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# DNA repair polymorphisms and the risk of stomach adenocarcinoma and severe chronic gastritis in the EPIC-EURGAST study

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**Background** The contribution of genetic variation in DNA repair genes to gastric cancer (GC) risk remains essentially unknown. The aim of this study was to explore the relative contribution of DNA repair gene polymorphisms to GC risk and severe chronic atrophic gastritis (SCAG).

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**Method** A nested case control study within the EPIC cohort was performed including 246 gastric adenocarcinomas and 1175 matched controls. Controls with SCAG ($n = 91$), as defined by low pepsinogen A (PGA) levels, and controls with no SCAG ($n = 1061$) were also compared. Twelve polymorphisms at DNA repair genes (MSH2, MLH1, XRCC1, OGG1 and ERCC2) and TP53 gene were analysed. Antibodies against *Helicobacter pylori* were measured.

**Results** No association was observed for any of these polymorphisms with stomach cancer risk. However, ERCC2 K751Q polymorphism was associated with an increased risk for non-cardial neoplasm [odds ratio (OR) = 1.78; 95% confidence interval (CI) 1.02–3.12], being ERCC2 K751Q and D312N polymorphisms associated with the diffuse type. ERCC2 D312N (OR = 2.0; 95% CI 1.09–3.65) and K751Q alleles (OR = 1.82; 95% CI 1.01–3.30) and XRCC1 R399Q (OR = 1.69; 95% CI 1.02–2.79) allele were associated with an increased risk for SCAG.

**Conclusion** Our study supports a role of ERCC2 in non-cardial GC but not in cardial cancer. A concordant result was observed for subjects with low PGA levels. XRCC1 allele was associated also with SCAG. This is the first prospective study suggesting that individual variation in DNA repair may be relevant for gastric carcinogenesis, a finding that will require further confirmation validation in larger independent studies.

**Keywords** Gastric carcinoma, DNA repair, polymorphism, genetic susceptibility, *H. pylori*

## Introduction

A steady decline in the incidence of gastric cancer (GC) has been observed in most countries in the last decades. However, GC remains the second most common cause of cancer death in the world.[1] *Helicobacter pylori* (Hp) infection is an established risk factors of non-cardial GC.[2] Tobacco smoking is causally associated with GC[3] while dietary factors are thought to have an important role in gastric carcinogenesis.[4,5]

DNA damage is critical to carcinogenesis.[6] Exposure to carcinogens, undue DNA replication, loss of bases due to spontaneous disintegration of chemical bonds and exposure to endogenous reactants such as alkyl groups, metal cations and oxygen-reactive species, can induce mutations. When cells fail to adequately repair the acquired damage, carcinogenesis may occur. A wide spectrum of genetic and epigenetic alterations in oncogenes and tumour suppressor genes underlie gastric tumourigenesis. These aberrations are already detected in metaplasia occurring in severe chronic atrophic gastritis (SCAG), a putative predisposing factor for GC.[7–12]

Four important pathways in DNA repair have been described: (i) base excision repair (BER)—it repairs small chemical adducts (methylated or oxygenated bases), usually of endogenous origin; (ii) mismatch repair (MMR)—that repairs single base substitutions usually secondary to errors occuring during DNA replication; (iii) nucleotide excision repair (NER)—that removes more than one base in response to adducts, such as those of heterocyclic aromatic amines (HAA) or polycyclic aromatic hydrocarbons (PAH) and (iv) double-strand break repair (DSBR). P53 plays a key role in maintaining genomic stability by participating at the signal transduction pathway and is a major genome guardian molecule in response to DNA damage.[13]

Genetic variation in DNA repair genes has been postulated as an important contributor to the aetiology of GC.[14,15] However, there is scarce information regarding GC and DNA repair gene polymorphisms. Inconsistent results have been observed regarding XRCC1 194Arg and 399Gln alleles.[16,17] A negative association was observed for XRCC1 399Gln variant for cardial cancer,[18] while no association was found for XRCC1, XPD, MGMT and XRCC3 variants[19,20] and OGG1 variant alleles.[21–23]

The aim of this study is to describe the effect of genetic variation in DNA repair genes on the risk of GC in a nested case–control study conducted within a large cohort study: the European Prospective Investigation into Cancer and Nutrition (EPIC).[24] In this study, we have explored a series of polymorphisms in genes that are relevant to three DNA repair pathways: BER (XRCC1 and OGG1), NER (ERCC2)

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and MMR (MSH2 and MLH1). Also a common TP53 polymorphism was included. Genes and polymorphisms were selected according to prior data on functional effect or reports of association to malignancies, to increase the likelihood of positive findings.

