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XRCC1, *XRCC3*, *XPD* gene polymorphisms, smoking and ^{32}P-DNA adducts in a sample of healthy subjects

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DNA repair genes have an important role in protecting individuals from cancer-causing agents. Polymorphisms in several DNA repair genes have been identified and individuals with non-dramatic reductions in the capacity to repair DNA damage are observed in the population, but the impact of specific genetic variants on repair phenotype and cancer risk has not yet been clarified. In 308 healthy Italian individuals belonging to the prospective European project EPIC, we have investigated the relationship between DNA damage, as measured by ^{32}P-DNA adduct levels, and three genetic polymorphisms in different repair genes: *XRCC1*-Arg399Gln (exon 10), *XRCC3*-Thr241Met (exon 7) and *XPD*-Lys751Gln (exon 23). DNA adduct levels were measured as relative adduct level (RAL) per 10^9 normal nucleotides by DNA ^{32}P-post-labelling assay in white blood cells from peripheral blood. Genotyping was performed by PCR–RFLP analysis. The *XRCC3*-241Met variant was significantly associated with higher DNA adduct levels, whereas *XRCC1*-399Gln and *XPD*-751Gln were associated with higher DNA adduct levels only in never-smokers. *XRCC3*-241Met homozygotes had an average DNA adduct level of 11.44 ± 1.48 (±SE) compared with 7.69 ± 0.88 in Thr/Met heterozygotes and 6.94 ± 1.11 in Thr/Thr homozygotes (F = 3.206, P = 0.042). Never-smoking *XRCC1*-399Gln homozygotes had an average DNA adduct level of 15.60 ± 5.42 compared with 6.16 ± 0.97 in Gln/Arg heterozygotes and 6.78 ± 1.10 in Arg/Arg homozygotes (F = 5.237, P = 0.007). A significant odds ratio (3.81, 95% CI 1.02–14.16) to have DNA adduct levels above median value was observed for *XPD*-751Gln versus *XPD*-751Lys never-smoking homozygotes after adjustment for several confounders. These data show that all the analysed polymorphisms could result in deficient DNA repair and suggest a need for further investigation into the possible interactions between these polymorphisms, smoking and other risk factors.

Abbreviations: BER, base excision repair; EPIC, European Prospective Investigation into Cancer and Nutrition; NER, nucleotide excision repair; OR, odds ratio; RAL, relative adduct level; SE, standard error; WBC, white blood cells; XPD, xeroderma pigmentosum-D gene; XRCC 1–3, X-ray repair cross complementing groups 1–3.

Introduction

Several studies have documented that the genes involved in DNA repair and maintenance of genome integrity are critically involved in protecting against mutations that lead to cancer and/or inherited genetic disease (1–3). Molecular epidemiology studies have shown that inheritance of genetic variants at one or more loci results in a reduced DNA repair capacity and an increase in the individual risk of cancer (4–9). These polymorphisms, although generally associated with a slight increase in the risks of cancer, are highly prevalent in the population, and therefore the attributable risks for cancer could be high. In addition, a large number of investigations have shown that phenotypic tests, indirectly related to repair, are predictive of cancer occurrence (10).

In the present study of 308 healthy adults from the Italian EPIC cohort (11–12), we have correlated DNA damage, measured by ^{32}P-DNA adduct levels, with genetic polymorphisms in three DNA repair genes, representing three different repair pathways. The proteins involved are members of multiprotein complexes, in which amino acid residues at protein–protein interfaces or in the active site may determine protein function. XRCC1 (X-ray repair cross-complementing) plays a role in the base excision repair (BER) pathway, interacts with DNA polymerase β, PARP and DNA ligase III (13) and has a BCRT domain, characteristic of proteins involved in cycle checkpoint functions and responsive to DNA damage (14). Ionizing radiation and alkylating agents cause DNA base damage and strand breaks that elicit the BER system (15–16). The Arg399Gln polymorphism resides at the C-terminal side of the PARP-interacting domain and within a relatively non-conserved region between conserved residues of the BRCT domain.

XRCC3 participates in DNA double-strand break/recombination repair and is a member of an emerging family of Rad-51-related proteins (17) that probably participate in homologous recombination to maintain chromosome stability and repair DNA damage (18–22). XRCC3 is shown to interact directly with HsRad51, and like Rad55 and Rad57 in yeast, may cooperate with HsRad51 during recombinational repair (23). The T241M substitution in XRCC3 is a non-conservative change, but it does not reside in the ATP-binding domains, which are the only functional domains that have been identified in the protein at this time (24).

XPD is involved in the nucleotide-excision repair (NER) pathway (25), which recognizes and repairs a wide range of structurally unrelated lesions such as bulky adducts and thymidine dimers (26–28). XPD works as an ATP-dependent 5′-3′ helicase joined to the basal transcription factor IIH (TFIIH) complex (29–30). The XPD-Lys751Gln substitution

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does not reside in any known or hypothesized helicase/ATPase domains (24,31).

