratories with Fam and Vic fluorophors.

OGG1-Ser326Cys polymorphism has been genotyped by the primer extension technique (PE) on a denaturing high performance liquid chromatography (DHPLC) instrument (29) (Varian, Walnut Creek, CA) to avoid possible TaqMan mistyping due to the existence of other nucleotide substitution in codon 326. The primer extension technique, or single base extension, consists of a specific elongation of a primer by the dideoxynucleotide complementary to the base allelic substitution; the rate of appropriate dideoxynucleotide incorporation completely overwhelmed misincorporations, and heterozygotes were easily detectable after separation of the extended primers through a DHPLC column. Technical details on primers/probes and experimental conditions for TaqMan and PE/DHPLC analysis for genotyped SNPs are published on line as supplementary table.

DNA typing quality control

A methodological validation was performed at the ISI Foundation laboratory, including a comparison between PCR–RFLP, DHPLC and TaqMan assay (28). Moreover, at least 10% of the genotyping have been randomly repeated for each polymorphism. Concordance was in the range of 99–100% for all the comparisons; discordant genotypes were excluded from the analysis. Another extensive evaluation of genotyping methods has been performed in the Cambridge laboratory using 864 DNA samples of mixed quality on the *BRCA2*-Asn372His genotype by allele specific oligonucleotides (ASO) and by Taqman (28). If the two results did not agree, a forced RsaI digest was additionally used.

Statistical analysis

We computed odds ratios (OR) and 95% confidence intervals (CI) in conditional logistic regression models for matched groups. All association analyses, at the level of individual SNPs, were performed assuming recessive or co-dominant model of inheritance of traits associated with alleles. In the 'recessive' model, disease risks were compared between subjects who were

homozygous for the rare allele and all others. In the 'co-dominant' model, disease risks were related to the number of copies of an allele (0–2) carried by the individuals. A dominant model has not been tested due to the general biological implausibility of this hypothesis, avoiding, at the same time, unnecessary multiple comparisons. If the single allelic dose was sufficient to show the same phenotype in heterozygotes and variant homozygotes, we should expect a strong effect of the SNP in terms of changes in activity/expression of the gene, which is generally not observed with phenotypic assays, being characteristic of more severe mutations rather than low penetrant SNPs. We tested the association between polymorphisms and a number of other variables, including dietary variables, BMI, physical exercise and educational level, but none was identified as a potential confounder. The stepwise method was employed to select the set of polymorphisms independently associated with the outcomes. In this procedure the significance level to keep a variable in the model was set to 0.1. We further performed the analysis of each polymorphism stratified by gender, passive smoking (ETS) and previous smoking (former smokers) habits. We tested for interaction under a multiplicative model between gender, smoking (ETS and former smokers) and each genotype by including an interaction term in the logistic regression models. D' linkage disequilibrium (LD) values were calculated in the control population using the maximum likelihood method (Arlequin ver. 2.000) and haplotype frequencies were estimated in cases and controls. Crude ORs and 95% CI were computed from contingency tables on derived absolute frequencies in cases and controls to identify possible unfavourable (at risk)/favourable (protective) haplotypes; each haplotype was compared with the sum of the remaining in order to increase the statistical power and due to the difficulty to define strong *a priori* reference haplotypes. We also used a Bayesian approach proposed by Wacholder *et al.* (30,31) to estimate the false positive report probability (FPRP) of positive results; this method requires the estimation (from previous biochemical or molecular information and/or results from meta-analyses) of the prior probabilities that the specific SNPs are associated with the disease under study. We gave a high prior probability (of 0.25) when (i) the biological plausibility was high and (ii) the existing epidemiological evidence of association with cancer was fair; a prior probability of 0.1 was given when the prior epidemiological evidence was poor but biological plausibility was high, and a prior probability of 0.01 when both were poor. The available epidemiological evidence did not allow higher prior probabilities. On this basis, we decided to consider a prior probability of 0.25 for XRCC1 codon 399 and for XRCC3 codon 241, 0.1 for XPD codon 751 and OGG1 codon 326, and $\leq$0.01 for all the other genes/polymorphisms, with the estimated statistical power to detect an OR of 1.5 or of 2.0 (or 0.67 and 0.5, respectively, if observed ORs were <1) and α level equal to the observed *P*-value. Given the many comparisons, we preferred to be conservative using a cut point of 0.2 for FPRP, even though we cannot exclude higher thresholds. All the analyses were performed by the SAS package for personal computers (SAS, Cary, NC).

Results

We analysed 22 DNA repair SNPs (Table I) in 567 cancer patients (bladder cancer, 124; lung cancer, 116; oral/pharyngeal cancer, 43; laryngeal cancer, 39; leukaemias, 169; and COPD, 77) and in 1094 controls in the framework of the case–control Gen-Air project nested within the EPIC study. Oral/pharyngeal cancers and laryngeal cancers were pooled and defined as upper aero-digestive cancer (UADC). All polymorphisms studied were in Hardy–Weinberg equilibrium in controls except for *XRCC1*-Pro206Pro ($P = 0.038$) and *LIG4* codon 3 ($P \ll 0.0001$); whereas, the *XRCC1*-Pro206Pro deviation can be disregarded, the second could strongly affect haplotype reconstruction, thus *LIG4* results must be interpreted with caution. A genotyping error seems unlikely as 10% of samples have been re-genotyped with 99% of concordance. Genotype frequencies and ORs (95% CI) related to the variant alleles are shown in Table II for controls and cases (stratified by tumour type and COPD).