# Materials and methods

## The EPIC study

The EPIC cohort consists of 521 457 subjects (368 010 women and 153 447 men), mostly aged 35–70 years, recruited between 1992 and 1998 in 23 centres, in 10 European countries: Denmark, France, Greece, Germany, Italy, The Netherlands, Norway, Spain, Sweden and the UK. Eligible subjects, usually from the general population residing in a given geographical area, were invited to participate in the study by mail or by personal contact. Those who accepted signed an informed consent form. Then questionnaires on diet and lifestyle, anthropometric measurements and blood samples were obtained.[24] Follow-up is based upon population cancer registries in most countries, except in France, Germany and Greece, where it is mainly achieved by active contact with study subjects and review of health insurance and pathology reports. In this study, the follow-up was complete until December 2000 or December 2001 for countries using cancer registry data and December 2002 for the remaining three countries. Participating subjects in the EPIC study are Caucasians.

## Nested case–control study

Only subjects having blood collected were considered. Prevalent GC cases ($n = 138$) and 2403 subjects lost for follow-up were excluded. The study subjects were selected from the EPIC cohort according to a nested case–control design and were used for genotyping as well as for the analysis of Hp antibodies and pepsinogens. Cases were all subjects newly diagnosed during the follow-up of cancer of the stomach, defined by code C16 of the International Statistical Classification of Diseases, 10th Revision (ICD-10). An independent panel of pathologists reviewed original slides and/or cuts from paraffin blocks as well as pathology reports provided by each EPIC centre, in order to confirm and validate the diagnosis, tumour site and morphology. Initially, 290 GC cases with available blood samples were identified; four cases of cancer located in gastric stump as well as 31 tumours other than adenocarcinoma were excluded. For each new incident case, up to four control subjects were randomly selected among cohort members alive and free of cancer at the time of diagnosis of the case, matched by centre, gender, age (±2.5 years) and date of blood collection (±45 days). Three cases without available controls with the matching conditions and six cases without information on the studied genes were excluded. Thus, the final population for GC risk assessment included 246 GC and 1175 matched controls (Table 1). The whole set of controls with genetic information was used to describe the genotype frequencies and to compute Hardy–Weinberg equilibrium (HWE) tests, linkage disequilibrium (LD) measures and to ascertain the presence of severe chronic atrophic gastritis (SCAG) by means of pepsinogen A (PGA) levels (Table 1). The proportion of males was 49% for non-cardial and 74% for cardial cases (and matched controls), while 65% of case–control sets were from Central and North Europe and 35% from Mediterranean countries participating in EPIC.

## Laboratory assays

### *DNA extraction*

Genomic DNA from patients and controls was extracted from a 0.5 ml aliquot of buffy coat, which had been kept frozen since blood extraction and processing. With the exception of Mälmo samples, all other DNAs were extracted at the IARC by use of the Puregene DNA Purification System adapted to the Gentra Autopure LS DNA preparation platform (Gentra Systems, Minneapolis, USA). DNA samples were pipetted to 96-well plates for DNA concentration measurement with PicoGreen dsDNA quantitation assay and kit (Molecular Probes, Inc, The Netherlands), drying and further distribution. DNA from the frozen buffy coat straws from Mälmo samples was extracted by the phenol–chlorophorm method, and also distributed dried in 96-well plates. Before use, dried DNAs were reconstituted with water to a final concentration of 20 ng/µl for the IARC samples and 2 ng/µl, for the Mälmo Samples, and kept frozen.

**Table 1** Description of the cases and controls participating in the EPIC-EURGAST study

| Characteristics | Cases | | Controls |
|---|---|---|---|
| No. of gastric adenocarcinoma | 255 | | |
| **Excluded** | | | |
| Cases without controls | 3 | | |
| Cases without genotyping information | 6 | | |
| Included in the analysis | 246 | | 1175 |
| Non-cardia | 128 | Pepsinogen <22 µG/l (SCAG) | 91 |
| Cardia (including GEJ) | 69 | Pepsinogen >22 µG/l | 1061 |
| Unspecified location | 49 | Pepsinogen NA | 23 |

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*Genotyping analysis*

Polymorphisms at DNA repair genes and at TP53 gene (Table 2) were analysed at the ICO Laboratory in a LightCycler™ instrument by melting curve analysis of a fluorescently labelled sensor probe specific for each analysed variant, following manufacturer instructions (Roche Diagnostics, Mannheim, Germany). Melting curve analysis of some polymorphisms (MSH2: IVS12-6T>C, ERCC2: K751Q, XRCC1: R399Q and R194W) was also performed in a LightTyper instrument (Roche Diagnostics), after amplification in a GeneAmp®PCR System 9700 (Applied Biosystems).

