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Polymorphic DNA repair and metabolic genes: a multigenic study on gastric cancer

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Risk factors for gastric cancer (GC) include inter-individual variability in the inflammatory response to *Helicobacter pylori* infection, in the ability of detoxifying DNA reactive species and repairing DNA damage generated by oxidative stress and dietary carcinogens. To evaluate the association between polymorphic DNA repair genes and GC risk, a case–control study including 314 histologically confirmed GC patients and 548 healthy controls was conducted in a GC high-risk area in Tuscany, Italy. Polymorphic variants of base excision repair (*APE1*-D148E, *XRCC1*-R194W, *XRCC1*-R399Q and *OGG1*-S326C), nucleotide excision repair (*XPC*-PAT, *XPA*-23G>A, *ERCC1*-19007T>C and *XPD*-L751Q), recombination (*XRCC3*-T241M) and alkylation damage reversal (*MGMT*-L84F) were tested for their potential role in the development of GC by using logistic regression models. The same population was also characterised for *GSTT1* and *GSTM1* variant alleles to search for possible functional interactions between metabolic and DNA repair genotypes by two-way interactions using multivariate logistic models. No significant association between any single DNA repair genotype and GC risk was detected with a borderline association with the *XPC*-PAT homozygous genotype [odds ratio (OR) = 1.42; 95% confidence interval (CI) 0.94–2.17]. Gene–gene interaction analysis revealed combinations of unfavourable genotypes involving either multiple DNA repair polymorphisms or DNA repair and *GST*-specific genotypes. The combination of the *XPC*-PAT and the *XPA* variant alleles significantly increased GC risk (OR = 2.15; 95% CI 1.17–3.93, *P* = 0.0092). A significant interaction was also found between the *APE1* wild-type genotype and either the single *GSTT1* (OR = 4.90; 95% CI 2.38–10.11, *P* = 0.0079) or double *GSTM1-GSTT1* null (OR = 7.84; 95% CI 3.19–19.22, *P* = 0.0169) genotypes or the *XPA*-mutant allele (OR = 3.56; 95% CI 1.53–8.25, *P* = 0.0012). These findings indicate that a complex interaction between host factors such as oxidative stress, antioxidant capacity and efficiency of multiple DNA repair pathways underlies the inter-individual variability in GC risk.

Introduction

Gastric cancer (GC) still represents the fourth most common cancer in the world and the second leading cause of cancer death, although a marked decline in incidence and mortality rates occurred in most industrialised countries (1).

Overall, GC is considered a multistep and multifactorial process involving different components (dietary, genetic and infectious) (2). One of the proposed mechanisms of GC development involves oxidative stress, including inflammation induced by *Helicobacter pylori* infection. Inflammation results in the generation of DNA-damaging reactive oxygen species (ROS) as reported in *H.pylori*-infected gastric mucosa cells (3). Individual differences in the intensity of the inflammatory response (4,5) and in the metabolic capacity of detoxifying oxidative species (6) have been shown to contribute to GC risk. On the same line, several epidemiological studies have reported the protective effects of dietary antioxidants in stomach carcinogenesis, although results are not entirely consistent (7). The intake of antioxidants via the diet, particularly fresh fruit and vegetables, may decrease GC risk through the inactivation of ROS, thus potentiating the antioxidant cell capacity. Conversely, the available evidence supports a positive association between intake of nitrite and nitrosamine, meat and processed meat and GC (reviewed in ref. 8).

The type of damage induced by ROS and/or dietary carcinogens includes pre-mutagenic lesions that may lead to mutations in cancer-related genes. Cells are provided of four main repair pathways to prevent mutation fixation. The base excision repair (BER) is the preferential mechanism for repair of endogenous/oxidative damage, nucleotide excision repair (NER) for bulky adducts, mismatch repair and recombination are mainly involved in the repair of lesions persisting at replication. Inherited functional polymorphisms in DNA repair genes may influence the host capacity to repair DNA damage,

thus leading to increased cancer risk (9). Some studies have provided evidence of an increased GC risk for carriers of specific DNA repair gene alleles (10–14). However, lack of association has been observed in other population-based studies in some cases by analysis of the same variants (15–20).

In Italy, as in most industrialised countries, GC mortality has been decreasing since 1950, but specific high-risk areas, such as the North-Eastern Tuscany, are still present (with incident and mortality rates higher than Italian rates) (21).

Although *H.pylori* infection is the major GC risk factor, the infection *per se* does not account for GC susceptibility of subjects from these high-risk areas (5,22).

Genetic polymorphisms that can modulate the inflammatory response and oxidative stress-related carcinogenesis may play an important role.

The aim of this study is to evaluate the effect of selected polymorphisms in DNA repair genes belonging to different repair pathways either alone or in combination with polymorphisms in *GST* genes involved in ROS detoxification on the risk of GC in a population residing in a high-risk Italian area (21). A subset of this same population has been previously characterised for polymorphisms in genes involved in the inflammatory response and antioxidative capacity (5,6). The selected repair genes mostly belonged to BER and NER because of their major role in the removal of DNA damage induced by oxidative stress and/or diet carcinogens and their polymorphisms (namely, *APE1*-D148E, *XRCC1*-R194W, *XRCC1*-R399Q, *OGG1*-S326C, *XPC*-PAT, *XPA*-23G>A, *ERCC1*-19007T>C, *XPD*-L751Q, *XRCC3*-T241M and *MGMT*-L84F) were selected according to frequency, functional effects and association to cancer.

Materials and methods

Study population

The study was performed comparing a series of GC patients and healthy adults both residing in an area of ~50 miles surrounding the city of Florence in North-Eastern Tuscany (Central Italy), where a high risk of GC has been traditionally reported (21).

