*BRIEF ARTICLE*

# Association between *EGF* +61A/G polymorphism and gastric cancer in Caucasians

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## Abstract

**AIM:** To investigate the association between epidermal growth factor (*EGF*) +61A/G polymorphism and susceptibility to gastric cancer, through a cross-sectional study.

**METHODS:** Polymerase chain reaction resctriction fragment lenght polymorphism analyses were used to genotype *EGF* +61 in 207 patients with gastric lesions (162 patients with gastric adenocarcinomas, 45 with atrophy or intestinal metaplasia) and 984 controls. All subjects were Caucasian.

**RESULTS:** Genotype distribution was 23.5% for GG and 76.5% for GA/AA in the control group, 18.4% for GG and 68.6% for GA/AA in the entire group with gastric lesions and 17.9% for GG and 82.1% for GA/AA in the group with gastric adenocarcinoma. No statistically significant associations were found between *EGF* +61 variants and risk for developing gastric cancer [odds ratios (OR) = 1.41, 95% confidence intervals (CI): 0.90-2.21, $P$ = 0.116]. However, the stratification of individuals by gender revealed that males carrying A alleles (*EGF* +61A/G or AA) had an increased risk for developing gastric cancer as compared to GG homozygous males (OR = 1.55, 95% CI: 1.05-2.28, $P$ = 0.021).

**CONCLUSION:** In summary, we found that males who were A carriers for *EGF* +61 had an increased risk for developing gastric cancer. This result may be explained by the suggestion that women secrete less gastric acid than men.

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## INTRODUCTION

Growth factors activate the complex processes of cellular signalling, promoting cell changes[1,2]. They are "positive signals" in the cell and are regulated by "negative signals" which control amplitude and duration[2]. The balance between these signals is all-important in cell homeostasis[2]. Epidermal growth factor (EGF) performs a key role in promoting cell survival[3]. After binding to its receptor (EGFR), it induces a signalling cascade that culminates in change of gene transcription[3-5]. EGFR signalling is not only important in cellular proliferation but also contributes to several other cellular processes involved in cancer progression, including angiogenesis, metastatic spread, and inhibition of apoptosis[6].

EGFR (also known as ErbB1) belongs to the family of ErbB (from avian erythroblastic leukaemia viral oncogene homologue) receptors which are involved in the development of several human cancers[7]. The increase in EGFR signalling may be caused by overexpression of EGFR, increased concentration of ligand(s) (through autocrine/paracrine processes), the presence of aberrant receptors due to gene alteration, and alterations in molecules that control receptor signalling output[6,8]. EGFR and ErbB-2 are frequently overexpressed in gastric carcinomas[9].

*EGF* gene has a polymorphism in position +61 which consists of the substitution of adenine (A) for guanine (G). AA genotype carriers have lower levels of EGF expression than individuals with the GG or AG genotypes[10]. Ethnic differences in the distribution of the *EGF* gene have been reported, and several studies have been carried out regarding the role of *EGF* genotypes in susceptibility to gastric cancer in Asian populations and in other organs.

In this cross-sectional study we analysed the association between this *EGF* polymorphism and the risk for gastric cancer in a high incidence Caucasian population.

## MATERIALS AND METHODS

### Subjects

This cross-sectional study was performed in 1191 individuals, including 207 patients histologically diagnosed with gastric lesions followed at the Portuguese Institute of Oncology-Porto (IPO-P), and a control group of 984 individuals without cancer disease history, all from the northern region of Portugal. All individuals provided informed consent according to the Declaration of Helsinki, and the patients in both groups were of Caucasian ethnicity.

Patients were further divided according to the type of lesions at histopathological diagnosis following multiple endoscopic biopsies. Patients included those who displayed lesions such as high-grade dysplasia and intestinal-type invasive gastric adenocarcinoma (*n* = 162) and patients with non-dysplastic lesions associated with gastric

adenocarcinoma such as atrophy or intestinal metaplasia (*n* = 45), who had received standardized follow-up since 2001. The group of patients with gastric adenocarcinoma included 73 females and 89 males (55%) with a median age at diagnosis of 54 years (mean 54.3 years, standard deviation 11.8 years), and the group of patients with atrophy or intestinal metaplasia included 25 females and 20 males (44%) with a median age at diagnosis of 59 years (mean 59.7 years, standard deviation 10.8 years).