The variant allele frequencies in 1094 controls were as follows: *APEX*-148Glu = 0.48; *OGG1*-326Cys = 0.21; *PCNA*-3′-UTR C = 0.11; *XRCC1*-194Trp = 0.07; *XRCC1*-206Pro = 0.45; *XRCC1*-399Gln = 0.34; *ERCC1*-118Asn = 0.40; *ERCC2*/XPD-312Asn = 0.39; *ERCC2*/XPD-751Gln = 0.41; *MGMT*-84Phe = 0.14; *BRCA1*-871Leu = 0.33; *BRCA2*-372His = 0.28; *LIG4*-3Val = 0.08; *LIG4*-9Ile = 0.16; *NBS1*-185Gln = 0.31; *RAD51*-5′UTRG→C C = 0.07; *RAD51*-5′UTRG→T T = 0.42; *RAD52*-3'UTR T = 0.42; *XRCC2*-188His = 0.08; *XRCC3*-IVS6 -14 G = 0.29; *XRCC3*-241Met = 0.40; *TP53*-72Pro = 0.24. All the gene frequencies were in agreement with previous studies in Caucasian populations (http://perseus.isi.it/huge; http://www.ncbi.nlm.nih.gov/SNP/).

Few polymorphisms are individually associated with the diseases that we considered when analysed by either univariate (Table II) or multivariate analyses; results observed at uni variate analyses were not further considered if significance was limited only to the heterozygotes, unless significances were confirmed by the multivariate analyses. Among the many comparisons (Table II), the following significant associations were found Table II: *XRCC1*-399 Gln/Gln variant homozygotes (OR = 2.20, 95% CI = 1.16–4.17) and *XRCC3*-241 Met/Met homozygotes (OR = 0.51, 95% CI = 0.27–0.96) in leukaemia, whereas for *XRCC1*-206 Pro/Pro (G/G) variant homozygotes the association was borderline (OR = 0.57, 95% CI = 0.32–1.01).

Stepwise multivariate analysis

Multivariate analyses were perfomed with stepwise selection of variables, using both a co-dominant and a recessive model. The first model did not reveal any significant association for lung and bladder cancers, but confirmed the protective effect for the *XRCC1*-206Pro (G) allele (OR = 0.65, 95% CI = 0.44–0.94) in the leukaemia group. A significant decreased risk emerged for the *APEX*-148Glu allele (OR = 0.59, 95% CI = 0.35–0.99) in UADC. The recessive model in the stepwise multivariate analysis revealed a protective effect of the *XRCC1*-399Gln/Gln in lung cancer (OR = 0.22, 95% CI = 0.05–0.98), and confirmed the opposite effect (OR = 2.47, 95% CI = 1.02–6.02) in the leukaemia group.

ETS, former smoking and gender

Table III shows the distribution of ETS, former smoking and gender in the current population. As we previously published (32), in the nested case–control study exposure to ETS was associated with increased risks (odds ratio 1.70, 1.02 to 2.82, for respiratory diseases; 1.76, 0.96 to 3.23, for lung cancer alone). Odds ratios were consistently higher in former smokers than in those who had never smoked. Except for leukaemia, all the other cancers are strongly related to smoking, and gender is a risk factor for most of the cancers, justifying a selection of these variables for the interaction analysis.

After stratification by ETS, no significant association has been found for individual polymorphisms both in the exposed and non-exposed group, neither for each single kind of tumour or COPD, nor for the overall tumours. Among former smokers, an increased risk of lung cancer has been found for XPD-751Gln (C) carriers (CC versus AA, OR = 2.53, 95% CI = 1.00–6.44 and CA versus AA, OR = 2.84, 95% CI = 1.27–6.37, respectively; $P = 0.007$ for interaction), and also a possible increased risk of UADC for *XRCC3*-241Met/Met carriers (OR = 10.11, 95% CI = 1.02–99.93), although the CI is very wide due to subgrouping. In addition, the previously described associations with leukaemia seems to be limited to the never-smoker group: an increased risk for *XRCC1*-399Gln/Gln variant homozygotes (OR = 2.57, 95% CI = 1.19–5.52) and a decreased risk for XRCC3-241Met/Met homozygotes (OR = 0.33, 95% CI = 0.14–0.77; $P = 0.007$ for interaction).

Table II. Case–control distribution of genotypes for the different DNA repair gene polymorphisms