Specific primers and hybridization probes were designed by TIB-MOLBIOL (Berlin, Germany), or in-house by use of the LightTyper Probe Design software from Roche Diagnostics, according to the gene or cDNA sequences published in the GeneBank or EMBL data bases. All PCR primers as well as the 3′-fluorescein and the 5′LC-Red640 or 5′LC-Red705 probes were synthesized by TIB-MOLBIOL and can be provided by the authors upon request. A minimum of 10 test DNAs, different from the EurGast ones, were used to standardize all the Light Cycler genotyping protocols, which are available upon request. The results obtained were confirmed by a second genotyping method, such as restriction analysis, SSCP analysis or direct DNA sequencing of a new PCR product. As quality control, 10% of the samples were reanalysed using the same technique for all single nucleotide polymorphisms (SNPs). Concordance rate was 99.6% (100% for 8 SNPs, 99% for 3 and 98% for 1). Genes and polymorphisms have been named according to the HUGO Gene Nomenclature commitee (http://www.genomic.unimelb.edu.au; http://snp500cancer.nci.nih.gov/home.cfm). Polymorphisms have been identified according to the ID numbering of the dbSNP database of the NCBI (http://www.ncbi.nlm.nih.gov/SNP).

## Hp antibodies and PGA levels

Quantification of anti-Hp antibodies in plasma stored sample (0.5 ml straw) of all cases and controls included in the nested study was done by ELISA using the lysate of the Hp CCUG strain.[25] A cut-off value of 100 EU was defined using serum samples from individuals negative for Hp infection as determined by clinical, microbiological and serological assays (western blotting). Serum samples giving EU values above 100 were considered as positive for anti-Hp IgG antibodies. In previous experiments, this assay exhibited specificity and sensitivity >90%. The observed prevalence of Hp seropositivity was 83.7% among cases and 68.7% among controls.[25] Serum PGA was also measured as biomarkers of severe chronic atrophic gastritis using a RIA procedure (Sorin, Saluggia, Italy). SCAG was defined as PGA levels lower than 22 μg/l.[27]

## Statistical methods

Each polymorphism was tested in controls to ensure the fitting with HWE. Pair-wise LD for polymorphisms within the same gene was measured using $r^2$. To test the hypothesis of association between genetic polymorphisms and cardial or non-cardial GC, multivariate methods based on multiple conditional logistic regression analyses[26] were used after adjusting for Hp infection, education, weight, height, physical activity at work and leisure time, tobacco smoking status, number of cigarettes by day, intake of vegetables, fresh fruits, red and processed meat and energy.

Analyses were performed initially under a co-dominant inheritance model (three genotypes separated, results not shown). Then, simplified models were chosen: a dominant model—heterozygotes grouped with the homozygotes for the minor allele when both genotypes had a similar effect—or a recessive model—heterozygotes grouped with the homozygotes for the major allele when both genotypes had a similar effect. Reference genotype was defined as the homozygous more prevalent allele (wild-type) in dominant models and as the homozygous wild-type combined with the heterozygous genotype, when recessive models were considered. The remaining genotypes were classified as variant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the variant compared to the wild-type genotype group that was set as the reference category. Comparisons between controls with and without SCAG were made using unconditional logistic regression, adjusting for the same set of variables used in the cardial and non-cardial models and by sex, centre, age and date of blood extraction.

# Results

## Genotype frequencies

The frequencies of the genotypes for each polymorphism studied are shown in Table 2. These frequencies are in agreement with a previous study of the EPIC cohort.[28] All polymorphisms were in HWE among controls with the exception of XRCC1 R194W. This led us to exclude this variant from any further analyses. Polymorphisms within ERCC2 and MLH1 genes were in some LD ($r^2 = 0.44$ for ERCC2 and between 0.51 and 0.76 for the three MLH1 variants when considered as pairs).