The lack of any observed variation in known functional domains of these proteins is not surprising, given that amino acid substitutions in these critical domains could cause loss of function and disease or embryo lethality. Biochemical and biological characterization of these variants, especially the non-conservative amino acid substitutions, and molecular epidemiology studies on cancer will provide insights into the potential for these variants to be cancer susceptibility alleles.

Two of the polymorphisms analysed, *XRCC3*-Thr241Met and *XRCC1*-Arg399Gln, are non-conservative amino acid changes with a potential functional relevance, even though their effect on phenotype has to be elucidated. The *XPD*-Lys751Gln polymorphism is a conservative substitution which has been chosen because of its high frequency.

We report here the relationship between the above polymorphisms and DNA damage, measured by ^{32}P-DNA adduct levels in peripheral leucocytes, in 308 healthy adults from the Italian EPIC cohort (11,12).

Materials and methods

Subjects

The Italian section of the large European project EPIC (11), a prospective study on diet and cancer, is based on 47 749 volunteers of both sexes (age 35–64 years) enrolled between 1993 and 1998 in five centres across different areas of the country: Varese and Turin (12 083 and 10 604 volunteers) in Northern Italy; Florence (13 597) in Central Italy; Ragusa (6403) and Naples (5062 women) in Southern Italy. An informed consent form was signed by all subjects prior to enrollment in the study. Detailed information about diet and life-style habits has been recorded for each subject and a blood sample has been collected and stored in liquid nitrogen.

A random sample of 308 subjects (153 men), stratified by age, sex and area of residence, was selected from the three main geographical areas where the study was conducted (Northern Italy: Varese, 53 and Turin, 53; Central Italy: Florence, 100; Southern Italy: Ragusa, 76 and Naples, 26). For a complete description of the different sociodemographic and anthropometric characteristics of the sample see Palli *et al.* (12).

One buffy coat straw has been retrieved for each subject included in this sample and shipped on dry ice to the study laboratory at the National Cancer Institute (IST) in Genoa for DNA extraction by phenol–chloroform (32). Aliquots of the DNA samples were then shipped to Turin University for DNA repair polymorphism analysis.

Detection of DNA adducts

All analyses concerning white blood cell (WBC) DNA adducts were carried out at IST, Genoa, as described in Palli *et al.* (12). Leukocyte DNA adducts levels were measured as relative adduct labelling (RAL)$\times 10^9$ normal nucleotides, using the nuclease P1 modification of the ^{32}P-post-labelling technique; the detection limit was 0.1 adduct per 10^9 normal nucleotides (12). The analyses were carried out blind prior to decoding. One standard was routinely included in the analyses, i.e. benzo[*a*]pyrene DNA adducts, from liver of mice treated intraperitoneally with 0.06 mg/kg B[*a*]P for 24 h (30). The average levels of B[*a*]P DNA adducts were 51 ± 1.0 (SE) per 10^9 nucleotides.

Polymorphism analysis

Polymerase chain reaction (PCR) followed by enzymatic digestion was used for the genotyping of the *XRCC1*-Arg399Gln, *XPD*-Lys751Gln and *XRCC3*-Thr241Met polymorphisms (24). All of the PCR reactions were performed in a total reaction volume of 20 µl containing 10 ng genomic DNA, 0.4 U *Taq* polymerase (PE Applied Biosystems) in 1× PCR buffer, 1.5 mM $MgCl_2$, 50 mM dNTPs and 250 nM each primer. Thermal cycling conditions were as follows: initial denaturation step at 95°C for 3 min, 35 cycles of PCR consisting of 95°C for 20 s, 20 s at the appropriate annealing temperature and 72°C for 20 s, followed by a final extension step at 72°C for 5 min.

The *XRCC1*-Arg399Gln polymorphism, a G→A transition in exon 10 (position 28 152) was determined using the following primers: sense, 5′-CAAGTACAGCCAGGTCCTAG-3′; antisense, 5′-CCTTCCCTCATCT-GGAGTAC-3′, 55°C annealing temperature for the PCR reaction. The 248 bp PCR product was digested with *Nci*I (Promega): the Arg allele was cut into 89 and 159 bp fragments (Gln allele not digested).

The *XPD*-Lys751Gln polymorphism, a A→C transversion in exon 23 (position 35 931) was determined using the following primers: sense, 5′-CTGCTCAGCCTGGAGCAGCTAGAATCAGAGGAGACGCTG-3′; antisense, 5′-AAGACCTTCTAGCACCACCG-3′, 67°C annealing temperature for the PCR reaction. The 161 bp PCR product was digested with *Pst*I (Promega): the Lys allele was cut into 41 and 120 bp fragments (Gln allele not digested).

