The interleukin-8-251*T/*A polymorphism is not associated with risk for gastric carcinoma development in a Portuguese population

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It has been demonstrated that polymorphisms within inflammation-related genes are associated with risk of gastric carcinoma in *Helicobacter pylori*-infected individuals. Recently, several studies have reported conflicting results regarding the association between the interleukin *(IL)8*-251*T/*A polymorphism and risk of gastric carcinoma. In this study, we performed a case–control analysis, including 693 controls, 187 chronic gastritis cases and 333 gastric carcinoma cases, to determine the association between the *IL8*-251 polymorphism and risk of chronic gastritis and gastric carcinoma in the northern Portugal population. We found no significant association between the *IL8*-251 polymorphism and increased risk of chronic gastritis or gastric carcinoma, in agreement with that reported in other populations of white origin. The retrospective analysis of published data shows that the association between the *IL8*-251 polymorphism and risk of gastric carcinoma tends to be reproducible in populations of Asian origin. The estimated effect of the polymorphism under analysis was not significantly different in subgroups of gastric carcinoma cases defined by histologic type and anatomic site of the tumours, and by sex and age of the participants. In conclusion our results indicate that although the *IL8*-251 polymorphism might be a relevant host susceptibility factor for gastric carcinoma development, this association is likely to be ethnic-specific. *European Journal of Cancer Prevention* 17:28–32 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2008, **17**:28–32

Keywords: cancer, inflammation, interleukin-8, *Helicobacter pylori*, stomach

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Received 4 November 2006 **Accepted** 10 January 2007

Introduction

Patients infected with *Helicobacter pylori*, a stomach colonizing bacterium, are at increased risk of developing gastric carcinoma (GC) (Parsonnet *et al.*, 1991; Reed, 1996; Uemura *et al.*, 2001). The risk of developing this type of tumour relates to the physiological and histological changes that *H. pylori* infection induces in the stomach (Correa, 1992; Sipponen, 1994; Kuipers *et al.*, 1995; Carneiro *et al.*, 2001). Although there is evidence showing that *H. pylori* infection plays a crucial role in the pathogenesis of GC, a striking difference exists between the number of infected individuals and the number that go on to develop malignancy. Hence, progression towards disease is likely to depend on the combined effects of bacterial pathogenicity, host susceptibility and environmental factors.

It has been demonstrated that human genetic polymorphisms in inflammation-linked genes are associated with risk of development of *H. pylori*-related GC. The best studied examples are the interleukin (IL) 1B-511, IL1RN VNTR and the tumour necrosis factor alpha-308 polymorphisms (El Omar *et al.*, 2000, 2003; Machado *et al.*, 2001, 2003; Figueiredo *et al.*, 2002). These findings led to the hypothesis that *H. pylori* infection in individuals with the aforementioned alleles may result in increased production of gastric proinflammatory cytokines, leading to severe and sustained inflammation, gastric atrophy and hypochlorhydria, and ultimately to the development of GC (El-Omar *et al.*, 2000; Machado *et al.*, 2001; El-Omar, 2001).

Recently, a number of studies have implied the *IL8*-251*T/*A polymorphism to also be associated with increased risk of GC (Savage *et al.*, 2004; Lu *et al.*, 2005; Ohyauchi *et al.*, 2005; Taguchi *et al.*, 2005; Lee *et al.*, 2005). The IL-8 cytokine is involved in recruitment and activation of immune cells in the gastric mucosa, thus

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playing a major role in the gastric inflammatory response to *H. pylori* infection (Bodger and Crabtree, 1998). This association finds further support in functional evidence showing that the *IL8*-251*T/*A polymorphism affects the transcriptional activity of the *IL8* gene (Hull *et al.*, 2000; Ohyauchi *et al.*, 2005; Taguchi *et al.*, 2005; Lee *et al.*, 2005). In other studies, however, the association between the *IL8*-251*T/*A polymorphism and risk of GC could not be replicated (Kamangar *et al.*, 2006; Savage *et al.*, 2006). Interestingly, all the studies reporting positive associations were conducted on populations of Asian origin (Savage *et al.*, 2004; Lu *et al.*, 2005; Ohyauchi *et al.*, 2005; Taguchi *et al.*, 2005; Lee *et al.*, 2005), whereas studies reporting negative findings were conducted on white populations (Kamangar *et al.*, 2006; Savage *et al.*, 2006).