## Association with GC

None of the analysed polymorphisms was associated with overall GC risk (Table 3). ERCC2 K751Q polymorphism increased the risk for non-cardial neoplasm (OR = 1.78; 95% CI 1.02–3.12) following a recessive model (Table 3). On the other hand, no association was observed when cardial carcinomas were considered. The presence of R72P TP53 polymorphism did not associate with an increased risk of

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**Table 2** Frequency of DNA repair polymorphisms in cardial and non-cardial adenocarcinoma and controls with and without SCAG in the EPIC-EURGAST study

| | | | GC cases | | | | | | Controls | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stomach | | Cardia | | Non-cardia | | No SCAG | | SCAG | | Total | |
| Gene | Polymorphism | Genotype | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| MSH2 | IVS1 + 9C > G | Total | 243 | | 68 | | 127 | | 1049 | | 90 | | 1162 | |
| | rs2303426 | CC | 95 | 39.1 | 23 | 33.8 | 55 | 43.3 | 387 | 36.9 | 38 | 42.2 | 434 | 37.3 |
| | | CG | 112 | 46.1 | 34 | 50.0 | 55 | 43.3 | 510 | 48.6 | 37 | 41.1 | 558 | 48.0 |
| | | GG | 36 | 14.8 | 11 | 16.2 | 17 | 13.4 | 152 | 14.5 | 15 | 16.7 | 170 | 14.6 |
| MSH2 | IVS12-6T > C (2006-6T > C) | Total | 242 | | 69 | | 125 | | 1059 | | 91 | | 1173 | |
| | rs2303428 | TT | 200 | 82.6 | 58 | 84.1 | 105 | 84.0 | 865 | 81.7 | 76 | 83.5 | 959 | 81.8 |
| | | TC | 40 | 16.5 | 11 | 15.9 | 18 | 14.4 | 188 | 17.8 | 14 | 15.4 | 205 | 17.5 |
| | | CC | 2 | 0.8 | 0 | 0.0 | 2 | 1.6 | 6 | 0.6 | 1 | 1.1 | 9 | 0.8 |
| MLH1 | IVS5 + 79A > G (453 + 79A > G) | Total | 245 | | 69 | | 128 | | 1053 | | 91 | | 1166 | |
| | rs4234259 | AA | 66 | 26.9 | 19 | 27.5 | 35 | 27.3 | 296 | 28.1 | 32 | 35.2 | 336 | 28.8 |
| | | AG | 128 | 52.2 | 37 | 53.6 | 69 | 53.9 | 523 | 49.7 | 41 | 45.1 | 573 | 49.1 |
| | | GG | 51 | 20.8 | 13 | 18.8 | 24 | 18.8 | 234 | 22.2 | 18 | 19.8 | 257 | 22.0 |
| MLH1 | I219V (Ex8-23A > G, 655A > G) | Total | 244 | | 69 | | 127 | | 1049 | | 89 | | 1161 | |
| | rs1799977 | AA | 102 | 41.8 | 32 | 46.4 | 54 | 42.5 | 472 | 45.0 | 38 | 42.7 | 520 | 44.8 |
| | | AG | 122 | 50.0 | 31 | 44.9 | 65 | 51.2 | 445 | 42.4 | 41 | 46.1 | 495 | 42.6 |
| | | GG | 20 | 8.2 | 6 | 8.7 | 8 | 6.3 | 132 | 12.6 | 10 | 11.2 | 146 | 12.6 |
| MLH1 | IVS14-19A > G (1668-19A > G) | Total | 245 | | 69 | | 128 | | 1057 | | 90 | | 1170 | |
| | rs9876116 | AA | 63 | 25.7 | 19 | 27.5 | 34 | 26.6 | 305 | 28.9 | 30 | 33.3 | 343 | 29.3 |
| | | AG | 131 | 53.5 | 40 | 58.0 | 68 | 53.1 | 527 | 49.9 | 42 | 46.7 | 579 | 49.5 |