The control subjects included 524 females and 460 males (46.7%) randomly recruited from the Blood Donor Bank of IPO-P and Hospital de S. Marcos, Braga, and had no current or history of neoplastic disease. The median age was 45 years (mean 46.2 years, standard deviation 11.1 years).

DNA was extracted from peripheral blood samples obtained by a standard venipuncture technique using EDTA-containing tubes, as previously described in studies from our group[11,12].

### EGF +61A/G genotype analysis

The +61A/G polymorphism was genotyped by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis, as previously described[10]. Briefly, amplification was carried out in a 50 μL reaction mixture containing: 1 × Taq Buffer, 1.5 mmol/L of $MgCl_2$, 0.2 mmol/L of dNTPs, 0.3 μmol/L of each primer and 1U Taq DNA polymerase. The cycling conditions were: 95℃ for 5 min, followed by 35 amplification cycles at 94℃ for 60 s, 55℃ for 60 s and 72℃ for 60 s, followed by one elongation step at 72℃ for 5 min. A 242 base pair (bp) fragment was amplified using primers: F-5′TGTCACTAAAGGAAAGGAGGT3′ and R-5′TTCACAGAGTTTAACAGCCC3′. The A61G variation was identified with the restriction enzyme *Alu* I. Two units of restriction enzyme were added to 10 μL of PCR products in a final volume of 15 μL. The incubation was performed at 37℃ overnight. The products were separated on 3% agarose gels with 0.5% ethidium bromide and photographed under UV illumination.

After destruction of the recognition site by the restriction enzyme, the A allele produced 4 fragments: 15, 34, 91 and 102 bp, while the G allele produced 3 fragments: 15, 34 and 193 bp. In the gel only fragments 91, 102 and 193 bp were visible.

### Statistical analysis

The computer software SPSS for Windows (version 15.0) and Epi Info (version 6.04) were used for all statistical analyses. The $\chi^2$ test was used to compare differences between categorical variables, and verify that the observed allele distribution in the control group was in Hardy-Weinberg equilibrium. A 5% level of significance was used in the analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the relationship between the polymorphic variants and gastric lesions.

## RESULTS

The frequencies of EGF genotypes in the gastric lesions

**Table 1  Associations between *EGF* +61A/G variants and clinicopathological parameters in patients with gastric lesions[1] *n* (%)**

| | Cases | Controls | OR (95% CI) | *P* |
|---|---|---|---|---|
| Gastric lesions[1] (*n* = 207) | | | | |
| Genotypes | | | | |
| GG | 38 (18.4) | 231 (23.5) | 1 | |
| GA | 104 (50.2) | 449 (45.6) | 1.41 (0.92-2.15) | 0.096 |
| AA | 65 (31.4) | 304 (30.9) | 1.30 (0.82-2.06) | 0.237 |
| GA + AA | 169 (68.6) | 753 (76.5) | 1.36 (0.92-2.04) | 0.109 |
| Atrophy or intestinal metaplasia (*n* = 45) | | | | |
| Genotypes | | | | |
| GG | 9 (20.0) | 231 (23.5) | 1 | |
| GA | 20 (44.4) | 449 (45.6) | 1.44 (0.49-2.76) | 0.743 |
| AA | 16 (35.6) | 304 (30.9) | 1.35 (0.55-3.37) | 0.478 |
| GA + AA | 36 (80.0) | 753 (76.5) | 1.23 (0.56-2.78) | 0.590 |
| Gastric adenocarcinoma (*n* = 162) | | | | |
| Genotypes | | | | |
| GG | 29 (17.9) | 231 (23.5) | 1 | |
| GA | 84 (51.9) | 449 (45.6) | 1.49 (0.93-2.40) | 0.082 |
| AA | 49 (30.2) | 304 (30.9) | 1.28 (0.77-2.16) | 0.317 |
| GA + AA | 133 (82.1) | 753 (76.5) | 1.41 (0.90-2.21) | 0.116 |

[1]Gastric lesions: atrophy or intestinal metaplasia and gastric adenocarcinoma. OR: Odds ratios; CI: Confidence intervals.