GC cases. The original series of 188 GC cases (5,6) identified in a GC high-risk area including Florence and the surrounding rural district in the period 1995–1997 was expanded with an additional series of 128 GC consecutives cases identified in the same area in the period 2000–2005, while admitted in the Surgery Departments of the main hospitals. Each case, after the signature of an informed consent form, was invited to donate a blood sample. All patients were affected by adenocarcinoma of the stomach, histologically confirmed. Overall, 316 GC cases were enrolled and detailed information was collected on 314 subjects. Results of genetic analyses were not available for the whole series with a few results missing for each genotype.

Population controls. Two series of healthy adults were randomly selected from the municipality lists of two areas in North-Eastern Tuscany in the frame of an on-going multi-site cancer epidemiology project: the City of Florence and one nearby rural area (5,6). Of 700 randomly selected subjects, 553 subjects (79.0%), after signature of an informed consent form, accepted to participate into the study and provided a blood sample. Detailed information was collected on 548 subjects. Results of genetic analyses were not available for the whole series with a few results missing for each genotype.

Blood collection

The blood samples collected from both GC cases and controls were processed at Cancer Prevention and Research Institute (Florence) in the same day of collection. Red blood cells, buffy coat, serum and plasma were separated, divided in aliquots and stored at −80°C. Two aliquots of buffy coat and plasma for each study subject were retrieved and shipped on dry ice to Istituto Superiore di Sanità (ISS) (Rome) and Institute for Scientific Interchange Foundation (ISI) (Turin) for DNA extraction and genetic analyses and to ISS (Rome) for *H.pylori* serology.

DNA extraction

Genomic DNA was obtained from frozen buffy coat, using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

DNA genotyping

XPC polymorphisms. To determine the *XPC*-PAT polymorphism, intron 9 of the *XPC* gene was amplified as described by Khan *et al*. (23) (Table I). The polymerase chain reaction (PCR) products were resolved on a 1.5% agarose gel stained with ethidium bromide. After electrophoresis, homozygous $PAT^{-/-}$ genotypes were represented by 266-bp ('S' or '−' allele) DNA band, whereas homozygous $PAT^{+/+}$ genotypes were represented by a 344-bp ('L' or '+' allele) fragment. Heterozygous genotypes displayed a combination of both alleles (266 and 344 bp).

The intron 11 (-5 CA) single-nucleotide polymorphism (SNP) was detected by PCR amplification of the acceptor site as described by Khan *et al*. (24) (supplementary Appendix A is available at *Mutagenesis* Online). The polymorphic position was analysed by FauI restriction endonuclease (SibEnzyme) digestion of the 203-bp PCR products. The C at position -5 of XPC intron 11 creates a FauI site. FauI digestion converts a 203-bp PCR product into two fragments of 43 and 160 bp.

Taqman genotyping. 5′ Nuclease Assay (TaqMan) was used to genotype *APE1*-D148E, *XRCC1*-R194W, *XRCC1*-R399Q, *OGG1*-S326C, *XPD*-L751Q, *XPA*-23G>A, *ERCC1*-19007T>C, *MGMT*-L84F and *XRCC3*-T241M. Information on TaqMan primers, probes and PCR conditions for genotyped SNPs are reported in Appendix A, available at *Mutagenesis* Online.

GSTM1-GSTT1 polymorphisms. Wild-type (+/+), heterozygous (+/−) and null (−/−) genotypes of *GST* gene were analysed simultaneously by using a multiplex PCR in which multiple genes are co-amplified in the same reaction tube. The method used was by Abdel-Rahman *et al*. (25) with minor modifications (6).

H.pylori serology

The enzyme-linked immunosorbent assay was used to investigate the presence of IgG antibodies to *H.pylori* (Helory 192; Eurospital, Trieste, Italy) and of *CagA* protein (Helori CTX; Eurospital). Our cases and controls were considered to be *H.pylori* positive if one of the two tests was positive. Plasma samples were kept frozen (−80°C) until tested.

Statistical analysis

For each characteristic of the study populations (sex, area of residence, *H.pylori* seroprevalence and GC family history), the differences in the distribution between cases and controls were tested using χ^2 test. Comparison of genotype frequencies between cases and controls was also performed by χ^2 test. Because the *XRCC1*-R194W*TT genotype was extremely rare (<0.5%), it was combined with the *XRCC1*-R194W*CT genotype, assuming a dominant model of inheritance.

Table I. Distribution of the study population according to selected individual characteristics

Characteristics	Cases *N* (%)	Controls *N* (%)	Chi-square *P*-value
Sex			
Male	177 (56.4)	270 (49.3)	
Female	137 (43.6)	278 (50.7)	0.045
Area of residence			
Urban	133 (42.4)	357 (65.1)	
Rural	181 (57.6)	191 (34.9)	<0.0001
H.pylori serology[a]			
Negative	39 (13.0)	196 (36.0)	
Lysate positive	90 (30.1)	132 (24.3)	<0.0001
CagA positive	170 (56.9)	216 (39.7)	
GC family history			
Negative	262 (83.4)	499 (91.1)	
Positive	52 (16.6)	49 (8.9)	0.0008
Smoking history[a]			
Current-smoker	46 (16.8)	173 (31.6)	
Ex-smoker	107 (39.0)	148 (27.0)	
Never-smoker	121 (44.2)	227 (41.4)	<0.0001

[a]Some data are missing.

Hardy–Weinberg equilibrium of alleles at individual loci was assessed by χ^2 test with one degree of freedom (26).