In this study, our goal was to perform a case–control analysis, including 693 controls, 187 chronic gastritis cases and 333 GC cases, to determine the association between the *IL8*-251*T/*A polymorphism and risk of chronic gastritis and GC in the northern Portugal population.

Methods

Study population

A total of 1213 participants from the north of Portugal were enrolled in this study, comprising 333 GC patients, 187 individuals with chronic gastritis and 693 unselected controls. The control group consisted of 293 healthy blood donors and 400 community controls from a representative sample of the noninstitutionalized adult population of Porto, Portugal, assembled as part of an ongoing health and nutrition survey (median age: 45 years; range, 18–83 years; male : female ratio, 0.9 : 1). A detailed description of the selection procedures and participants was published previously (Ramos *et al.*, 2004).

Individuals with chronic gastritis (median age 42 years; range, 24–62 years; male : female ratio, 14.6 : 1) were recruited among shipyard workers who had undergone standard gastroscopy as part of a screening program for premalignant lesions of the gastric mucosa. Individuals with evidence for past or present peptic ulcer disease were excluded from this study. Patients with GC (median age 58 years; range, 24–90 years; male : female ratio, 1.5 : 1) were diagnosed at Hospital S. João, Porto, Portugal. A detailed description of the histopathological procedures was described previously (Machado *et al.*, 2003). Briefly, GCs were classified as intestinal (46.0%), diffuse (29.0%) and atypical (25.0%). Cardia GC corresponded to 13.9% of the cases and noncardia GC to 86.1% of the cases. As reported previously (Estevens *et al.*, 1993; Machado *et al.*, 2003), 98% of the controls and individuals with chronic gastritis were positive for *H. pylori* in this cohort. Among GC cases the positivity rate reached 61%. The procedures followed in this study were in accordance with the institutional ethical standards. All the samples enrolled in this study were delinked and unidentified from their donors.

IL8-251*T/*A genotyping

Genomic DNA was extracted from gastric antral biopsies using the method described by Boom *et al.* (1990). Briefly, biopsy specimens were homogenized in guanidinium isothiocyanate using a sterile micropestle. DNA was captured onto silica particles, washed and eluted in 100 µl of 10 mmol/l Tris–HCl (pH 8.3). Genomic DNA from blood samples of control individuals and from GC patients was isolated using standard proteinase K digestion and phenol/chloroform extraction. Genotyping was performed by the 5′-nuclease PCR assay (Taq Man). Taqman primers, probes and conditions are available upon request. In a subset of 50 samples the genotypes were confirmed by direct sequencing.

IL8-251*T/*A luciferase assay

The effect of the *IL8*-251 polymorphism in the transcriptional activity of the *IL8* promoter was measured by a standard luciferase reporter assay. The *IL8* promoter (position −750 to −1) with either a T or an A at position −251 was cloned into the pGL3 vector (Invitrogen, Carlsbad, California, USA), and transfected into GC cell lines AGS and GP202. The two promoter sequences had no sequence differences other than the −251 when checked by direct sequencing. The pSV-β-galactosidase vector (Promega, Madison, Wisconsin, USA) was used as a control for transfection efficiency.

Statistics

Evidence for deviation from Hardy–Weinberg equilibrium of alleles at individual loci was assessed by exact tests using the program GENEPOP (Raymond and Rousset, 1995). The association between different genotypes and GC was assessed through the odds ratios and respective 95% confidence intervals, estimated by logistic regression analysis. Average luciferase expression levels were calculated after triplicate measurements for each cell line and *IL8*-251 allele, and compared by Student's *t*-test. Differences were considered significant when $P < 0.05$.