Gene–genotype (amino acid)	Controls		Lung			Bladder			Leukaemia			UADC			COPD		
	N	(%)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)
XPD cod. 312																	
G/G (Asp/Asp)	418	38.2	49	42.2		48	38.7		71	42.0		32	39.0		31	40.3	
A/G (Asn/Asp)	506	46.3	48	41.4	1.05 (0.60–1.82)	60	48.4	0.98 (0.60–1.59)	76	45.0	0.77 (0.50–1.18)	46	56.1	1.35 (0.76–2.41)	32	41.6	0.56 (0.30–1.08)
A/A (Asn/Asn)	170	15.5	19	16.4	0.94 (0.48–1.85)	16	12.9	0.88 (0.43–1.80)	22	13.0	0.71 (0.39–1.31)	4	4.9	0.34 (0.09–1.24)	14	18.2	0.83 (0.34–2.02)
XPD cod. 751																	
A/A (Lys/Lys)	397	36.3	37	31.9		50	40.3		70	41.4		34	41.5		30	39.0	
A/C (Lys/Gln)	504	46.1	58	50.0	1.50 (0.86–2.60)	58	46.8	0.78 (0.47–1.29)	79	46.8	0.76 (0.49–1.18)	39	47.6	1.07 (0.59–1.94)	31	40.3	0.69 (0.37–1.29)
C/C (Gln/Gln)	193	17.6	21	18.1	1.23 (0.63–2.42)	16	12.9	0.55 (0.27–1.11)	20	11.8	0.59 (0.32–1.07)	9	11.0	0.62 (0.25–1.53)	16	20.8	1.03 (0.45–2.38)
PCNA 3′UTR																	
G/G	871	79.7	95	81.9		99	79.8		133	79.2		65	79.3		61	79.2	
C/G	210	19.2	21	18.1	1.07 (0.56–2.05)	25	20.2	0.89 (0.49–1.60)	35	20.8	1.03 (0.63–1.69)	17	20.7	1.05 (0.51–2.15)	15	19.5	1.05 (0.51–2.16)
C/C	12	1.1	0	0.0	—	0	0.0	—	0	0.0	—	0	0.0	—	1	1.3	2.00 (0.12–31.97)
XRCC1 cod. 194																	
C/C (Arg/Arg)	951	86.9	98	84.5		108	87.1		145	85.8		78	95.1		67	87.0	
C/T (Arg/Trp)	141	12.9	16	13.8	0.94 (0.46–1.90)	16	12.9	1.02 (0.52–2.01)	23	13.6	1.43 (0.80–2.58)	4	4.9	0.50 (0.17–1.53)	10	13.0	0.63 (0.26–1.54)
T/T (Trp/Trp)	2	0.2	2	1.7	—	0	0.0	—	1	0.6	2.21 (0.14–35.59)	0	0.0	—	0	0.0	—
XRCC1 cod. 206																	
A/A (Pro/Pro)	342	31.3	36	31.0		41	33.1		62	36.7		24	29.3		29	37.7	
A/G (Pro/Pro)	508	46.5	58	50.0	1.53 (0.90–2.60)	56	45.2	0.91 (0.53–1.54)	78	46.2	0.74 (0.48–1.14)	39	47.6	1.02 (0.54–1.91)	36	46.8	1.01 (0.54–1.88)
G/G (Pro/Pro)	243	22.2	22	19.0	0.81 (0.41–1.60)	27	21.8	0.84 (0.46–1.51)	29	17.2	0.57 (0.32–1.01)	19	23.2	1.02 (0.47–2.19)	12	15.6	0.54 (0.22–1.35)
XRCC1 cod. 399																	
G/G (Arg/Arg)	484	44.2	51	44.0		54	43.6		67	39.6		34	41.5		30	39.0	
A/G (Gln/Arg)	482	44.1	58	50.0	1.37 (0.83–2.25)	53	42.7	0.96 (0.59–1.56)	74	43.8	0.98 (0.63–1.52)	38	46.3	1.29 (0.70–2.37)	39	50.7	1.88 (0.95–3.73)
A/A (Gln/Gln)	128	11.7	7	6.0	0.50 (0.20–1.21)	17	13.7	1.15 (0.55–2.40)	28	16.6	2.20 (1.16–4.17)	10	12.2	1.30 (0.49–3.49)	8	10.4	0.88 (0.31–2.50)
XRCC3-IVS6-14																	
A/A	554	50.7	53	45.7		60	48.4		92	54.4		46	56.8		37	48.1	
A/G	447	40.9	54	46.6	1.02 (0.62–1.68)	47	37.9	0.85 (0.52–1.39)	66	39.1	1.01 (0.68–1.51)	28	34.6	0.41 (0.21–0.80)	31	40.3	1.32 (0.67–2.59)
G/G	91	8.3	9	7.8	0.76 (0.31–1.86)	17	13.7	1.31 (0.61–2.81)	11	6.5	0.90 (0.43–1.90)	7	8.6	0.62 (0.22–1.77)	9	11.7	2.78 (0.93–8.31)
XRCC3 cod. 241																	
C/C (Thr/Thr)	383	35.0	44	37.9		46	37.1		61	36.1		29	35.4		34	44.2	
C/T (Thr/Met)	544	49.7	56	48.3	0.87 (0.51–1.47)	61	49.2	0.97 (0.59–1.60)	90	53.3	1.14 (0.73–1.77)	39	47.6	1.07 (0.59–1.96)	31	40.3	0.61 (0.33–1.11)
T/T (Met/Met)	167	15.3	16	13.8	1.35 (0.63–2.93)	17	13.7	0.96 (0.46–2.02)	18	10.7	0.51 (0.27–0.96)	14	17.1	1.91 (0.75–4.83)	12	15.6	0.76 (0.31–1.88)
APEX cod. 148																	
T/T (Asp/Asp)	309	28.2	33	28.5		31	25.0		42	24.9		28	34.2		19	24.7	
G/T (Glu/Asp)	526	48.1	56	48.3	0.97 (0.57–1.64)	69	55.7	1.38 (0.79–2.40)	82	48.5	1.29 (0.79–2.08)	36	43.9	0.68 (0.35–1.30)	37	48.1	1.23 (0.60–2.53)
G/G (Glu/Glu)	259	23.7	27	23.3	0.83 (0.44–1.54)	24	19.4	0.96 (0.49–1.88)	45	26.6	1.61 (0.94–2.76)	18	22.0	0.59 (0.28–1.25)	21	27.3	1.47 (0.62–3.50)
ERCC1 cod. 118																	
T/T (Asn/Asn)	402	36.8	48	41.4		56	45.2		61	36.1		27	32.9		29	37.7	
C/T (Asn/Asn)	503	46.0	49	42.2	0.63 (0.37–1.09)	52	41.9	0.79 (0.48–1.30)	82	48.5	1.15 (0.75–1.76)	38	46.3	1.56 (0.81–3.01)	33	42.9	0.93 (0.50–1.73)
C/C (Asn/Asn)	188	17.2	19	16.4	0.71 (0.36–1.40)	16	12.9	0.67 (0.33–1.37)	26	15.4	0.82 (0.45–1.48)	17	20.7	1.79 (0.80–4.01)	15	19.5	0.80 (0.32–1.99)
MGMT cod. 84																	
C/C (Leu/Leu)	803	73.5	91	78.5		90	72.6		128	75.7		54	65.9		62	80.5	
C/T (Leu/Phe)	268	24.5	23	19.8	0.73 (0.40–1.34)	31	25.0	1.17 (0.69–1.98)	36	21.3	0.73 (0.45–1.19)	26	31.7	1.44 (0.77–2.68)	12	15.6	0.62 (0.30–1.29)
T/T (Phe/Phe)	22	2.0	2	1.7	0.77 (0.13–4.41)	3	2.4	4.47 (0.45–44.30)	5	3.0	0.77 (0.23–2.52)	2	2.4	0.87 (0.17–4.52)	3	3.9	1.54 (0.30–7.82)