Multiple logistic regression models were performed to assess the association between each DNA repair polymorphism and GC risk by odds ratios (ORs) and their 95% confidence intervals (CIs) controlling for potential confounders (sex; age: <60 versus ≥60 years; area of residence: urban versus rural; *H.pylori* serology: negative versus positive and GC family history in first-degree relatives: negative versus positive). The analysis was performed with separate logistic regression models based on different inheritance model: dominant, recessive and multiplicative codominant effect (26). Additional regression analyses were performed in the subgroups of *H.pylori*-positive subjects ($n = 608$) and ever-smokers ($n = 474$) to evaluate the effect of polymorphisms involved in oxidative DNA damage repair among subjects with differential exposure to factors that produce oxidative damage.

Two-way interactions between all pairs of DNA repair polymorphisms in Hardy–Weinberg equilibrium ($n = 10$) and between these polymorphisms and metabolic polymorphisms were investigated by using multivariate logistic models (26).

The rejection of null hypothesis of no interaction was performed by Likelihood Ratio test comparing models with and without the interaction term in the presence of individual genotypes effects. We performed these tests on the multiplicative codominant model. Therefore, test for SNP–SNP interactions is a χ^2 with one degree of freedom.

Two-way interactions between all pairs of unlinked DNA repair polymorphisms ($n = 9$) were tested also with 'linkage disequilibrium (LD)-based statistic' proposed by Zhao *et al.* (27).

The large number of interactions analysed could lead to false positive results, therefore, we calculated the *q*-values proposed by Storey and Tibshirani (28). The *q*-values were calculated using the R library *q*-value.

Finally, we evaluated the interactions between smoking history and DNA repair and metabolic polymorphisms using multivariate logistic models (26).

The analyses were carried out using Stata 9.0 (StataCorp. 2005. Stata Statistical Software: Release 9; StataCorp LP, College Station, TX) and R (R Foundation for Statistical Computing, R Development Core Team. R: A Language and Environment for Statistical Computing. 2008, http://www.R-project.org).

Results

Individual characteristics

The distribution of selected individual characteristics in cases and controls is shown in Table I. The mean age at diagnosis (±SD) of the GC cases was 68.8 years (±9.9) and males were 56.4% (177/314). The mean age of the controls at recruitment was 55.5 years (±7.0) with 49.3% being male. GC cases reported a higher prevalence of GC family history among their first-degree relatives ($P = 0.0008$) were more often *H.pylori* seropositive ($P < 0.0001$) and were more often residing in a rural area ($P < 0.0001$). A significant difference between cases and controls emerged also by smoking history ($P < 0.0001$), although this information is not available for 40 GC cases.

Genotype frequencies

The genotype frequency distribution of the analysed DNA repair gene polymorphisms are shown in Table II. No significant associations with the GC risk emerged in univariate analysis. The distribution of the analysed genotypes among our population controls was consistent with the Hardy–Weinberg equilibrium, and the observed frequency of different genotypes was similar to those previously observed in Caucasian populations (16,29,30).

The distribution of *XPC*-PAT polymorphism was compared with *XPC* intron11A>C polymorphism results in a subgroup of 121 controls showing a perfect correlation with the *XPC*-PAT genotype (except one subject). PAT^-/intron 11 C and PAT^+/intron 11 A haplotypes are in almost complete LD in our population ($D' = 1$, 95% CI 0.89–1, $r^2 = 0.23$); thus, we further considered only *XPC*-PAT in the statistical analyses.

Table III shows the distribution of *GSTM1* and *GSTT1* genotypes in our series. The frequencies of the two polymorphisms in the control group are in agreement with previously reported data in Caucasians (31). A significant difference between cases and controls with respect to null *GSTT1* ($P = 0.001$) and double null (*GSTM1-GSTT1)* genotypes ($P = 0.0001$) was found. In univariate analysis significant associations with the GC risk emerged for *GSTT1* null (OR = 1.79; 95% CI 1.25–2.56) and double null subjects (OR = 2.44; 95% CI 1.54–3.87).

Genotype analysis

Logistic regression analysis was performed for all SNPs in the context of different models after adjustment for several potential confounders. The estimated ORs and 95% CI are shown in Table IV. Multivariate analyses based on different models (dominant, recessive or multiplicative codominant) did not show any significant association with GC risk. Only a suggestion for an increased GC risk associated with the *XPC*-$PAT^{+/+}$ genotype emerged (OR = 1.42; 95% CI 0.94–2.17).

The associations between DNA repair SNPs and GC risk were also evaluated among 608 *H.pylori*-positive subjects. No

Table II. Distribution of the study population according to DNA repair genotypes[a]