The *XRCC3*-Thr241Met polymorphism, a T→C transition in exon 7 (position 18 067) was determined using the following primers: sense, 5′-GCCTGGTGGTCATCGACTC-3′; antisense, 5′-ACAGGGCTCTGGAA-GGCACTGCTCAGCTCACGCA<u>C</u>C-3′ (underlined base modifies primer sequence introducing a restriction site in the presence of the T nucleotide), 60°C annealing temperature for the PCR reaction. The 136 bp PCR product was digested with *Nco*I (Promega): the Thr allele was cut into 39 and 97 bp fragments (Met allele not digested).

As DNA typing quality control for the three polymorphisms, a methodological validation has been performed (33) including a comparison among PCR–RFLP, direct sequencing, and denaturing high performance liquid chromatography (DHPLC) by using the primer extension technique (34). Due to the small amount of DNA available for the EPIC subjects, we used a set of 50 individuals belonging to a cardiovascular disease study to check the presence of false negatives/positives genotyped by the restriction digestion method. All DHPLC typings confirmed the PCR–RFLP results.

Statistical methods

The significance of the differences among genotypes for ^{32}P-post-labeling DNA adduct levels was estimated both by non parametric tests, i.e. Mann–Whitney and Kruskal–Wallis rank sum tests, and by Student's *t*-test and ANOVA *F*-test when differences between two or more groups were compared.

The chi-square statistic with Yates correction, or the Fisher exact test when appropriate, were used to test associations of categorical data. Multivariate logistic regression analysis was carried out to calculate odds ratios (OR) adjusted for different possible confounders (age, sex, body mass index, centre of origin, month and year of blood drawing, smoking status) (12) by using a dichotomous variable for DNA adduct levels (above/below 4.9 per 10^9 RAL, median value; or, detectable/undetectable measurements).

Values of $P \leqslant 0.05$ were considered significant. All the analysis were performed by the statistical package SPSS (version 5.0.1).

Results

Sample characteristics

A complete description of ^{32}P-DNA adduct levels according to different sociodemographic and anthropometric characteristics of the sample is reported in Palli *et al.* (12).

No significant difference has been observed in ^{32}P-DNA adduct levels among current smokers (7.92 ± 1.24), ex-smokers (8.78 ± 1.11) and never-smokers (7.74 ± 1.00), whereas a significant difference ($P < 0.001$) exists among the centres participating to the study: Varese (6.29 ± 1.14), Turin (7.33 ± 1.43), Florence (10.98 ± 1.15), Ragusa (5.22 ± 1.35) and Naples (10.81 ± 2.26). We investigated whether these differences could be associated with a different distribution of repair genotypes among regions of residence, but no statistically significant difference in genotype frequency occurred (Figure 1).

DNA repair gene frequencies

Genotype and allele frequencies for the three polymorphisms analysed were calculated by direct counting and genotype distributions were in Hardy–Weinberg equilibrium. Overall genotype frequencies are reported in Table I. Allele frequencies were as follows: *XRCC1*-399Arg/Gln, 0.66/0.34; *XRCC3*-241Thr/Met, 0.58/0.42 and *XPD*-751Lys/Gln, 0.59/0.41.

A difference in the genotype distribution across separate categories of DNA adduct levels was observed only for the *XRCC3*-Thr241Met polymorphism (Table I). When subjects were classified in separate groups according to the presence/absence of detectable DNA adducts ($\chi^2 = 5.622$, $P = 0.06$), and above/below median DNA adduct levels ($\chi^2 = 11.819$, $P = 0.003$), a significant odds ratio (OR) emerged by the

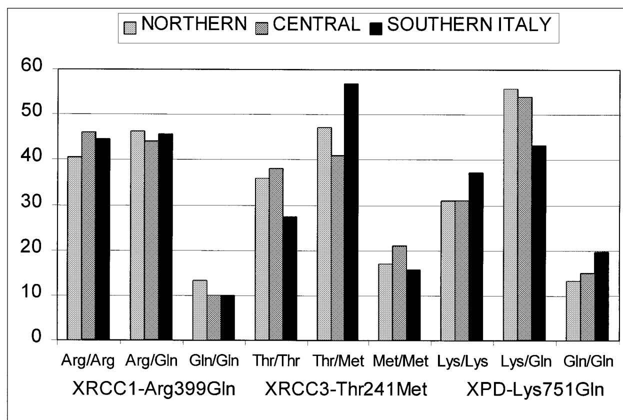

Fig. 1. Genotype frequencies (percentages on *y* axis) for the three DNA repair polymorphisms in the three Italy regions of residence: Northern (Varese and Torino), Central (Florence) and Southern Italy (Ragusa and Naples).

comparison of *XRCC3*-241Met homozygotes with *XRCC3*-241Thr homozygotes (crude OR 2.73, 95% CI 1.16–6.42 and OR 3.19, 95% CI 1.58–6.42, respectively). To take into account the effects of several confounders (age, sex, BMI, centres, month and year of blood drawing and smoking status) multivariate logistic regression analyses were also carried out; OR values were only slightly modified and still significant (adjusted OR 2.68, 95% CI 1.07–6.68; OR 4.01, 95% CI 1.82–8.83, respectively).