| | | GG | 51 | 20.8 | 10 | 14.5 | 26 | 20.3 | 225 | 21.3 | 18 | 20.0 | 248 | 21.2 |
| XRCC1 | R399Q (Ex10-4G > A) | Total | 245 | | 69 | | 128 | | 1059 | | 91 | | 1173 | |
| | rs25487 | GG | 100 | 40.8 | 21 | 30.4 | 58 | 45.3 | 433 | 40.9 | 30 | 33.0 | 473 | 40.3 |
| | | GA | 114 | 46.5 | 39 | 56.5 | 54 | 42.2 | 491 | 46.4 | 45 | 49.5 | 545 | 46.5 |
| | | AA | 31 | 12.7 | 9 | 13.0 | 16 | 12.5 | 135 | 12.7 | 16 | 17.6 | 155 | 13.2 |
| XRCC1 | R194W (Ex6-22C > T) | Total | 245 | | 69 | | 128 | | 1061 | | 91 | | 1175 | |
| | rs1799782 | CC | 224 | 91.4 | 63 | 91.3 | 116 | 90.6 | 938 | 88.4 | 80 | 87.9 | 1039 | 88.4 |
| | | CT | 20 | 8.2 | 6 | 8.7 | 11 | 8.6 | 114 | 10.7 | 10 | 11.0 | 126 | 10.7 |
| | | TT | 1 | 0.4 | 0 | 0.0 | 1 | 0.8 | 9 | 0.8 | 1 | 1.1 | 10 | 0.9 |
| XRCC1 | L190L (Ex6-32C > G) | Total | 245 | | 69 | | 128 | | 1061 | | 91 | | 1175 | |
| | rs2307170 | CC | 242 | 98.8 | 68 | 98.6 | 127 | 99.2 | 1039 | 97.9 | 88 | 96.7 | 1150 | 97.9 |
| | | CG | 3 | 1.2 | 1 | 1.4 | 1 | 0.8 | 22 | 2.1 | 3 | 3.3 | 25 | 2.1 |
| | | GG | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| OGG1 | S326C (Ex6-315C > G) | Total | 243 | | 68 | | 127 | | 1026 | | 89 | | 1138 | |
| | rs1052133 | CC | 156 | 64.2 | 44 | 64.7 | 79 | 62.2 | 621 | 60.5 | 57 | 64.0 | 688 | 60.5 |
| | | CG | 76 | 31.3 | 20 | 29.4 | 41 | 32.3 | 352 | 34.3 | 27 | 30.3 | 391 | 34.4 |
| | | GG | 11 | 4.5 | 4 | 5.9 | 7 | 5.5 | 53 | 5.2 | 5 | 5.6 | 59 | 5.2 |
| ERCC2 | K751Q (Ex23 + 61A > C) | Total | 245 | | 69 | | 128 | | 1058 | | 91 | | 1172 | |
| | rs13181 | AA | 99 | 40.4 | 36 | 52.2 | 48 | 37.5 | 407 | 38.5 | 33 | 36.3 | 447 | 38.1 |
| | | AC | 105 | 42.9 | 25 | 36.2 | 54 | 42.2 | 504 | 47.6 | 40 | 44.0 | 555 | 47.4 |
| | | CC | 41 | 16.7 | 8 | 11.6 | 26 | 20.3 | 147 | 13.9 | 18 | 19.8 | 170 | 14.5 |
| ERCC2 | D312N (Ex10-16G > A) | Total | 244 | | 69 | | 127 | | 1028 | | 85 | | 1135 | |
| | rs1799793 | GG | 110 | 45.1 | 36 | 52.2 | 53 | 41.7 | 415 | 40.4 | 24 | 28.2 | 444 | 39.1 |
| | | GA | 96 | 39.3 | 26 | 37.7 | 51 | 40.2 | 476 | 46.3 | 43 | 50.6 | 532 | 46.9 |
| | | AA | 38 | 15.6 | 7 | 10.1 | 23 | 18.1 | 137 | 13.3 | 18 | 21.2 | 159 | 14.0 |
| TP53 | R72P (Ex4 + 119G > C) | Total | 245 | | 69 | | 128 | | 1056 | | 90 | | 1168 | |
| | rs1042522 | GG | 124 | 50.6 | 38 | 55.1 | 60 | 46.9 | 588 | 55.7 | 50 | 55.6 | 650 | 55.7 |
| | | GC | 109 | 44.5 | 29 | 42.0 | 59 | 46.1 | 399 | 37.8 | 36 | 40.0 | 444 | 38.0 |
| | | CC | 12 | 4.9 | 2 | 2.9 | 9 | 7.0 | 69 | 6.5 | 4 | 4.4 | 74 | 6.3 |