Table II. Continued

Gene-genotype (amino acid)	Controls		Lung			Bladder			Leukaemia			UADC			COPD		
	N	(%)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)
OGG1 cod. 326																	
C/C (Ser/Ser)	673	61.5	66	56.9		78	62.9		102	60.4		55	67.1		47	61.0	
C/G (Ser/Cys)	371	33.9	46	39.7	1.13 (0.71–1.80)	40	32.3	0.98 (0.60–1.59)	59	34.9	1.25 (0.83–1.90)	25	30.5	0.61 (0.34–1.10)	23	29.9	0.85 (0.46–1.59)
G/G (Cys/Cys)	50	4.6	4	3.5	0.47 (0.13–1.74)	6	4.8	0.74 (0.24–2.25)	8	4.7	1.64 (0.62–4.37)	2	2.4	—	7	9.1	2.33 (0.64–8.51)
BRCA1 cod. 871																	
T/T (Pro/Pro)	367	43.8	43	47.3		50	49.5		59	44.0		22	34.9		28	50.9	
C/T (Leu/Pro)	383	45.7	38	41.8	0.95 (0.53–1.69)	42	41.6	1.01 (0.59–1.74)	56	41.8	0.72 (0.45–1.16)	29	46.0	1.05 (0.49–2.24)	20	36.4	1.24 (0.56–2.71)
C/C (Leu/Leu)	88	10.5	10	11.0	0.95 (0.39–2.30)	9	8.9	0.94 (0.36–2.46)	19	14.2	1.79 (0.82–3.89)	12	19.1	1.82 (0.68–4.85)	7	12.7	0.83 (0.25–2.83)
BRCA2 cod. 372																	
A/A (Asn/Asn)	431	51.4	58	64.4		52	51.0		69	50.7		36	57.1		25	45.5	
A/C (Asn/His)	349	41.6	25	27.8	0.66 (0.35–1.23)	48	47.1	1.10 (0.64–1.91)	56	41.2	1.05 (0.65–1.69)	23	36.5	0.69 (0.34–1.40)	21	38.2	1.32 (0.56–3.07)
C/C (His/His)	59	7.0	7	7.8	2.33 (0.55–9.80)	2	2.0	0.26 (0.05–1.23)	11	8.1	0.87 (0.38–1.99)	4	6.4	1.31 (0.31–5.54)	9	16.4	2.67 (0.71–10.06)
NBS1 cod. 185																	
C/C (Glu/Glu)	404	48.0	41	44.6		43	42.6		64	47.1		24	38.1		31	56.4	
C/G (Glu/Gln)	350	41.6	43	46.7	1.27 (0.70–2.31)	47	46.5	1.75 (1.00–3.07)	58	42.7	1.04 (0.65–1.66)	31	49.2	1.78 (0.83–3.84)	21	38.2	0.83 (0.39–1.76)
G/G (Gln/Gln)	88	10.5	8	8.7	1.04 (0.37–2.90)	11	10.9	1.17 (0.49–2.79)	14	10.3	0.58 (0.25–1.30)	8	12.7	1.89 (0.61–5.90)	3	5.5	0.54 (0.10–2.91)
RAD51 5'UTR																	
G/G	734	86.5	82	88.2		90	88.2		117	84.8		53	85.5		47	85.5	
C/G	110	13.0	11	11.8	0.58 (0.28–1.24)	11	10.8	1.17 (0.50–2.74)	21	15.2	1.84 (0.96–3.53)	8	12.9	1.63 (0.55–4.81)	8	14.6	0.60 (0.21–1.76)
C/C	5	0.6	0	0.0	—	1	1.0	2.00 (0.12–31.97)	0	0.0	—	1	1.6	2.29 (0.14–37.67)	0	0.0	—
RAD51 5'UTR																	
C/C	272	32.2	29	31.5		37	36.6		58	42.0		22	34.9		15	27.3	
C/T	429	50.8	44	47.8	1.12 (0.59–2.13)	46	45.5	0.70 (0.39–1.25)	54	39.1	0.61 (0.36–1.05)	27	42.9	0.49 (0.21–1.16)	28	50.9	1.01 (0.40–2.55)
T/T	144	17.0	19	20.7	1.34 (0.60–3.00)	18	17.8	0.78 (0.36–1.66)	26	18.8	0.93 (0.47–1.81)	14	22.2	0.80 (0.30–2.14)	12	21.8	2.41 (0.81–7.16)
RAD52 5'UTR																	
G/G	278	33.4	25	28.1		34	33.7		52	38.5		18	30.0		10	18.9	
G/T	401	48.2	39	43.8	1.01 (0.51–1.99)	52	51.5	1.23 (0.69–2.22)	62	45.9	0.85 (0.51–1.40)	34	56.7	1.42 (0.67–2.97)	30	56.6	0.90 (0.30–2.67)
T/T	153	18.4	25	28.1	1.64 (0.74–3.64)	15	14.9	0.90 (0.43–1.91)	21	15.6	0.69 (0.33–1.47)	8	13.3	1.31 (0.46–3.76)	13	24.5	1.68 (0.56–5.01)
XRCC2 cod. 188																	
G/G (Arg/Arg)	698	83.7	80	86.0		83	83.0		116	84.1		56	88.9		50	92.6	
A/G (His/Arg)	130	15.6	12	12.9	0.59 (0.25–1.44)	17	17.0	1.34 (0.65–2.74)	21	15.2	1.09 (0.59–2.01)	7	11.1	0.67 (0.22–2.02)	4	7.4	0.55 (0.15–2.05)
A/A (His/His)	6	0.7	1	1.1	0.54 (0.03–9.05)	0	0.0	—	1	0.7	—	0	0.0	—	0	0.0	—
LIG4 cod. 3																	
C/C (Ala/Ala)	748	88.5	85	91.4		91	89.2		117	84.8		58	93.6		52	96.3	
C/T (Ala/Val)	66	7.8	8	8.6	1.27 (0.45–3.61)	6	5.9	0.77 (0.26–2.23)	16	11.6	1.54 (0.74–3.20)	3	4.8	1.09 (0.23–5.21)	1	1.9	0.31 (0.03–2.67)
T/T (Val/Val)	31	3.7	0	0.0	—	5	4.9	1.72 (0.49–6.03)	5	3.6	1.00 (0.30–3.28)	1	1.6	0.59 (0.05–7.43)	1	1.9	0.26 (0.03–2.33)
LIG4 cod. 9																	
C/C (Thr/Thr)	570	70.8	64	72.7		64	64.7		89	68.5		48	80.0		44	80.0	
C/T (Thr/Ile)	211	26.2	23	26.1	1.38 (0.73–2.62)	31	31.3	1.06 (0.57–1.97)	37	28.5	1.13 (0.67–1.93)	10	16.7	0.64 (0.26–1.56)	11	20.0	0.39 (0.14–1.09)
T/T (Ile/Ile)	24	3.0	1	1.1	—	4	4.0	1.38 (0.36–5.24)	4	3.1	0.86 (0.25–2.91)	2	3.3	0.37 (0.04–3.35)	0	0.0	—
TP53 cod. 72																	
G/G (Arg/Arg)	480	57.2	41	45.1		58	56.9		74	53.6		36	57.1		29	52.7	
C/G (Pro/Arg)	309	36.8	43	47.3	1.93 (1.06–3.51)	38	37.3	1.04 (0.59–1.83)	56	40.6	0.94 (0.60–1.48)	24	38.1	0.90 (0.43–1.88)	23	41.8	1.13 (0.53–2.44)
C/C (Pro/Pro)	50	6.0	7	7.7	0.89 (0.32–2.47)	6	5.9	1.65 (0.46–5.85)	8	5.8	0.73 (0.22–2.43)	3	4.8	0.91 (0.15–5.49)	3	5.5	1.05 (0.19–5.93)