	Cases *N* (%)	Controls *N* (%)	*P*-value[b]	OR (95% CI)
APE1-D148E				
TT	103 (34.6)	208 (38.1)		1[c]
GT	147 (49.3)	243 (44.5)		1.22 (0.88–1.69)
GG	48 (16.1)	95 (17.4)	0.40	1.02 (0.66–1.59)
XRCC1-R399Q				
GG	123 (42.6)	250 (45.8)		1[c]
AG	137 (47.4)	233 (42.7)		1.20 (0.87–1.63)
AA	29 (10.0)	63 (11.5)	0.41	0.94 (0.56–1.57)
XRCC1-R194W				
CC	259 (87.8)	466 (85.3)		1[c]
CT + TT	36 (12.2)	80 (14.7)	0.53	0.83 (0.55–1.25)
OGG1-S326C				
CC	192 (63.3)	325 (59.6)		1[c]
CG	101 (33.2)	191 (35.0)		0.90 (0.66–1.22)
GG	11 (3.5)	29 (5.4)	0.41	0.64 (0.29–1.38)
XPC-PAT				
S	88 (28.8)	148 (27.1)		1[c]
LS	149 (48.7)	292 (53.3)		0.86 (0.61–1.21)
L (PAT^+)	69 (22.5)	107 (19.6)	0.39	1.08 (0.71–1.65)
XPA-23G>A				
GG	134 (47.2)	249 (47.6)		1[c]
GA	115 (40.5)	215 (41.1)		0.99 (0.72–1.37)
AA	35 (12.3)	59 (11.3)	0.91	1.10 (0.67–1.80)
ERCC1-19007T>C				
TT	99 (34.6)	179 (34.0)		1[c]
TC	135 (47.2)	249 (47.3)		0.98 (0.70–1.37)
CC	52 (18.2)	98 (18.7)	0.98	0.96 (0.62–1.49)
XPD-L751Q				
AA	90 (30.5)	177 (32.4)		1[c]
AC	157 (53.2)	284 (52.0)		1.09 (0.78–1.52)
CC	48 (16.3)	85 (15.6)	0.85	1.11 (0.70–1.56)
XRCC3-T241M				
CC	95 (32.3)	189 (34.6)		1[c]
CT	148 (50.3)	268 (49.1)		1.10 (0.79–1.53)
TT	51 (17.4)	89 (16.3)	0.78	1.14 (0.73–1.78)
MGMT-L84F				
CC	210 (72.2)	395 (73.6)		1[c]
CT	77 (26.5)	131 (24.4)		1.11 (0.79–1.55)
TT	4 (1.3)	11 (2.0)	0.66	0.68 (0.16–2.35)

[a]Some data on each genotype are missing.
[b]*P*-value from chi-square.
[c]Reference category.

qualitative differences were found respect to the analysis performed in the whole population, with a borderline effect of the *XPC*-PAT$^{+/+}$ genotype (OR = 1.55; 95% CI 0.98–2.46). No heterogeneity of effects between *H.pylori*-positive and -negative subjects was found using Likelihood Ratio tests for interaction. Furthermore, the *GSTM1-GSTT1* double null subjects seemed to be at increased risk in the *H.pylori*-positive subgroup (OR = 2.28; 95% CI 1.41–3.68) as compared to the *H.pylori*-negative subgroup (OR = 1.44; 95% CI 0.55–3.82) although a formal Likelihood Ratio tests of interaction showed no evidence of heterogeneity of effects ($P = 0.270$). These findings can be explained by the fact that 87% of our cases were *H.pylori* positive with a consequent low power to detect heterogeneity of effects between *H.pylori*-positive and -negative subjects.

Similarly, the association between DNA repair SNPs and GC risk was unchanged when the analysis was performed on the subgroup of 474 ever-smokers subjects as compared to the whole population. Only the recessive effect of *XRCC1*-R399Q became protective 0.35 (0.15–0.81). No heterogeneity of effects between the two groups of ever-smokers and never-smokers was found using Likelihood Ratio tests for interaction.

Gene–gene interactions

Two-way interactions between all DNA repair and *GST* polymorphisms studied were investigated using multivariate logistic models. Since the genetic risk models are uncertain for most of the SNPs considered, we performed these analyses on the codominant models. Among the several two-way interactions evaluated, four interactions (between *APE1*-D148E and *XPA*, *APE1*-D148E and *GSTT1*, *APE1*-D148E and double null (*GSTM1-GSTT1*) and *XPC*-PAT and *XPA*-23G>A) were statistically significant ($P = 0.0012$, $P = 0.0079$, $P = 0.0169$ and $P = 0.0092$, respectively). Two interactions (between *APE1*-D148E and *XPA*-23G>A and *XPC*-PAT and *XPA*-23G>A) were highly significant according to Zhao method, also after taking into account the multiple comparisons (q-value = 0.024 and q-value = 0.022, respectively).

The effect size of each genotype combination for the four significant interactions by logistic models is shown in Table V. Higher GC risk values were found for subjects wild type for *APE1*-D148E and carrying the variant genotype A/A of *XPA*-23G>A polymorphism (OR = 3.56; 95% CI 1.53–8.25) or lacking GSTT1 activity (OR = 4.90; 95% CI 2.38–10.11) or lacking both GSTM1 and GSTT1 activity (OR = 7.84; 95% CI 3.19–19.22). An increased GC risk emerged also for subjects carrying the 'G' allele variant of *APE1*-D148E and lacking GSTT1 activity (OR = 1.84; 95% CI 1.04–3.28) or both *GSTT1-GSTM1* gene (OR = 2.32; 95% CI 1.12–4.82). Finally, a significant increased GC risk was found for subjects carrying the variant + of *XPC*-PAT polymorphism and the allele 'A' of *XPA*-23G>A polymorphism (OR = 2.15; 95% CI 1.17–3.93).

In the subgroup of *H.pylori*-positive subjects ($n = 608$), the four above mentioned two-way interactions (between *APE1*-D148E and *XPA*-23G>A, *APE1*-D148E and *GSTT1*, *APE1*-D148E and double null (*GSTM1-GSTT1*) and *XPC*-PAT and *XPA*-23G>A) remained statistically significant ($P = 0.0089$, $P = 0.0068$, $P = 0.044$ and $P = 0.032$, respectively). The same was observed in the subgroup of ever-smokers ($n = 474$) ($P = 0.0002$, $P = 0.0052$, $P = 0.0084$ and $P = 0.0032$ for the four interactions, respectively).

Finally, we tested the interactions between smoking status and DNA repair polymorphisms. A significant interaction was found between smoking and the following polymorphisms: *XPC*-PAT ($P = 0.0247$) with an increased GC risk among current-smokers for subjects carrying the variant '+' (OR = 2.03; 95% CI 1.09–3.77); *OGG1*-S326C ($P = 0.0138$) with a reduced GC risk among current-smokers for the 'G' variant (OR = 0.38; 95% CI 0.17–0.85); *XPD*-L751Q and *OGG1*-S326C ($P = 0.002$) with a reduced GC risk among current-smokers for the combined effects of the two polymorphisms (OR = 0.17; 95% CI 0.04–0.67).