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non-cardial carcinoma and cardial carcinoma under a dominant model.

### Association with SCAG

Both ERCC2 alleles showed an increased risk for SCAG (D312N OR = 2.00 95% CI 1.09–3.65; K751Q OR = 1.82 95% CI 1.01–3.30) (Table 3). XRCC1 R399Q variant was also associated with an increased risk for SCAG (OR = 1.69 95% CI 1.02–2.79). No association was observed between R72P TP53 polymorphisms and the risk of SCAG.

### Association with histological type of GC

For non-cardial cases, we tested the association of ERCC2 and TP53 polymorphisms with the intestinal and diffuse type. The K751Q (OR = 3.55; 95% CI 1.24–10.2) and D312N (OR = 6.43; 95% CI 1.65–25.0) ERCC2 variants were associated with higher risk of carcinomas of the diffuse type, although tests for heterogeneity regarding intestinal type were $P = 0.17$ and $P = 0.10$, respectively (Table 4). No differences were observed regarding TP53 R72P polymorphism and histological type (Table 4). The remaining variants analysed did not show any association with the histological type. The small sample size precluded this type of analysis in cardial neoplasms. Finally, we tested if the association between genetic variant and GC and SCAG risk was modified by Hp status and no significant interaction was observed (data not shown).

## Discussion

In recent years, much attention has been paid to the potential role of variations in DNA repair capability and cancer risk.[29] However, a low number of studies have addressed the role of GC risk and DNA repair individual variability. This is the first nested case–control study within a cohort attempting to evaluate the association between individual susceptibility in DNA repair and GC risk in Western countries. The study is based on a relatively large, of confirmed adenocarcinoma cases, validated by a panel of pathologists. The prospective design has allowed an adequate control of putative genotype selection due to diseases as well as tumour burden, a potential confounder when evaluating DNA repair.

None of the analysed polymorphisms was associated with overall GC risk. However, K751Q ERCC2 variant has been shown to modestly increase non-cardial GC risk while no effect was observed in cardial neoplasms. Both ERCC2 variants were also associated with an increased risk of diffuse type non-cardial cancer and with SCAG, further suggesting that this association may be biologically relevant. ERCC2/XPD is a component of the core transcription factor IIH, a key player of the NER pathway involved in the removal of bulky DNA lesions such as those of HAA and PAH.[6]

The two variants analysed of ERCC2 result in amino acid changes that may impair DNA repair efficiency,[30,31] although their functional relevance has been recently challenged.[32] These common polymorphisms have been associated with cancer of the bladder,[33] lung[34] and prostate,[35] although results are conflicting.[32,36] A number of genetic aberrations including K-*ras*[9] and *TP53*[7] mutations, microsatellite instability[10] or mutations in genes involved in genomic instability maintenance such as polymerase β[37] have been reported in metaplasia occurring in atrophic gastritis. Thus, it is plausible to suggest that DNA repair impairment may enhance cancer risk by increasing the probability of acquiring mutations early during tumour progression. Nonetheless, the association with ERCC2 variants is intriguing. It is well known that HA and PAH are formed by cooking meat at high temperature.[38] While no study has specifically addressed the association between HA and PAH exposure and GC risk, we have previously observed that red and processed meat intake increases the risk of non-cardial cancer.[5]

The p53, a known tumour suppressor protein, plays a key role in DNA damage sensing and TP53 alterations are present in a high proportion of GC.[39] The TP53 polymorphism most frequently studied has been R72P, which was found to affect protein degradation by the E6 oncoprotein following human papilloma virus infection.[40] We did not observe an association of this polymorphism and the risk of non-cardial carcinoma and cardial carcinoma or SCAG. Another study has addressed this issue in Caucasian population also with negative results.[41] On the other hand, association of this variant with non-cardial carcinomas has been reported in Mexican[42] and Asian[43] populations but not in another Asian study.[44]

We did not confirm the reported association between GC risk and XRCC1 variants that may impair the ability to repair nitrosamine-induced DNA adducts also associated with red and processed meat intake. In a Chinese population,[16] an association was observed between XRCC1 variants[30,45,46] and GC risk that was mainly restricted to cardial cancer. Other study reported an association of an XRCC1 haplotype, including the 194, 280 and 399 variants, with distal gastric carcinoma.[17] However, in our study the R399Q variant associated with an increase in SCAG.

*OGG1* S326G variant has been shown to decrease the ability to repair 8-oxoguanine typical of oxidative damage, which has been associated with chronic Hp infection.[47] This variant affecting BER has been previously associated with increased risk of lung[48] and oesophageal[49] cancer. In line with previous studies in Asian[21–23] and Brazilian populations,[22] we have observed no differences in GC risk. The association observed by Tsukino with chronic gastritis[21] was not replicated in our study.