OR and 95% CI from conditional logistic regression.

Table III. Cases and controls stratified by country, gender, smoking status and ETS

	Cases		Controls	
	N	(%)	*N*	(%)
Age (mean ± SD)	568	61.3 ± 7.9	1094	61.2 ± 8.1
Country				
France	9	(30.0)	21	(70.0)
Italy	63	(32.6)	130	(67.4)
Spain	48	(29.8)	113	(70.2)
United Kingdom	195	(38.9)	306	(61.1)
The Netherlands	45	(33.1)	91	(66.9)
Greece	30	(33.3)	60	(66.7)
Germany	95	(32.7)	196	(67.4)
Denmark	83	(31.9)	177	(68.1)
Gender				
Male	293	(34.0)	568	(66.0)
Female	275	(34.3)	526	(65.7)
Smoking status				
Never	300	(33.5)	595	(66.5)
Former	268	(34.9)	499	(65.1)
ETS (someone regularly smokes at home/work)				
No	29	(14.2)	175	(85.8)
Yes	28	(13.7)	177	(86.3)

Cases and controls were matched for all the variables except for ETS ($P = 0.87$).

A differential risk associated with gender seems also to be apparent, with an increased leukaemia risk among males for *BRCA1*-871Leu/Leu carriers (OR = 4.17, 95% CI = 1.25–13.92; $P = 0.018$ for interaction) and for *ERCC1*-118Asn/Asn (OR = 3.35, 95% CI = 1.23–9.13; $P = 0.03$ for interaction) among upper aero-digestive cancers. On the other hand, a protective effect on bladder cancer has been observed for XPD-751Gln/Gln female carriers (OR = 0.21, 95% CI = 0.05–0.86) and an increased risk for females heterozygotes for *MGMT*-84Leu/Phe (OR = 3.08, 95% CI = 1.18–8.02) and for *NBS1*-185Glu/Gln (OR = 2.88, 95% CI = 1.15–7.26).

No significant results have been observed for any of the polymorphisms in relation to air pollution expressed as either residence nearby heavy traffic roads or individual pollutants.

Haplotype analysis

We reconstructed maximum likelihood haplotypes for *RAD51*, *XRCC1*, *XPD*/*ERCC1*, *XRCC3* and *LIG4* genes (Table IV), and estimated haplotype frequencies and ORs (95% CI) for cases and controls. D' LD measures for the control population showed that the *XRCC1* and *XPD*/*ERCC1* genes are on two different LD blocks, although they are physically very close; thus, we reconstructed haplotypes separately. A significantly increased risk associated with the *XPD*/*ERCC1*-GAT haplotype was observed in bladder cancers (OR = 1.38, 95% CI = 1.06–1.79) and leukaemias (OR = 1.28, 95% CI = 1.02–1.61), even though a non-significant higher risk was evident for lung and UADC (Table IV). We found a slightly elevated odds ratio associated with the *XRCC3*-AC haplotype for leukaemias (OR = 1.28, 95% CI = 1.02–1.62). A reduced risk was observed for the *XRCC1*-TAG haplotype (OR = 0.69, 95% CI = 0.50–0.97) in the UADC. None of the associations with haplotypes were statistically significant in lung cancer and COPD.

False positive report probability

On the basis of biological evidence and results from meta-analyses (see Introduction and Statistical methods), we considered a prior probability of 0.25 for *XRCC1* codon 399 and for *XRCC3* codon 241, 0.1 for *XPD* codon 751 and *OGG1* codon 326, and ≤0.01 for all the other genes/polymorphisms. Table V shows the FPRPs for the statistically significant associations we observed from univariate and multivariate analyses, and from the haplotype analysis. None of the single SNP associations seems to be a truly positive association with disease at the prior probability levels defined above (Table V), with a statistical power to detect an OR of 1.5 or 0.67 and using a stringent FPRP cut point of 0.2, even though the *XRCC1*-206 polymorphism was associated at a prior probability level of 0.25 with leukaemia. However, considering an FPRP cut point of 0.5 most of the associations were likely to be true associations (Table V). Moreover, we identified three possible interesting haplotypes with FPRP ≤ 0.2 (Table V): an increased risk for *XPD-ERCC1*-GAT both in bladder cancer (prior probability = 0.1) and leukaemia (prior probability = 0.25), and an increased risk for *XRCC3*-AC in leukaemia (prior probability = 0.1); a decreased risk was observed for *XRCC1*-TAG in UADC but only with OR = 0.5 and a prior probability = 0.5.