Table III. Distribution of the study population according to *GSTT1* and *GSTM1* genotypes[a]

	Cases *N* (%)	Controls *N* (%)	*P*-value[b]	OR (95% CI)
GSTT1				
Not null	218 (73.7)	455 (83.3)		1[c]
Null	78 (26.3)	91 (16.7)	0.001	1.79 (1.25–2.56)
GSTM1				
Not null	130 (43.9)	271 (49.6)		1[c]
Null	166 (56.1)	275 (50.4)	0.113	1.26 (0.94–1.69)
GSTT1-GSTM1				
Other	246 (83.1)	504 (92.3)		1[c]
Double null	50 (16.9)	42 (7.7)	0.0001	2.44 (1.54–3.87)

[a]Some data on each genotype are missing.
[b]*P*-value from chi-square.
[c]Reference category.

Discussion

In the current study, we evaluated the association between selected DNA repair polymorphisms alone or in combination with metabolic polymorphisms and overall GC risk in

Table IV. Associations between selected DNA repair SNPs and gastric cancer risk: ORs[a] and 95% CIs models

	Allele (wild-type > mutated)	Dominant	Recessive	Multiplicative codominant
APE1-D148E	T>G	1.06 (0.74–1.51)	0.96 (0.61–1.52)	1.02 (0.80–1.30)
XRCC1-R399Q	G>A	1.09 (0.77–1.53)	0.70 (0.41–1.22)	0.96 (0.75–1.24)
XRCC1-R194W	C>T	0.69 (0.42–1.13)	—	—
OGG1-S326C	C>G	0.98 (0.69–1.39)	0.47 (0.20–1.06)	0.90 (0.67–1.20)
XPC-PAT	S>L	1.09 (0.75–1.59)	1.42 (0.94–2.17)	1.17 (0.92–1.50)
XPA-23G>A	G>A	1.05 (0.75–1.49)	1.31 (0.73–2.28)	1.09 (0.84–1.41)
ERCC1-19007T>C	T>C	0.86 (0.60–1.24)	0.97 (0.62–1.50)	0.93 (0.73–1.18)
XPD-L751Q	A>C	0.89 (0.62–1.29)	0.99 (0.62–1.57)	0.94 (0.73–1.21)
XRCC3-T241M	C>T	1.03 (0.72–1.47)	1.20 (0.76–1.89)	1.06 (0.83–1.36)
MGMT-L84F	C>T	1.15 (0.78–1.69)	0.84 (0.21–3.33)	1.10 (0.78–1.57)

[a]From a multivariate logistic analysis including terms for age, sex, area of residence, *H.pylori* seropositivity and GC family history.

Table V. Estimated ORs and 95% CI* associated with the genotype combination of the four significant two-way interactions

Two-way interaction		Cases *N* (%)	Controls *N* (%)	OR (95% CI)
APE1-D148E	*XPA*-23G>A			
T/T	G/*	82 (28.9)	175 (33.5)	
T/T	A/A	20 (7.0)	22 (4.2)	3.56 (1.53–8.25)
G/*	G/*	167 (58.8)	289 (55.3)	1.20 (0.81–1.78)
G/*	A/A	15 (5.3)	37 (7.0)	0.71 (0.32–1.59)
APE1-D148E	*GSTT1*			
T/T	Not null	65 (22.4)	176 (27.2)	
T/T	Null	33 (11.4)	32 (5.0)	4.90 (2.38–10.11)
G/*	Not null	148 (51.0)	279 (58.7)	1.43 (0.95–2.16)
G/*	Null	44 (15.2)	59 (9.1)	1.84 (1.04–3.28)
APE1-D148E	*GSTM1-GSTT1*			
T/T	Other	74 (25.5)	194 (35.5)	
T/T	Double null	24 (8.3)	14 (2.6)	7.84 (3.19–19.22)
G/*	Other	166 (57.2)	310 (56.8)	1.32 (0.89–1.94)
G/*	Double null	26 (9.0)	28 (5.1)	2.32 (1.12–4.82)
XPC-PAT	*XPA*-23G>A			
S + LS	G/G	109 (38.7)	194 (37.1)	
S + LS	A/*	109 (38.7)	228 (43.6)	0.84 (0.56–1.24)
L (PAT^+)	G/G	24 (8.5)	55 (10.5)	0.77 (0.40–1.47)
L (PAT^+)	A/*	40 (14.1)	46 (8.8)	2.15 (1.17–3.93)

Asterisks indicate genotypes calculated with a multivariate logistic analysis including terms for age, sex and area of residence, *H.pylori* seropositivity and GC family history.

a population residing in a high-risk area in Central Italy (5,6). The selection of polymorphisms was guided by biological plausibility, previously reported association with cancer and, possibly, knowledge of function.