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**Table 3** DNA repair polymorphisms and OR of cardia and non-cardial adenocarcinoma and SCAG in the EPIC-EURGAST study

| | | | **Stomach**[a] | | | | | | **Cardia**[b] | | | | | | **Non-Cardia**[a] | | | | | | **SCAG (PGA < 22)**[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **Polymorphism** | **Model** | Effect | Ref | OR | CI 95% | | *P*-value | Effect | Ref | OR | CI 95% | | *P*-value | Effect | Ref | OR | CI 95% | | *P*-value | Effect | Ref | OR | CI 95% | | *P*-value |
| MSH2 | IVS1 + 9C > G | Dominant | 148 | 95 | 0.95 | 0.70 | 1.30 | 0.75 | 45 | 23 | 1.48 | 0.77 | 2.85 | 0.24 | 72 | 53 | 0.83 | 0.53 | 1.30 | 0.40 | 51 | 37 | 0.84 | 0.52 | 1.35 | 0,47 |
| MSH2 | IVS12-6T > C (2006-6T > C) | Dominant | 42 | 200 | 1.08 | 0.73 | 1.61 | 0.70 | 11 | 58 | 1.05 | 0.48 | 2.31 | 0.90 | 20 | 103 | 1.07 | 0.57 | 2.00 | 0.83 | 15 | 74 | 1.09 | 0.58 | 2.03 | 0.79 |
| MLH1 | IVS5 + 79A > G (453 + 79A > G) | Dominant | 179 | 66 | 1.12 | 0.81 | 1.56 | 0.50 | 50 | 19 | 0.92 | 0.47 | 1.81 | 0.81 | 92 | 34 | 1.27 | 0.78 | 2.07 | 0.35 | 59 | 30 | 0.82 | 0.50 | 1.34 | 0.43 |
| MLH1 | I219V (Ex8-23A > G, (655A > G) | Dominant | 142 | 102 | 1.18 | 0.87 | 1.60 | 0.28 | 37 | 32 | 0.98 | 0.53 | 1.79 | 0.94 | 72 | 53 | 1.30 | 0.83 | 2.03 | 0.25 | 51 | 36 | 1.11 | 0.69 | 1.80 | 0.66 |
| MLH1 | IVS14-19A > G (1668-19A > G) | Dominant | 182 | 63 | 1.19 | 0.85 | 1.67 | 0.31 | 50 | 19 | 0.88 | 0.44 | 1.77 | 0.73 | 93 | 33 | 1.34 | 0.82 | 2.19 | 0.25 | 60 | 28 | 0.87 | 0.52 | 1.44 | 0.58 |
| XRCC1 | R399Q (Ex10-4G > A) | Dominant | 145 | 100 | 1.00 | 0.74 | 1.35 | 0.97 | 48 | 21 | 1.36 | 0.70 | 2.61 | 0.36 | 68 | 58 | 0.67 | 0.43 | 1.05 | 0.08 | 61 | 28 | **1.69** | **1.02** | **2.79** | **0.04** |
| XRCC1 | L190L (Ex6-32C > G) | Dominant | 3 | 242 | 0.75 | 0.21 | 2.65 | 0.65 | 1 | 68 | 0.69 | 0.07 | 6.84 | 0.75 | 1 | 125 | 0.36 | 0.04 | 3.34 | 0.37 | 3 | 86 | 1.92 | 0.51 | 7.20 | 0.33 |
| OGG1 | S326C (Ex6-315C > G) | Dominant | 87 | 156 | 0.88 | 0.65 | 1.19 | 0.41 | 24 | 44 | 0.94 | 0.50 | 1.77 | 0.85 | 46 | 79 | 0.86 | 0.54 | 1.37 | 0.53 | 32 | 55 | 0.81 | 0.49 | 1.32 | 0.40 |
| ERCC2 | K751Q (Ex23 + 61A > C) | Recessive | 41 | 204 | 1.35 | 0.90 | 2.02 | 0.15 | 8 | 61 | 0.82 | 0.31 | 2.16 | 0.68 | 25 | 101 | **1.78** | **1.02** | **3.12** | **0.04** | 18 | 71 | **1.82** | **1.01** | **3.30** | **0.05** |
| ERCC2 | D312N (Ex10-16G > A) | Recessive | 38 | 206 | 1.19 | 0.78 | 1.82 | 0.43 | 7 | 62 | 0.93 | 0.34 | 2.56 | 0.89 | 22 | 103 | 1.40 | 0.76 | 2.60 | 0.28 | 18 | 65 | **2.00** | **1.09** | **3.65** | **0.03** |
| TP53 | R72P (Ex4 + 119G > C) | Dominant | 121 | 124 | 1.25 | 0.92 | 1.70 | 0.15 | 31 | 38 | 1.46 | 0.78 | 2.72 | 0.24 | 67 | 59 | 1.46 | 0.90 | 2.35 | 0.12 | 39 | 49 | 1.07 | 0.67 | 1.70 | 0.79 |

Effect: number of cases in the effect category.
Ref: number of cases in the reference category.
[a]Conditional logistic regression, adjusted by Hp infection, education, weight, height, physical activity at work and leisure time, tobacco smoking, number of cigarettes, intake of vegetables, fresh fruits, red and processed meat and energy. Matched by sex, centre, age and date of blood extraction.
[b]Unconditional logistic regression. Also adjusted by sex, centre, age and date of blood extraction.