Discussion

Most of the analysed DNA repair polymorphisms did not show any significant difference in the genotype distribution between cases and controls. However, several potential associations emerged from our data. Some associations were not stable in both univariate and multivariate analyses, but this was expected when considering different models of inheritance. Moreover, with the accumulation of evidence on DNA repair gene polymorphism data, some SNPs seem to have opposite risk trends in different cancer sites. This is the case of the XRCC1-399Gln/Gln which, with a recessive model in a stepwise multivariate analysis, revealed a strong protective effect on lung cancer risk, but had the opposite effect on risk of leukaemia (31,33,34). These results could simply indicate a chance association although a possible explanation could be the tissue specific balance between apoptotic signal and repair effects in the different tissues. Less efficient repair variants of specific repair pathways can result in a protective signal (accumulation of damage, cell-cycle block and apoptosis) in some tissues (e.g. lung) whereas in others (e.g. blood cells) they are risk factors (unrepaired or abortive attempt to repair damage and subsequent mutation). For many of the analysed polymorphisms there is insufficient functional information to formulate strong *a priori* hypotheses, but association studies suggested different risks for some cancer sites (http://perseus.isi.it/huge).

The *XRCC1*-Pro206Pro polymorphism was associated with a significantly reduced risk of leukaemia for carriers of the G variant allele, but this SNP does not lead to an amino acid substitution. For this reason, this polymorphism is unlikely to be the direct cause of a functional change (35). However, the relatively high frequency (0.38) of the minor allele gives the opportunity for linkage disequilibrium studies. As far as we know, this variant has been tested only in breast cancer (21), but no significant association was detected. The contrasting results reported for the well-studied *XRCC1*-Arg194Trp and *XRCC1*-Arg399Gln polymorphisms in different cancer sites (5,6; http://perseus.isi.it/huge) suggest that other

Table IV. Case–control distribution of estimated haplotypes for the different DNA repair gene polymorphisms

Haplotype	Controls		Lung			Bladder			Leukaemia			Oral/respiratory			COPD		
	N	(%)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)	*N*	(%)	OR (95%CI)
RAD51																	
G G	848	50.4	91	49.5	0.96 (0.71–1.30)	107	53.0	1.11 (0.83–1.48)	149	54.0	1.15 (0.89–1.49)	60	48.4	0.92 (0.64–1.33)	50	45.5	0.82 (0.56–1.21)
G T	714	42.5	82	44.6	1.09 (0.80–1.43)	82	40.6	0.93 (0.69–1.25)	106	38.4	0.84 (0.65–1.10)	54	43.6	1.05 (0.72–1.51)	52	47.3	1.22 (0.83–1.79)
C G	120	7.1	11	6.0	0.83 (0.44–1.57)	13	6.4	0.90 (0.50–1.62)	21	7.6	1.07 (0.66–1.74)	10	8.1	1.14 (0.58–2.24)	8	7.3	1.02 (0.49–2.15)
	1682		184			202			276			124			110		
XRCC1																	
C A A	736	33.7	71	30.5	0.87 (0.65–1.16)	87	35.1	1.07 (0.81–1.40)	129	38.1	1.21 (0.96–1.54)	58	35.4	1.08 (0.77–1.50)	55	35.7	1.10 (0.78–1.54)
C A G	312	14.3	39	16.9	1.22 (0.85–1.75)	35	14.1	0.99 (0.68–1.44)	48	14.3	1.00 (0.72–1.38)	25	15.2	1.08	29	18.8	1.39 (0.91–2.12)
C G A	1	0.1	0	0.0	—	0	0.0	—	1	0.3	5.56 (0.46–67.39)	0	0.0	—	0	0.0	—
C G G	993	45.4	102	44.0	0.94 (0.72–1.24)	110	44.4	0.96 (0.74–1.25)	135	39.9	0.80 (0.63–1.01)	77	47.0	1.06 (0.77–1.46)	60	39.0	0.77 (0.55–1.07)
T A A	1	0.1	1	0.5	11.06 (0.76–161)	0	0.0	—	0	0.0	—	0	0.0	—	0	0.0	—
T A G	143	6.5	19	8.1	1.26 (0.77–2.08)	16	6.5	0.99 (0.58–1.68)	25	7.4	1.14 (0.73–1.77)	4	2.4	0.36 (0.13–0.98)	10	6.5	0.99 (0.51–1.93)
	2186		232			248			338			164			154		
XPD-ERCC1																	
A A C	99	4.6	4	1.9	0.40 (0.15–1.06)	8	3.2	0.70 (0.34–1.46)	16	4.7	1.02 (0.59–1.77)	11	7.0	1.57 (0.83–2.96)	6	3.8	0.83 (0.35–1.93)
A A T	23	1.0	1	0.5	0.47 (0.07–3.09)	5	1.8	1.78 (0.64–4.90)	0	0.0	—	0	0.0	—	2	1.1	1.02 (0.21–5.02)
A C C	459	21.0	51	22.0	1.07 (0.77–1.48)	52	20.9	0.99 (0.72–1.37)	72	21.2	1.01 (0.77–1.34)	31	18.7	0.87 (0.58–1.30)	33	21.4	1.02 (0.69–1.53)
A C T	264	12.1	29	12.7	1.05 (0.70–1.58)	28	11.1	0.91 (0.60–1.38)	33	9.7	0.78 (0.53–1.14)	12	7.3	0.57 (0.31–1.04)	20	12.7	1.06 (0.65–1.73)
G A C	240	11.0	21	9.0	0.80 (0.50–1.28)	20	8.0	0.70 (0.43–1.13)	38	11.3	1.03 (0.72–1.49)	20	12.0	1.11 (0.68–1.81)	19	12.2	1.13 (0.68–1.86)
G A T	935	42.8	106	45.6	1.12 (0.85–1.47)	126	50.7	1.38 (1.06–1.79)	165	48.8	1.28 (1.02–1.61)	76	46.3	1.15 (0.84–1.58)	65	42.1	0.97 (0.70–1.35)
G C C	81	3.7	11	4.6	1.26 (0.66–2.41)	4	1.8	0.47 (0.18–1.24)	8	2.5	0.66 (0.32–1.36)	10	6.2	1.72 (0.88–3.37)	5	3.6	0.95 (0.39–2.30)
G C T	85	3.9	9	3.8	0.97 (0.48–1.97)	6	2.5	0.62 (0.27–1.44)	6	1.9	0.47 (0.21–1.06)	4	2.6	0.65 (0.24–1.75)	5	3.2	0.83 (0.33–2.08)
	2186		232			248			338			164			154		
XRCC3																	
A C	681	31.2	72	31.0	0.99 (0.74–1.33)	72	29.0	0.90 (0.68–1.20)	124	36.7	1.28 (1.01–1.62)	54	33.3	1.10 (0.79–1.55)	50	32.5	1.06 (0.75–1.50)
A T	874	40.0	88	37.9	0.92 (0.69–1.21)	95	38.3	0.93 (0.71–1.22)	126	37.3	0.89 (0.70–1.13)	66	40.7	1.03 (0.75–1.43)	55	35.7	0.83 (0.59–1.17)
G C	628	28.7	72	31.0	1.12 (0.83–1.50)	81	32.7	1.20 (0.91–1.59)	88	26.0	0.87 (0.67–1.13)	42	25.9	0.87 (0.60–1.25)	49	31.8	1.16 (0.81–1.64)
G T	1	0.1	0	0.0	—	0	0.0	—	0	0.0	—	0	0.0	—	0	0.0	—
	2184		232			248			338			162			154		
LIG4																	
C C	1313	82.3	151	85.8	1.30 (0.84–2.02)	154	77.6	0.75 (0.52–1.07)	211	81.0	0.92 (0.66–1.28)	104	86.5	1.38 (0.81–2.38)	96	88.8	1.72 (0.93–3.16)
C T	166	10.4	17	9.7	0.92 (0.55–1.56)	28	14.3	1.44 (0.94–2.21)	27	10.5	1.02 (0.66–1.56)	11	9.3	0.88 (0.47–1.67)	9	8.4	0.79 (0.39–1.59)
T C	26	1.6	0	0.0	—	5	2.7	1.69 (0.66–4.33)	4	1.7	1.05 (0.38–2.91)	2	1.8	1.11 (0.27–4.52)	1	1.0	0.60 (0.08–4.25)
T T	91	5.7	8	4.6	0.79 (0.37–1.65)	11	5.4	0.94 (0.49–1.80)	18	6.8	1.20 (0.71–2.03)	3	2.4	0.40 (0.12–1.32)	2	1.8	0.30 (0.07–1.27)
	1596		176			198			260			120			108		