DNA repair genotypes, smoking status and DNA adduct levels

Stratifying the analysis by smoking status (Table II), the difference in the distribution of subjects with DNA adduct levels above/below the median value was still significant for the *XRCC3* polymorphism (Met/Met versus Thr/Thr genotypes) in never-smokers (χ^2 = 7.68, P = 0.021), both as crude and adjusted estimates (OR 4.32, 95% CI 1.38–13.57 and OR 5.34, 95% CI 1.44–19.75, respectively). In ex-smokers the OR adjusted estimate, 5.81 (95% CI 1.10–30.72), was statistically significant, but it was not in current smokers, OR 3.64 (95% CI 0.68–19.51).

Interestingly enough, in the never-smoker group also the *XPD*-Gln and *XRCC1*-Gln homozygotes seem to be at higher risk of having DNA adducts above the median compared with wild-type homozygotes (adjusted OR 3.81, 95% CI 1.02–14.16 and 3.00, 95% CI 0.80–11.28, respectively).

Very similar results emerged from the analysis of mean and median adducts in the overall sample and in the different groups according to smoking status (Table III, Figure 2A–C). The *XRCC3*-241Met variant was significantly associated with higher DNA adduct levels, whereas *XRCC1*-399Gln was associated exclusively in never-smokers. *XRCC3*-241Met homozygotes had an average DNA adduct level of 11.44 ± 1.48 (±SE) compared with 7.69 ± 0.88 in Thr/Met heterozygotes and with 6.94 ± 1.11 in Thr/Thr homozygotes (F = 3.206, P = 0.042). Never-smoking *XRCC1*-399Gln homozygotes had an average DNA adduct level of 15.60 ± 5.42 compared with 6.16 ± 0.97 in Gln/Arg heterozygotes and with 6.78 ± 1.10 in Arg/Arg homozygotes (F = 5.237, P = 0.007). A higher DNA–adduct level was observed in *XPD*-751Gln never-smoking homozygotes (10.14 ± 2.14) compared with Lys/Gln and Lys/Lys carriers (7.06 ± 1.03 and 7.76 ± 2.40, respectively) (Figure 2C), although the difference was not statistically significant.

In the whole series, homozygotes for one of the three polymorphic genotypes varied between 11.1 for *XRCC1* and 17.9% for *XRCC3*. Overall, 16/308 (5.2%) subjects resulted in homozygotes for the polymorphic variant at two of the three loci analysed; no triple homozygote was identified. In particular, four subjects were homozygotes for both the *XRCC1*-399Gln and *XRCC3*-241Met variants (4/308, 1.3%) and a possible interaction is suggested, the double unfavorable homozygote XRCC1/XRCC3 having higher ^{32}P-DNA adducts compared with the remaining genotypes (26.08 ± 7.74 versus 7.89 ± 0.62; t = –3.303, P = 0.001).

Discussion

In 308 healthy Italian EPIC individuals we have studied the relationship between three genetic polymorphisms of different DNA repair genes (*XRCC1*, *XRCC3* and *XPD*) and DNA damage, as measured by ^{32}P-DNA adduct levels. Our study suggests that all the analysed variants could result in deficient DNA repair.

The *XRCC1*-399Gln variant frequency (0.34) is similar to that reported by Lunn *et al.* (36) for the Caucasoid population (0.37), but different from the one estimated by Shen *et al.* (24) analysing DNA sequence in 12 individuals (0.25). The *XPD*-751Gln frequency is higher (0.41) than those estimated in the US (0.29) (24) and in the UK (0.30) (37), but similar to the frequency calculated by Duell *et al.* (38) in the US (0.39). Finally, in our sample the *XRCC3*-241Met frequency is similar (0.42) to that reported by Shen *et al.* (0.38) (24) and higher than that described by Winsey *et al.* (35) in the UK (0.30).

To investigate whether the *XRCC1*-Arg399Gln (exon 10), *XRCC3*-Thr241Met (exon 7) and *XPD*-Lys751Gln (exon 23) polymorphisms were associated with differences in DNA repair, which might be reflected in levels of genotoxic damage, we compared ^{32}P-DNA adduct levels in WBC from peripheral blood (Tables I–III). Considering the overall sample, a significant difference among genotypes and ^{32}P-DNA adduct levels (Table III) was only observed for the *XRCC3*-241Met variant (Thr/Thr = 6.94 ± 1.11, Thr/Met = 7.69 ± 0.88, Met/Met = 11.44 ± 1.48; F = 3.206, P = 0.042). Interestingly, when the population was split into current smokers, ex-smokers and never-smokers, a significant difference was also observed for *XRCC1* and *XPD* polymorphisms but only in never-smoking subjects (Tables II–III). Similar results were obtained by calculating the risks (OR) of having DNA adduct levels above the median value, according to different genotypes for each polymorphism (Table II).