**Table 4** Risk of diffuse and intestinal non-cardial adenocarcinoma regarding ERCC2 and p53 variants in the EPIC-EURGAST study

| | | | | | Non-cardial[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Diffuse ($n=53$ cases) | | | | Intestinal ($n=47$ cases) | | | | |
| Gene | Polymorphism | Model | Effect | Reference | OR | CI 95% | | $P$-value | OR | CI 95% | | $P$-value | $P^{\#}$ |
| ERCC2 | K751Q (Ex23+61A>C) | Recessive | CC | AA-AC | 3.55 | 1.24 | 10.2 | 0.02 | 1.59 | 0.61 | 4.12 | 0.34 | 0.17 |
| ERCC2 | D312N (Ex10-16G>A) | Recessive | AA | GG-GA | 6.43 | 1.65 | 25.0 | 0.01 | 0.76 | 0.23 | 2.47 | 0.65 | 0.10 |
| TP53 | R72P (Ex4+119G>C) | Dominant | GC-CC | GG | 1.42 | 0.60 | 3.36 | 0.43 | 1.75 | 0.72 | 4.25 | 0.21 | 0.37 |

[a]No possible comparisons in cardia due to the small number of diffuse cases.
Matched conditional regression, adjusted by Hp infection, education, weight, height, physical activity at work and leisure time, tobacco smoking, number of cigarettes, intake of vegetables, fresh fruits, red and processed meat and energy.
[#]$P$-value for a Wald statistic test for heterogeneity.

No previous studies have addressed the relative contribution of MMR gene variants to GC risk. MSI, a symptom of defective MMR repair, is a frequent event occuring in 14–47% of sporadic gastric tumours, associated with specific tumour features.[50,51] None of the variants analysed in the MSH2 and MLH1 genes has been associated with an increased GC risk. This observation included the MSH2 IVS12-6T>C variant previously suggested to affect exon 13 splicing and associated with colorectal cancer risk.[19,52–54] We have observed no associations with cardial GC and DNA repair polymorphism. Cardial cancer may be associated to distinct risk factors as shown by its increasing incidence in developed countries[55] and the lack of association with Hp infection.[56]

We are aware that this study has several limitations. Although its statistical power for GC analysis remains among the highest reported so far (80% power at the 5% significance level to detect main effects of genotypes with a frequency between 5% and 10% in controls for an OR of 1.5) the number of cases is low for the analysis of gene–environmental interaction.[57] For these reasons, our main conclusions are based upon the main effects of each SNP analysed in the whole set. Also, since many tests were performed some false positive results may be expected. However, it must be considered that all the genes and polymorphisms analysed were included because there was *a priori* hypothesis about its potential relationship with the disease. Thus, each test could be considered, to some extent, independent. For this reason, we decided not to apply any correction for multiple testing and to give more value to concordant observations in GC and SCAG risk. The limited number of polymorphisms per gene analysed has precluded haplotype analyses, which may have been more informative. Nonetheless, those SNPs most likely to carry information within these genes have been included. Finally, the identification of cases of SCAG was based on serum pepsinogen I levels that have previously shown a high sensitivity (89.5%) and specificity (91.5%) for screening of SCAG in the general population[58] and used to identify SCAG in the frame of epidemiological projects.[26]

The 5-year survival rate of GC is very low and the identification and better control of risk factors represent the most effective way for reducing the burden of these tumours. Results presented here point to a role of variants in ERCC2 in distal GC but not in cardial cancer that support the hypothesis of some different etiological pathways between both tumours. Regarding this polymosphism, there is a concordant result between distal tumours and the group with low PGA level (SCAG). It seems that XRCC1 variants are associated with low PGA level in Western population. This is the first prospective study showing that individual variations in DNA repair may be relevant to GC risk, a finding that will require further validation in larger independent studies before definitive conclusions can be drawn.

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**KEY MESSAGES**

- The contribution of genetic variation in DNA repair genes to GC risk remains essentially unknown.
- The EPIC is a large cohort study of over 500 000 people in 10 European countries devised to investigate the relationship between diet, metabolic and genetic factors, and cancer.
- We have explored a series of polymorphisms in genes that are relevant to three DNA repair pathways: BER (XRCC1 and OGG1), NER (ERCC2) and MMR (MSH2 and MLH1).

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