**Table 2  Associations between *EGF* +61A/G variants and gender**

| | | Controls | Cases | OR (95%CI) | *P* |
|---|---|---|---|---|---|
| GG | M | 117 | 14 | 1 | |
| | F | 114 | 15 | 1.04 (0.45-2.41) | 0.913 |
| AA/AG | M | 343 | 75 | 1 | |
| | F | 410 | 58 | 0.65 (0.44-0.95) | 0.021 |

OR: Odds ratios; CI: Confidence intervals.

group and the control group are presented in Table 1. The frequencies were 23.5% for GG and 76.5% for GA/AA in the control group, 18.4% for GG and 68.6% for GA/AA in the entire group with gastric lesions and 17.9% for GG and 82.1% for GA/AA in the group with gastric adenocarcinoma. As shown in Table 1, no increased risk of developing non-tumor gastric lesions (*P* = 0.109) or gastric adenocarcinoma (*P* = 0.116) was found for individuals who carried the A allele.

When adjustments were made for allele genotype and sex, we found that males who were A carriers had an increased risk of developing gastric cancer in comparison to females (OR = 1.55, 95% CI: 1.05-2.28, *P* = 0.021) (Table 2).

No other associations were found for the other characteristics tested.

## DISCUSSION

The EGF protein is a growth factor that activates signal transduction pathways promoting proliferation, migration and differentiation[13]. In particular, EGF is involved in regulating proliferation of mucosal cells in the gastrointestinal tract, stimulating mucus production, and inhibiting gastric secretion[14,15]. However, in gastric cancer, EGF displays oncogenic properties[16,17], and its overexpression is correlated with deep invasion, advanced malignancy stage, and poor patient prognosis[18].

In this cross-sectional study, we analysed the association between a functional polymorphism of the *EGF* gene (+61 A/G) and the risk for developing gastric cancer. Our data showed a statistically significant increased risk for developing gastric cancer in males who were A carriers (OR = 1.55, 95% CI: 1.05-2.28, *P* = 0.021); however, no statistically significant differences were found

when the entire cancer group was considered (male and female gastric adenocarcinoma patients, *P* = 0.116).

Salomon *et al*[9] reported that EGFR is overexpressed in 33% of gastric adenocarcinomas, compared to only 3.8% in the early stages of gastric carcinoma development or in non-malignant specimens. Nevertheless, EGFR expression was more frequent in well-differentiated advanced stage adenocarcinomas, and EGFR immunoreactivity was significantly higher in tumor stages III and IV as well as in metastatic carcinomas[9]. EGF and EGFR are expressed at a frequency of 42% and 41%, respectively, in poorly differentiated gastric carcinomas, and most frequently in tumors greater than 6 cm in size[9]. Although EGF and EGFR are associated with poor prognosis, less than half of gastric tumors have expression or overexpression of these proteins.

In our study, the most interesting result was the increased risk for gastric cancer in male patients. A sexual dimorphism in gastric acid secretion has been reported, with females secreting less gastric acid (approximately 40%) than males[19]. The mechanisms mediating this difference are unknown, but a role for oestrogens has been suggested which may inhibit gastric acid secretion through two oestrogen receptor (ER) subtypes present in the stomach[19]. EGF decreases FSH (follicle-stimulating hormone) and particularly inhibits the expression of hormones produced in the ovary (oestrogen and progesterone), acting in the evolution of ovarian follicles[20-26]. Therefore, in females with a lower expression of EGF (A carriers) we may consider that stomach cells may receive less information to proliferate. Nevertheless, in G carriers (with greater expression of EGF), the differences between genders are not significant, and according to our previous studies it is proposed that EGFR expression may be lower in G carriers, because EGF is involved in internalization of EGFR[27,28]. However, more work is required to elucidate the correct mechanism.