OR and 95% CI from contingency tables.

Table V. False positive report probability

	OR (95% CI)	*P*-value	OR = 1.5	Prior probability					OR = 2.0	Prior Probability				
			Power	0.500	0.250	0.100	0.010	0.001	Power	0.500	0.250	0.100	0.010	0.001
Univariate analysis														
Leukaemia														
XRCC1 cod. 399 A/A	2.20 (1.16–4.17)	0.016	0.120	**0.115**	0.281	0.540	0.928	0.992	0.385	**0.039**	**0.109**	0.268	0.801	0.976
XRCC1 cod. 206 G/G	0.57 (0.32–1.01)	0.054	0.296	**0.155**	0.354	0.622	0.948	0.995	0.673	**0.074**	**0.194**	0.420	0.888	0.988
XRCC3 cod. 241 T/T	0.51 (0.27–0.96)	0.037	0.203	**0.154**	0.353	0.621	0.947	0.995	0.524	**0.066**	**0.174**	0.388	0.875	0.986
Multivariate stepwise logistic regression (Co-dominant)														
Leukaemia														
XRCC1 cod. 206 G	0.65 (0.44–0.94)	0.022	0.446	**0.047**	**0.129**	0.308	0.830	0.980	0.918	**0.023**	**0.067**	**0.178**	0.704	0.960
UADC														
APEX cod. 148 G	0.59 (0.35–0.99)	0.046	0.322	**0.124**	0.299	0.561	0.934	0.993	0.735	**0.059**	**0.157**	0.359	0.860	0.984
Recessive														
Lung														
XRCC1 cod. 399 A/A	0.22 (0.05–0.98)	0.047	0.073	0.392	0.659	0.853	0.985	0.998	0.141	0.250	0.500	0.750	0.971	0.997
Leukaemia														
XRCC1 cod. 399 A/A	2.47 (1.02–6.02)	0.047	0.136	0.255	0.507	0.755	0.971	0.997	0.321	**0.127**	0.304	0.567	0.935	0.993
Haplotypes														
XRCC1-TAG														
UADC	0.36 (0.13–0.98)	0.046	0.114	0.286	0.545	0.783	0.975	0.998	0.260	**0.149**	0.344	0.612	0.945	0.994
XPD-ERCC-GAT														
Bladder	1.38 (1.06–1.79)	0.015	0.735	**0.020**	**0.059**	**0.157**	0.672	0.954	0.997	**0.015**	**0.044**	**0.121**	0.602	0.938
Leukaemia	1.28 (1.02–1.61)	0.035	0.912	**0.037**	**0.103**	0.256	0.791	0.975	1.000	**0.034**	**0.095**	0.239	0.776	0.972
XRCC3-AC														
Leukaemia	1.28 (1.01–1.62)	0.040	0.907	**0.042**	**0.117**	0.284	0.814	0.978	1.000	**0.038**	**0.107**	0.265	0.798	0.976

Prior probabilities ranging from 0.5 to 0.001, with the estimated statistical power to detect an OR of 1.5 or OR 2.0 (or 0.67 and 0.5, respectively, if observed ORs were <1) with α level equal to the observed *P*-value. Values given in boldface indicate the FPRP considering a cut point of 0.2.

polymorphisms and, in particular, a combination of SNPs in the *XRCC1* gene could be more important than single SNPs. The decreased lung cancer risk that we observed for *XRCC1*-399 Gln/Gln homozygotes seems to be in agreement with the opposite ORs in smokers and non-smokers reported by Zhou *et al*. (36) and David-Beabes *et al*. (37)

We found also an association between the *XRCC3*-241Met variant allele and a reduced risk of leukaemia. No results has been previously reported for leukaemia although an increased risk has been shown by our group (38) and by Stern *et al*. (39) in bladder cancer, and by Winsey *et al*. (40) in melanoma. However, a putative protective effect has been described by Shen *et al*. (41) in bladder cancer and no association has been reported for cancers of the lung (42–44), skin (45–47), acute myeloblastic leukaemia (48), breast cancer (21,47), and gastric carcinoma (49). More extensive investigations on the combined and haplotype effects of SNPs in the *XRCC3* gene are required to better clarify its possible involvement in cancer aetiology.