Since in the overall population we have not found a significant difference in ^{32}P-DNA adduct levels among current smokers (7.92 ± 1.24), ex-smokers (8.78 ± 1.11) and never-smokers (7.74 ± 1.00), it is interesting to observe such differences among genotypes when stratifying by smoking status (Table III, Figure 2A–C). In previous studies, a significant relationship has been described between smoking status and bulky DNA adduct levels, as determined by ^{32}P-post-labelling assay and other methods, in tissues in which smoking-related cancers may occur (39–43). Such evidence suggests that the difference in DNA adduct levels between smokers and non-smokers is more easily detectable in the target tissues of tobacco smoke (where higher adduct accumulation is expected), and less or not evident in WBC from peripheral blood, unless

Table I. Distribution of 308 Italian EPIC individuals according to detectable/undetectable levels of DNA adducts or for above/below DNA adduct median values, for three DNA repair genotypes, and for the wild-type allele homozygotes versus subjects with at least one variant allele

	All subjects (308) *n* = (%)	Undetectable adducts (83) *n* (%)	Detectable adducts (224) *n* (%)	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Below[a] (153) *n* (%)	Above[a] (155) *n* (%)	Crude OR (95% CI)	Adjusted OR[b] (95% CI)
***XRCC1*, exon 10 (codon 399)**									
Arg/Arg	134 (43.6)	37 (44.6)	97 (43.3)	1	1	66 (43.4)	68 (43.9)	1	1
Arg/Gln	139 (45.3)	40 (48.2)	99 (44.2)	0.94 (0.55–1.60)	0.95 (0.53–1.70)	71 (46.7)	68 (43.9)	0.92 (0.57–1.49)	0.90 (0.53–1.53)
Gln/Gln	34 (11.1)	6 (7.2)	28 (12.5)	1.78 (0.68–4.65)	1.87 (0.69–5.07)	15 (9.9)	19 (12.3)	1.22 (0.57–2.62)	1.45 (0.64–3.31)
Gln/Gln + Arg/Gln	173 (56.4)	46 (55.4)	127 (56.7)	1.05 (0.63–1.75)	1.08 (0.63–1.87)	86 (56.6)	87 (56.2)	0.98 (0.62–1.54)	1.02 (0.62–1.67)
		$\chi^2 = 1.755$[c]	$P = 0.416$			$\chi^2 = 0.536$[c]	$P = 0.765$		
***XRCC3*, exon 7 (codon 241)**									
Thr/Thr	104 (33.8)	33 (39.8)	71 (31.6)	1	1	59 (38.6)	45 (29)	1	1
Thr/Met	149 (48.4)	42 (50.6)	107 (47.6)	1.18 (0.68–2.04)	1.33 (0.73–2.41)	78 (51)	71 (45.8)	1.19 (0.72–1.97)	1.31 (0.75–2.30)
Met/Met	55 (17.9)	8 (9.6)	47 (20.8)	**2.73 (1.16–6.42)**	**2.68 (1.07–6.68)**	16 (10.5)	39 (25.2)	**3.19 (1.58–6.42)**	**4.01 (1.82–8.83)**
Met/Met + Thr/Met	204 (66.3)	50 (60.2)	154 (68.4)	1.43 (0.85–2.41)	1.54 (0.87–2.72)	94 (61.5)	110 (71.0)	1.53 (0.95–2.47)	**1.70 (1.00–2.89)**
		$\chi^2 = 5.622$[c]	$P = 0.060$			$\boldsymbol{\chi^2 = 11.819}$[c]	$\boldsymbol{P = 0.003}$		
***XPD*, exon 23 (codon 751)**									
Lys/Lys	102 (33.1)	28 (33.7)	74 (32.9)	1	1	54 (35.3)	48 (31)	1	1
Lys/Gln	157 (51)	42 (50.6)	115 (51.1)	1.04 (0.59–1.81)	1.06 (0.58–1.94)	77 (50.3)	80 (51.6)	1.16 (0.71–1.92)	1.19 (0.68–2.08)
Gln/Gln	49 (15.9)	13 (15.7)	36 (16.0)	1.05 (0.49–2.26)	1.25 (0.54–2.88)	22 (14.4)	27 (17.4)	1.38 (0.70–2.73)	1.53 (0.71–3.27)
Gln/Gln + Lys/Gln	206 (66.9)	55 (66.3)	151 (67.1)	1.04 (0.61–1.77)	1.11 (0.63–1.97)	99 (64.7)	107 (69.0)	1.21 (0.75–1.95)	1.25 (0.74–2.10)
			$\chi^2 = 0.021$[c]	$P = 0.990$			$\chi^2 = 0.908$[c]	$P = 0.635$	

[a]Below and above the median value of 4.9 per 10^9 RAL.
[b]Multivariate logistic regression: OR adjusted by age, sex, BMI, centres, month and year of blood drawing, repair genes and smoking status.
[c]3×2 Contingency table χ^2 test for the three different genotypes above/below RAL median value and between detectable/undetectable groups.
OR, odds ratio; CI, confidence interval. Statistically significant comparisons are marked in bold.