Others studies regarding the association between gastric cancer and *EGF* polymorphism in Asian populations have been reported[29-31]. Hamai *et al*[29] and Jin *et al*[30] associated A carriers with a lower risk of developing gastric cancer. However, Goto *et al*[31] did not find any differences between this polymorphism and gastric cancer. When analyzing the frequency of *EGF* +61 genotypes among these reports, one can observe that Asians present a significant difference in comparison to Caucasians (our study), namely in AA and GG genotypes (Table 3). In our results, the frequency of EGF genotypes in the control

**Table 3  Genotype distribution of *EGF* +61A/G polymorphism in different control populations reported in different case-control studies *n* (%)**

| | | Genotype | | |
|---|---|---|---|---|
| | *n* | AA | AG | GG |
| Asia | | | | |
| Jin *et al*[30] | 660 | 57 (8.6) | 289 (43.8) | 314 (47.6) |
| Goto *et al*[31] | 454 | 47 (10.4) | 188 (41.8) | 215 (47.8) |
| Hamai *et al*[29] | 230 | 25 (10.9) | 97 (42.1) | 108 (47.0) |
| Europe | | | | |
| Portugal (our data) | 984 | 304 (30.9) | 449 (45.6) | 231 (23.5) |
| Shahbazi *et al*[10] | 99 | 32 (32.3) | 47 (47.5) | 20 (20.2) |
| McCarron *et al*[32] | 310 | 121 (39.0) | 131 (42.3) | 58 (18.7) |
| Costa *et al*[34] | 570 | 173 (30.3) | 266 (46.7) | 131 (23.0) |
| Australia | | | | |
| James *et al*[33] | 2646 | 883 (33.4) | 1317 (49.8) | 446 (16.9) |

population was in agreement with other published studies with Caucasians populations[10,32-34]. Two recent meta-analyses have discussed the role of *EGF* polymorphism in susceptibility to cancer[35,36]. Future studies may confirm these results with adjustment for non-genetic putative risk factors for gastric cancer (ex: smoking, alcohol consumption, social class or *H. pylori*).

In conclusion, we found that female patients who were A carriers of *EGF* +61A/G had a decreased risk of developing gastric cancer. Furthermore, it has been suggested that women secrete less gastric acid than men[19], which is consistent with our hypothesis that different effects of *EGF* +61A/G variants may occur in males and females in relation to gastric cancer risk.

## COMMENTS

### Background
Epidermal growth factor (EGF) performs a key role in promoting cell survival. After binding to its receptor (EGFR), it induces a signalling cascade that culminates in change of gene transcription. EGFR signalling is not only important in cellular proliferation but also contributes to several other cellular processes involved in cancer progression, including angiogenesis, metastatic spread, and inhibition of apoptosis. EGFR (also known as ErbB1) belongs to the family of ErbB (from avian erythroblastic leukaemia viral oncogene homologue) receptors which are involved in the development of several human cancers. *EGF* gene has a polymorphism in position +61 which consists of the substitution of adenine (A) for guanine (G). AA genotype carriers have lower levels of EGF expression than individuals with the GG or AG genotypes. In this cross-sectional study, the authors analysed the associations between this EGF polymorphism and the risk for gastric cancer in a high incidence Caucasian population.

### Research frontiers
Although several gene loci have been associated with susceptibility to gastric cancer, the aetiology of gastric cancer is still unknown. The current study is the first to assess the impact of *EGF* gene polymorphisms and disease susceptibility in gastric cancer in a southern European Caucasian population.

### Innovations and breakthroughs
It is important to investigate the genetic variation in susceptibility to gastric cancer and to identify markers that will facilitate identification of individuals at risk of developing this disease. The results suggest that the stratification of individuals by gender revealed that males carrying A alleles (*EGF* +61A/G or AA) have an increased risk of developing gastric cancer as compared to GG homozygous males.

### Applications
The results of this study will help us to further understand the genetic determinants of gastric cancer.

### Peer review
This population based case-control study that adds to the existing evidence for a role of EGF/EGFR in pathogenesis of gastric carcinoma. The validity of the control population may be mildly contentious. The findings raise questions regarding the role of gender and sex hormones in gastric carcinoma. This is a small study with somewhat limited results, however it is well done, with a good sample size and with a good interpretation for the results observed. It can be relevant to the field.

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