The major contributor to GC is the *H.pylori* infection that by inducing chronic inflammation produces ROS and nitrogen species that may damage the adjacent epithelial cells. BER is the pathway of election for repair of lesions arising by cellular metabolism. The BER variants analysed in this study are common polymorphisms in key players of this pathway such as the DNA glycosylase *OGG1*-S326C variant that has been associated with decreased 8-oxoguanine repair (32), the *APE1*-D148E polymorphism that slightly decrease the activity of the major eukaryotic AP endonuclease (33) and the polymorphic variants *XRCC1*-R194W and *XRCC1*-R399Q both located in conserved domains of this scaffolding protein that potentially affect repair complex assembly and efficiency (34). Variants of NER proteins such as XPC (*XPC*-PAT) and XPA (*XPA*-A23G), which are involved in the recognition of bulky adducts and contribute to removal of oxidative DNA lesions (35–37) were also included. Because of their high frequency and association with cancer risk the *XPD*-L571Q (38), the *XRCC3*-241Met (39), the *ERCC1*-19007T>C (40) and the *MGMT*-L84F (41) variants were analysed too. No main effect on GC risk of the analysed DNA repair polymorphisms was identified. Only a borderline association between *XPC*-$PAT^{+/+}$ genotype and increased GC risk emerged: subjects homozygous for the PAT^+ allele are at an almost 1.5-fold increased risk of developing GC. These subjects have been shown to present a diminished DNA repair capacity of ultraviolet damage by a host-cell reactivation assay (42). We confirm that the PAT^+ allele is in LD with the intron 11 C/A polymorphism that leads to a high frequency of exon 12 skipping (24). The borderline association that we report might indicate that a dysfunction in bulky adducts and/or oxidative damage repair leads to increased GC risk. It should be noted that this study that includes 314 cases and 574 controls, under multiplicative codominant models and setting an alpha = 0.05, had a power of 80% to observe an OR of 1.75 for an SNP with 5% allele frequency and an OR of 1.40 for a SNP with 20% allele frequency.

The lack of an association of GC risk with single DNA repair polymorphisms has been reported by other studies. For instance, a recent nested case–control study within the European Prospective Investigation into Cancer and nutrition cohort including 246 gastric adenocarcinomas and 1175 matched controls analysing 12 polymorphisms at five DNA repair genes (*MSH2*, *MLH1*, *XRCC1*, *OGG1* and *ERCC2*) failed to show significant association for these polymorphisms (20). On the other hand, positive relationships with GC risk have been reported for *XRCC1* polymorphisms in a Chinese (43) and in a Korean (11) population and for specific genotypes (*XPA*-23A>G, *XPC*-PAT and *XRCC2*-C41657T) (13,14) in Chinese populations.

It should be considered that the apparent inconsistency of these reports may underlie differences in ethnicity, lifestyle and disease prevalence as well as possible limitations due to the relatively small sample size. Even more importantly, the low-penetrance genetic effects of single polymorphisms may largely depend on interaction with other polymorphisms and/or a particular environmental exposure.

In this study gene–gene interactions analysis revealed significantly increased GC risk for carriers of either multiple DNA repair polymorphisms or combinations of DNA repair and *GST* genotypes. Although the combined genotypes were relatively small, individuals with the unfavourable *XPC*-$PAT^{+/+}$ and *XPA*-23A/A genotypes showed a significantly higher GC risk. Interestingly, the same combined genotype was shown to be associated with significantly higher anti-benzo[*a*]pyrene diol epoxide (BPDE)-DNA adduct levels in a study conducted on polycyclic aromatic hydrocarbons-exposed workers (44) and with increased BPDE sensitivity in a twin study (45). We have shown that mutations in *XPC* lead to accumulation of oxidative damage in human cells (36) and the 'A' allele of *XPA*-23G>A has been associated with higher levels of sites sensitive to formamidopyrimidine DNA glycosylase that reveals oxidative DNA damage in lymphocytes of carriers (37). Polymorphisms in both genes might impair the ability to search for bulky adducts and/or oxidative DNA damage, thus leading to accumulation of DNA lesions and increased cancer risk. When considering the identification of an interaction between two genes, the power of this study was 80% to observe an OR_{int} of 4.25 when both genes have a 5% allele frequency and an OR_{int} of 1.8 when both genes have 20% allele frequency.

An important issue to consider, when the contribution of genes to the aetiology of cancer is discussed, is that polymorphisms may affect disease susceptibility only in the presence of a relevant exposure. The occurrence of *H.pylori* infection among cases was too high (87% positive) to allow the identification of possible differences between *H.pylori*-positive and -negative subjects. In a previous study, we have shown that double null subjects lacking both GSTM1 and GSTT1 activity are at increased risk of GC (6). Our interpretation of this finding was that since GSTs are involved in prevention of cellular damage by free radical, their deletion might be associated with high level of *H.pylori*-induced ROS, hence with enhanced endogenous DNA damage and thus increased susceptibility to inflammation-related cancer, such as stomach cancer. Interestingly, the GSTM1-GSTT1 double null subjects seemed to be at increased risk in the *H.pylori*-positive versus

H.pylori-negative subgroup suggesting that, under inflammation, the antioxidant response may impact on cancer risk. A significant interaction of the *APE1*-wild-type genotype with either the single G*STT1* or double *GSTM1-GSTT1* null genotype or the *XPA*-mutant allele, with the *APE1* being protective for cancer susceptibility, was also found. A recent study reported the characterisation of a promoter polymorphism in *APE1/Ref-1* gene that contributed to reduced lung cancer susceptibility by reducing the transcription efficiency of this gene (46). So, there is the possibility that the high expression of DNA repair genes may contribute to cancer risk by a mechanism of resistance to apoptosis in response to DNA damage. Moreover, *H.pylori* infection is associated with increased *APE1* expression in human cell lines and in gastric tissues from subjects with gastritis and gastric adenomas (47), and *APE1* overexpression increases genomic instability (48). Functional assays of *APE1* variants including monitoring of gene expression should be conducted to check whether combinations of specific polymorphisms might lead to increased genomic instability under increased intracellular ROS levels

In conclusion, this study indicates that the combined analysis of multiple polymorphisms is more discriminating than the use of a single-locus genotype in identifying individuals with a higher GC risk. Larger studies are needed to better validate the risk associated with these rare genotypes that identify DNA repair and oxidative metabolism unfavourable alleles as potential contributors to GC risk.