Table II. Distribution of 308 Italian EPIC individuals and DNA adducts levels above/below median for the three DNA repair genotypes, and for the wild-type allele homozygotes versus subjects with at least one variant allele according to the smoking status

	Never-smokers (131)			Ex-smokers (92)			Current smokers (81)		
	A/B[a]	OR (95%C.I.)		A/B[a]	OR (95%C.I.)		A/B[a]	OR (95%C.I.)	
		Crude	Adjusted[b]		Crude	Adjusted[b]		Crude	Adjusted[b]
XRCC1									
Arg/Arg	28/31	1	1	21/19	1	1	17/16	1	1
Arg/Gln	26/28	1.02 (0.57–2.62)	1.04 (0.41–2.61)	22/20	0.99 (0.41–2.36)	1.07 (0.37–3.06)	19/22	0.81 (0.32–2.03)	0.81 (0.26–2.50)
Gln/Gln +	12/6	2.21 (0.73–6.68)	3.00 (0.80–11.28)	4/6	0.60 (0.14–2.46)	0.69 (0.13–3.66)	3/3	0.94 (0.16–5.36)	1.41 (0.16–12.60)
Gln/Gln + Arg/Gln	38/34	1.23 (0.62–1.54)	1.27 (0.58–2.79)	26/26	0.90 (0.39–2.06)	0.96 (0.36–2.53)	22/25	0.82 (0.33–2.02)	0.93 (0.32–2.68)
XRCC3									
Thr/Thr	22/28	1	1	12/14	1	1	11/16	1	1
Thr/Met	27/32	1.07 (0.50–2.29)	1.04 (0.42–2.57)	23/26	1.03 (0.39–2.67)	2.02 (0.55–7.39)	21/20	1.52 (0.57–4.07)	2.31 (0.69–7.70)
Met/Met	17/5	**4.32 (1.38–13.57)**	**5.34 (1.44–19.75)**	12/5	2.79 (0.76–10.24)	**5.81 (1.10–30.72)**	7/6	1.69 (0.44–6.43)	3.64 (0.68–19.51)
Met/Met + Thr/Met	44/37	1.51 (0.74–3.07)	1.57 (0.69–3.54)	35/31	1.31 (0.53–3.27)	2.68 (0.77–9.38)	28/26	1.57 (0.61–3.99)	2.61 (0.83–8.23)
	$\boldsymbol{\chi^2 = 7.68}$[c]	***P* = 0.021**							
XPD									
Lys/Lys	19/24	1	1	16/18	1	1	12/12	1	1
Lys/Gln	34/35	1.22 (0.57–2.64)	1.08 (0.44–2.65)	23/19	1.36 (0.55–3.37)	2.13 (0.63–7.14)	22/23	0.95 (0.35–2.57)	0.80 (0.22–2.77)
Gln/Gln	13/6	2.73 (0.87–8.55)	**3.81 (1.02–14.16)**	8/8	1.12 (0.34–3.69)	2.07 (0.43–10.00)	5/7	0.71 (0.17–2.89)	0.40 (0.06–2.54)
Gln/Gln + Lys/Gln	47/41	1.44 (0.69–3.01)	1.55 (0.68–3.55)	31/27	1.29 (0.55–3.01)	1.92 (0.63–5.84)	27/30	0.90 (0.35–2.34)	0.72 (0.22–2.40)

[a]A/B, Above and below the 4.9 per 10^9 RAL median value.
[b]Multivariate logistic regression: OR adjusted by age, sex, BMI, centres, month and year of blood drawing, repair genes.
[c]3×2 Contingency table χ^2 test for the three different genotypes above/below the RAL median value.
OR, Odds ratio; CI, confidence interval. Statistically significant comparisons are marked in bold.