We also found that the *APEX*-148Glu variant allele was significantly associated with a reduced risk of UADC but there are no previous cancer association studies with this polymorphism. However, given the essential role played by this protein in BER and other pathways (4) we believe that it is important to investigate *APEX* polymorphisms in the future.

Notwithstanding the above results, after applying a method for the estimation of the number of false positive results, no polymorphism can be considered a true association, although with the power to detect an OR of 1.5 and a prior probability of 0.5 (as suggested by Wacholder *et al*. 2004, for smaller initial studies) most of the associations would not be considered likely false positive findings. However, it is also true that for some polymorphisms we could have been too much conservative in the estimation of false positive rate, in particular for those polymorphisms that are in linkage disequilibrium with other possible functional variants (e.g. *XRCC1*-206 polymorphism, Table V).

From a genetic perspective, in most multifactorial diseases, single polymorphisms in single genes are unlikely to alter the expression or function of specific proteins to the extent of producing a pathological phenotype. It is more likely that the combined effect of different SNPs in a gene produce a change in expression or protein function. Therefore, to look at the combination of putative functional SNPs, we further estimated haplotype frequencies for *RAD51*, *XRCC1*, *XPD*/*ERCC1*, *XRCC3* and *LIG4* genes; even though *XRCC1* and *XPD*/*ERCC1* are in the same chromosomal location (19q32.2) we did not find any significant LD between these two loci, as it has been described previously (20,23,25).

The most interesting result is the significantly increased risk of both bladder cancer and leukaemias associated with the *XPD*/*ERCC1*-GAT haplotype; a non-significant increased risk was also evident for the other cancers. These genes are included in the 19q13.2–13.3 chromosomal location that comprises two other genes (*RAI* and *ASEI*). This region seems to be the candidate for susceptibility to basal cell carcinoma (20,23) and post-menopausal breast cancer before the age of 55 years (25); in particular, the most significant results refer to the association with the polymorphisms in the *RAI* gene. Previous studies have found markers in *XPD* located near *RAI* to be associated with the development of melanoma, glioma and lung cancer (5,22,50,51). Thus it is possible that this limited region is important for other cancers as well. However, further studies are required to clarify whether the same haplotype could have different roles in different cancers as seems to be suggested by the accumulating data on single SNPs. In fact, we observed an increased risk of leukaemia for the *XRCC3*-AC haplotype, in agreement with Jacobsen *et al*. (52), who

showed that this haplotype was associated with the risk of lung cancer.

The observed discrepancies among studies could be due not only to cancer and/or population-specific differences, but also to the sample sizes and multiple subgroup analyses; additional work to select tagging SNPs in DNA repair genes in different populations is required in order to better define possible high-risk haplotypes in relation to different ethnic/population-specific distributions. We have undertaken a better characterization of our control population in terms of allele and haplotype distributions among countries and centres. Although the vast majority of our samples are Caucasians, no specific information on ethnic origins has been collected. However, current studies on linkage disequilibrium variation in Europe stress the need to test for both ethnic and population-specific differences.

Few studies have been published on *XRCC1* haplotypes and cancer (21,24,26,53) but unfortunately the results are not directly comparable due to the genotyping of different numbers of SNPs and of different polymorphisms. In particular, we analysed the *XRCC1*-Pro206Pro polymorphism, previously investigated only by Han *et al*. (21). Lee *et al*. (24) showed a protective effect on gastric cancer of the *XRCC1* haplotype 'A' (194Trp/280Arg/399Arg) corresponding to our T (A)G haplotype; they also found an increased risk for the haplotype 'D' corresponding to our C (G)G/C (A)G haplotypes.

Along similar lines, Han *et al*. (21) described the protective effect on breast cancer of the *XRCC1* haplotype defined by the *XRCC1*-194Trp variant, but the results were significant only when considering the single polymorphism. No significant association has been shown by Hao *et al*. (26) between oesophageal squamous cell carcinoma and *XRCC1* haplotypes, except for the *XRCC1*-77T→C polymorphism. Likewise, no association was found by Yin *et al*. (53) of *XRCC1* haplotypes with basal cell carcinoma.

Although on the basis of published data, no precise prior probability can be defined for haplotypes, we identified three possible interesting haplotypes (FPRP $\leq$ 0.2): increased risk for *XPD-ERCC1*-GAT both in bladder cancer and leukaemia, and increased risk for *XRCC3*-AC in leukaemia; a decreased risk was also observed for *XRCC1*-TAG in UADC but only with OR = 0.5 and a prior probability = 0.5.

Due to the limited contribution that single DNA repair SNPs seem to confer to the risk of UADC—at least in non-smokers—future studies should be designed to consider more SNPs of a specific gene trying to define possibly population-specific tagging SNPs, in order to confirm gene involvement in the (total or partial) absence of functional evidence. Our results suggest that a particular haplotype spanning the *XPD-ERCC1* region may modulate leukaemia and bladder cancer risk and possibly also other cancers. Further investigations of the combined effects of polymorphisms within these DNA repair genes, smoking and other risk factors may help to clarify the influence of genetic variation in the carcinogenic process. Larger collaborative studies are required to investigate the above cancers in a non-smoker population.

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