Supplementary data

Supplementary Appendix A at *Mutagenesis* Online.

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References

1. Parkin, D. M., Bray, F., Ferlay, J. and Pisani, P. (2005) Global cancer statistics, 2002. *CA Cancer J. Clin.*, **55**, 74–108.
2. Gonzalez, C. A., Sala, N. and Capella, G. (2002) Genetic susceptibility and gastric cancer risk. *Int. J. Cancer*, **100**, 249–260.
3. Obst, B., Wagner, S., Sewing, K. F. and Beil, W. (2000) Helicobacter pylori causes DNA damage in gastric epithelial cells. *Carcinogenesis*, **21**, 1111–1115.
4. El-Omar, E. M., Carrington, M., Chow, W. H. *et al.* (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, **404**, 398–402.
5. Palli, D., Saieva, C., Luzzi, I. *et al.* (2005) Interleukin-1 gene polymorphisms and gastric cancer risk in a high-risk Italian population. *Am. J. Gastroenterol.*, **100**, 1941–1948.
6. Palli, D., Saieva, C., Gemma, S. *et al.* (2005) GSTT1 and GSTM1 gene polymorphisms and gastric cancer in a high-risk italian population. *Int. J. Cancer*, **115**, 284–289.
7. Correa, P., Malcom, G., Schmidt, B., Fontham, E., Ruiz, B., Bravo, J. C., Bravo, L. E., Zarama, G. and Realpe, J. L. (1998) Review article: antioxidant micronutrients and gastric cancer. *Aliment. Pharmacol. Ther.*, **12** (Suppl. 1), 73–82.
8. Jakszyn, P. and Gonzalez, C. A. (2006) Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. *World J. Gastroenterol.*, **12**, 4296–4303.
9. Goode, E. L., Ulrich, C. M. and Potter, J. D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1513–1530.
10. Shen, H., Xu, Y., Qian, Y., Yu, R., Qin, Y., Zhou, L., Wang, X., Spitz, M. R. and Wei, Q. (2000) Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. *Int. J. Cancer*, **88**, 601–606.
11. Lee, S. G., Kim, B., Choi, J., Kim, C., Lee, I. and Song, K. (2002) Genetic polymorphisms of XRCC1 and risk of gastric cancer. *Cancer Lett.*, **187**, 53–60.
12. Ratnasinghe, L. D., Abnet, C., Qiao, Y. L., Modali, R., Stolzenberg-Solomon, R., Dong, Z. W., Dawsey, S. M., Mark, S. D. and Taylor, P. R. (2004) Polymorphisms of XRCC1 and risk of esophageal and gastric cardia cancer. *Cancer Lett.*, **216**, 157–164.
13. Wang, N., Dong, X. J., Zhou, R. M., Guo, W., Zhang, X. J. and Li, Y. (2009) An investigation on the polymorphisms of two DNA repair genes and susceptibility to ESCC and GCA of high-incidence region in northern China. *Mol. Biol. Rep.*, **36**, 357–364.
14. Dong, Z., Guo, W., Zhou, R., Wan, L., Li, Y., Wang, N., Kuang, G. and Wang, S. (2008) Polymorphisms of the DNA repair gene XPA and XPC and its correlation with gastric cardiac adenocarcinoma in a high incidence population in North China. *J. Clin. Gastroenterol.*, **42**, 910–915.
15. Shen, H., Wang, X., Hu, Z., Zhang, Z., Xu, Y., Hu, X., Guo, J. and Wei, Q. (2004) Polymorphisms of DNA repair gene XRCC3 Thr241Met and risk of gastric cancer in a Chinese population. *Cancer Lett.*, **206**, 51–58.
16. Huang, W. Y., Chow, W. H., Rothman, N., Lissowska, J., Llaca, V., Yeager, M., Zatonski, W. and Hayes, R. B. (2005) Selected DNA repair polymorphisms and gastric cancer in Poland. *Carcinogenesis*, **26**, 1354–1359.
17. Duarte, M. C., Colombo, J., Rossit, A. R., Caetano, A., Borim, A. A., Wornrath, D. and Silva, A. E. (2005) Polymorphisms of DNA repair genes XRCC1 and XRCC3, interaction with environmental exposure and risk of chronic gastritis and gastric cancer. *World J. Gastroenterol.*, **11**, 6593–6600.
18. Ye, W., Kumar, R., Bacova, G., Lagergren, J., Hemminki, K. and Nyren, O. (2006) The XPD 751Gln allele is associated with an increased risk for esophageal adenocarcinoma: a population-based case-control study in Sweden. *Carcinogenesis*, **27**, 1835–1841.
19. Ruzzo, A., Canestrari, E., Maltese, P. *et al.* (2007) Polymorphisms in genes involved in DNA repair and metabolism of xenobiotics in individual susceptibility to sporadic diffuse gastric cancer. *Clin. Chem. Lab. Med.*, **45**, 822–828.
20. Capella, G., Pera, G., Sala, N. *et al.* (2008) DNA repair polymorphisms and the risk of stomach adenocarcinoma and severe chronic gastritis in the EPIC-EURGAST study. *Int. J. Epidemiol.*, **37**, 1316–1325.
21. Parkin, D. M., Whelan, S. L., Ferlay, J., Teppo, L. and Thomas, D. B. (2002) *Cancer Incidence in Five Continents Volume VIII*. IARC Scientific Publications n. 155, Lyon, France, 362–363.
22. Palli, D., Masala, G., Del Giudice, G. *et al.* (2007) CagA+ Helicobacter pylori infection and gastric cancer risk in the EPIC-EURGAST study. *Int. J. Cancer*, **120**, 859–867.
23. Khan, S. G., Metter, E. J., Tarone, R. E., Bohr, V. A., Grossman, L., Hedayati, M., Bale, S. J., Emmert, S. and Kraemer, K. H. (2000) A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. *Carcinogenesis*, **21**, 1821–1825.
24. Khan, S. G., Muniz-Medina, V., Shahlavi, T., Baker, C. C., Inui, H., Ueda, T., Emmert, S., Schneider, T. D. and Kraemer, K. H. (2002) The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. *Nucleic Acids Res.*, **30**, 3624–3631.
25. Abdel-Rahman, S. Z., el-Zein, R. A., Anwar, W. A. and Au, W. W. (1996) A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. *Cancer Lett.*, **107**, 229–233.
26. Balding, D. J. (2006) A tutorial on statistical methods for population association studies. *Nat. Rev. Genet.*, **7**, 781–791.