Table III. Mean (± standard error) and median levels of DNA adducts per 10^9 normal nucleotides for the DNA repair genotypes, according to smoking status

	All subjects (308)			Never-smokers (131)			Ex-smokers (92)			Current smokers (81)		
	n (%)	Mean ± SE	Median	*n* (%)	Mean ± SE	Median	*n* (%)	Mean ± SE	Median	*n* (%)	Mean ± SE	Median
XRCC1												
Arg/Arg	134 (43.6)	7.07 ± 0.72	4.90	59 (45.0)	6.78 ± 1.10	4.40	40 (43.5)	7.57 ± 1.52	4.90	33 (41.3)	6.66 ± 1.14	6.05
Arg/Gln	139 (45.3)	8.36 ± 0.94	4.20	54 (41.2)	6.16 ± 0.97	4.15	42 (45.7)	10.14 ± 1.82	5.60	41 (51.3)	9.61 ± 2.24	3.60
Gln/Gln	34 (11.1)	11.31 ± 3.09	5.05	18 (13.8)	15.60 ± 5.42	7.62	10 (10.8)	7.96 ± 3.14	3.95	6 (7.4)	4.03 ± 1.91	3.00
					P = **0.007**[a]							
XRCC3												
Thr/Thr	104 (33.8)	6.94 ± 1.11	3.57	50 (38.2)	7.01 ± 1.94	3.90	26 (28.3)	8.61 ± 2.05	4.35	27 (33.4)	5.44 ± 1.25	2.90
Thr/Met	149 (48.4)	7.69 ± 0.88	4.20	59 (45.0)	6.76 ± 1.18	4.20	49 (53.2)	7.77 ± 1.46	3.90	41 (50.6)	8.93 ± 2.11	5.00
Met/Met	55 (17.8)	11.44 ± 1.48	6.70	22 (16.8)	11.98 ± 2.42	6.32	17 (18.5)	11.97 ± 2.97	8.30	13 (16.0)	9.88 ± 2.92	6.70
		P = **0.042**[a]			P = **0.047**[b]							
		P = **0.005**[b]										
XPD												
Lys/Lys	102 (33.1)	7.86 ± 1.23	4.20	43 (32.8)	7.76 ± 2.40	3.30	34 (37.0)	8.45 ± 1.93	3.90	24 (29.6)	7.31 ± 1.35	5.25
Lys/Gln	157 (51)	7.56 ± 0.72	4.90	69 (52.7)	7.06 ± 1.03	4.70	42 (45.7)	8.33 ± 1.43	4.90	45 (55.6)	7.58 ± 1.50	4.50
Gln/Gln	49 (15.8)	10.37 ± 1.94	6.10	19 (14.5)	10.14 ± 2.14	8.90	16 (17.3)	10.68 ± 3.31	5.35	12 (14.8)	10.42 ± 5.82	1.45

[a]ANOVA test.
[b]Kruskal–Wallis rank sum test for DNA adducts levels among several independent samples.
Statistically significant comparisons are marked in bold.