27. Zhao, J., Jin, L. and Xiong, M. (2006) Test for interaction between two unlinked loci. *Am. J. Hum. Genet.*, **79**, 831–845.
28. Storey, J. D. and Tibshirani, R. (2003) Statistical significance for genomewide studies. *Proc. Natl Acad. Sci. USA*, **100**, 9440–9445.
29. Shen, M., Hung, R. J., Brennan, P. *et al.* (2003) Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 1234–1240.
30. Marin, M. S., Lopez-Cima, M. F., Garcia-Castro, L., Pascual, T., Marron, M. G. and Tardon, A. (2004) Poly (AT) polymorphism in intron 11 of the XPC DNA repair gene enhances the risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1788–1793.
31. Garte, S., Gaspari, L., Alexandrie, A. K. *et al.* (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 1239–1248.
32. Bravard, A., Vacher, M., Moritz, E., Vaslin, L., Hall, J., Epe, B. and Radicella, J. P. (2009) Oxidation status of human OGG1-S326C polymorphic variant determines cellular DNA repair capacity. *Cancer Res.*, **69**, 3642–3649.
33. Tell, G., Quadrifoglio, F., Tiribelli, C. and Kelley, M. R. (2009) The many functions of APE1/Ref-1: not only a DNA repair enzyme. *Antioxid. Redox. Signal.*, **11**, 601–620.
34. Caldecott, K. W. (2003) XRCC1 and DNA strand break repair. *DNA Repair (Amst.)*, **2**, 955–969.
35. Shimizu, Y., Iwai, S., Hanaoka, F. and Sugasawa, K. (2003) Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.*, **22**, 164–173.
36. D'Errico, M., Parlanti, E., Teson, M. *et al.* (2006) New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J.*, **25**, 4305–4315.
37. Dusinska, M., Dzupinkova, Z., Wsolova, L., Harrington, V. and Collins, A. R. (2006) Possible involvement of XPA in repair of oxidative DNA damage deduced from analysis of damage, repair and genotype in a human population study. *Mutagenesis*, **21**, 205–211.
38. Benhamou, S. and Sarasin, A. (2002) ERCC2/XPD gene polymorphisms and cancer risk. *Mutagenesis*, **17**, 463–469.
39. Bishop, D. K., Ear, U., Bhattacharyya, A., Calderone, C., Beckett, M., Weichselbaum, R. R. and Shinohara, A. (1998) Xrcc3 is required for assembly of Rad51 complexes in vivo. *J. Biol. Chem.*, **273**, 21482–21488.
40. Zhou, W., Liu, G., Park, S., Wang, Z., Wain, J. C., Lynch, T. J., Su, L. and Christiani, D. C. (2005) Gene-smoking interaction associations for the ERCC1 polymorphisms in the risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 491–496.
41. Remington, M., Chtchetinin, J., Ancheta, K., Nghiemphu, P. L., Cloughesy, T. and Lai, A. (2009) The L84F polymorphic variant of human O6-methylguanine-DNA methyltransferase alters stability in U87MG glioma cells but not temozolomide sensitivity. *Neuro Oncol.*, **11**, 22–32.
42. Qiao, Y., Spitz, M. R., Guo, Z., Hadeyati, M., Grossman, L., Kraemer, K. H. and Wei, Q. (2002) Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. *Mutat. Res.*, **509**, 165–174.
43. Shen, H., Xu, Y., Qian, Y., Yu, R., Qin, Y., Zhou, L., Wang, X., Spitz, M. R. and Wei, Q. (2000) Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a chinese population (In Process Citation). *Int. J. Cancer*, **88**, 601–606.
44. Pavanello, S., Pulliero, A., Siwinska, E., Mielzynska, D. and Clonfero, E. (2005) Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B(a)PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. *Carcinogenesis*, **26**, 169–175.
45. Lin, J., Swan, G. E., Shields, P. G., Benowitz, N. L., Gu, J., Amos, C. I., de Andrade, M., Spitz, M. R. and Wu, X. (2007) Mutagen sensitivity and genetic variants in nucleotide excision repair pathway: genotype-phenotype correlation. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 2065–2071.
46. Lu, J., Zhang, S., Chen, D. *et al.* (2009) Functional characterization of a promoter polymorphism in APE1/Ref-1 that contributes to reduced lung cancer susceptibility. *FASEB J.*, **23**, 3459–3469.
47. Futagami, S., Hiratsuka, T., Shindo, T. *et al.* (2008) Expression of apurinic/apyrimidinic endonuclease-1 (APE-1) in H. pylori-associated gastritis, gastric adenoma, and gastric cancer. *Helicobacter*, **13**, 209–218.
48. Sossou, M., Flohr-Beckhaus, C., Schulz, I., Daboussi, F., Epe, B. and Radicella, J. P. (2005) APE1 overexpression in XRCC1-deficient cells complements the defective repair of oxidative single strand breaks but increases genomic instability. *Nucleic Acids Res.*, **33**, 298–306.