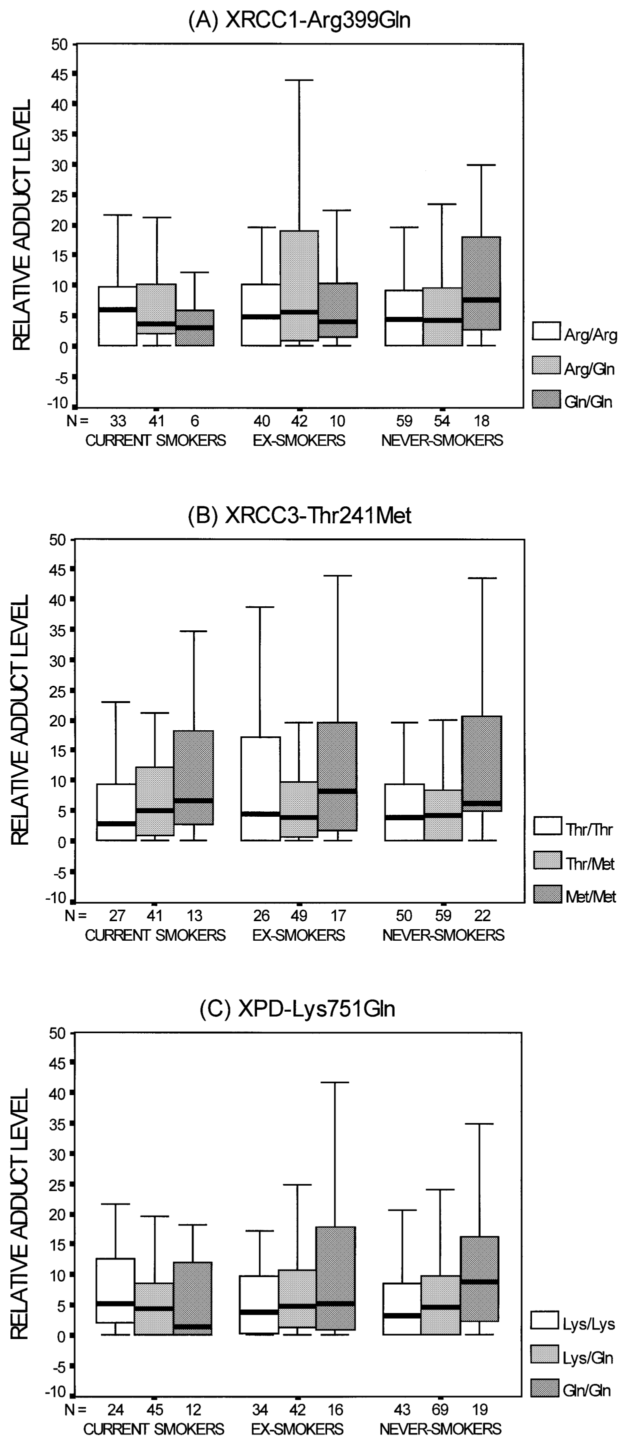

Fig. 2. Box plot representation of DNA adduct levels (RAL) per 10^9 normal nucleotides in the overall sample according to smoking status and to the different DNA repair genotypes: (**A**) *XRCC1*-Arg399Gln; (**B**) *XRCC3*-Thr241Met and (**C**) *XPD*-Lys751Gln. The box bounds the first and third quartiles (interquartile range), encompasses 50% of the data and includes the median (line within the box). Dispersion of the data above and below this range is marked by 'whiskers' that extend to the most extreme values within a 'fence' at 1.5 times the interquartile range.

we consider the effect of other variables, such as the different DNA repair genotypes.

Interestingly enough, analysing 41 nasal mucosa biopsies we observed the same behaviour in ^{32}P-DNA adduct levels for the same three DNA repair polymorphisms stratifying by smoking status, with the smoker group having higher mean adduct levels (unpublished data).

One possible explanation could be that smoking, both in WBC and target tissues of smoking, also alters the levels by triggering and up-regulating DNA repair enzymes, flattening or even reversing the difference among the analysed genotypes with possibly different repair efficiency (Figure 2A–C). Indeed, Wei *et al.* (44) showed that heavy smokers among both lung cancer patients and control subjects tended to have more proficient DNA repair capacity (DRC) in lymphocytes than lighter smokers, suggesting that cigarette smoking may, in fact, stimulate DRC in response to the DNA damage caused by tobacco carcinogens.

Previous studies showed that *XRCC1*-399Gln may be associated with increased DNA damage measured by aflatoxin B1–DNA adducts, glycophorin A (GPA) variant frequency (36), sister chromatid exchange and polyphenol DNA adducts (38). In contrast to our study, Lunn *et al.* (36) found a significant association for the NN GPA variant frequency in smoking subjects, while the never-smoking group analysed was very small (10 individuals). A positive association has also been found with squamous cell carcinoma of the head and neck (6), gastric cancer (45) and adenocarcinoma of the lung (46). Contrasting results have also been described (31) for *XPD*-Lys751Gln, in which the Lys/Lys *XPD*-751 genotype was found to be associated with reduced repair of X-ray-induced cytogenetic damage measured by chromatid aberrations. Another study by Dybdhal *et al.* (7) reported that individuals with the common allele (Lys751) had an elevated risk of basal cell carcinoma, whereas a recent study found no effect of XPD on polyphenol DNA adducts (38).

A possible explanation for these results is that amino acid variants in different domains of XPD may not only affect different protein interactions, resulting in the expression of different phenotypes (47), but also the same *XPD*-Lys751Gln polymorphism may have divergent effects in different DNA repair pathways and on different types of DNA damage. Moreover, this conservative substitution could be in linkage with another responsible *XPD* variant; in this case it is possible that different populations have different alleles in linkage disequilibrium with the responsible *XPD* variant.

In the present report we have found a clear association between the *XRCC3*-241Met variant and ^{32}P-DNA adduct levels in a sample of 308 EPIC subjects. This association indicates a possible role of the *XRCC3* gene in repairing bulky WBC DNA adducts, a novel function with respect to its known functions in DNA double-strand break/recombination repair. Few studies have been conducted regarding the *XRCC3* polymorphism's involvement in cancer: one study reported evidence of an association between a rare microsatellite polymorphism in the *XRCC3* gene and cancer in patients with varying radiosensitivity (48); another study from our group (33) has found an association of the *XRCC3*-241Met variant with bladder cancer, while Winsey *et al.* (35) found an association with melanoma.

The Thr241Met substitution in XRCC3 is a non-conservative change with possible biological implications for the functionality of the enzyme and/or the interaction with other proteins involved in DNA repair damage. To our knowledge, this is the first study to suggest that the *XRCC3*-Thr241Met polymorphism may be associated with the repair of bulky WBC DNA adduct lesions. These results must be confirmed in larger samples of different origin, in order to appreciate the

relatively small effect of polymorphisms with low penetrance, taking into account the effect of different exposures to environmental carcinogens.

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