Results

Genotype frequencies of the *IL8*-251 polymorphism in the control group did not deviate significantly from those expected under Hardy–Weinberg equilibrium ($P = 0.5$, Table 1). In the control population, the *IL8*-251*T allele had a frequency of 55%, similar to that described in other European populations (Hull *et al.*, 2000; Howell *et al.*, 2003). The *IL8*-251 genotype frequencies among controls, chronic nonatrophic gastritis patients, chronic

Table 1 ***IL8*-251*T/*A genotype frequencies in controls, gastritis and gastric carcinoma cases**

Genotypes	Controls (%)	Nonatrophic gastritis (%)	OR (95% CI)	Atrophic gastritis (%)	OR (95% CI)	Gastric carcinoma (%)	OR (95% CI)
TT	203 (29.3)	41 (34.5)	1 (referent)	21 (30.9)	1 (referent)	111 (33.3)	1 (referent)
TA	353 (50.9)	56 (47.0)	0.8 (0.5–1.2)	36 (52.9)	1.0 (0.6–1.7)	169 (50.8)	0.9 (0.7–1.2)
AA	137 (19.8)	22 (18.5)	0.8 (0.5–1.4)	11 (16.2)	0.8 (0.4–1.7)	53 (15.9)	0.7 (0.5–1.1)
Total	693	119		68		333	

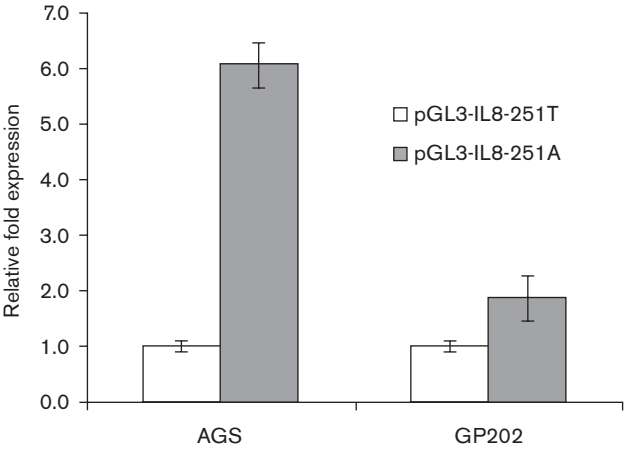
CI, confidence interval; OR, odds ratio.

atrophic gastritis patients and GC patients are summarized in Table 1.

No significant associations were found between the *IL8*-251 polymorphism and risk for development of chronic superficial gastritis, chronic atrophic gastritis or GC (Table 1). In logistic regression models that included age and sex, the adjusted odds ratios did not vary significantly, showing that the lack of association was not owing to differences in age or sex ratio between controls, individuals with chronic atrophic gastritis and GC cases. The estimated effects of the *IL8*-251 genotypes were not significantly different in subgroups of GC cases defined by histological type and anatomical site of the tumours.

In the luciferase reporter assay we observed that the *IL8*-251*A allele was significantly associated with a higher transcriptional level in both AGS ($P < 0.0001$) and GP202 ($P = 0.0001$) GC cell lines (Fig. 1).

Fig. 1



Effect of the *IL8*-251 polymorphism on the transcription level of the *IL8* promoter in gastric carcinoma cell lines AGS and GP202. Luciferase activity was calculated by normalizing against β-galactosidase background activity and expressed as a ratio between the A and the T alleles. Error bars represent standard deviation.

Discussion

In this study, we found no evidence for the existence of an association between the *IL8*-251*T/*A polymorphism and risk for developing chronic gastritis or GC. Our study was based on a larger sample size than any of the previously published studies, and had more than 90% power at the 1% significance level to detect a two-fold increase in risk of GC conferred by a genotype with a frequency of 20% in the general population, as seen for the *IL8*-251*A/*A genotype. These results are in accordance with those reported before for other populations of white origin, like the Polish (Savage *et al.*, 2006), and the Finnish (Kamangar *et al.*, 2006) (Table 2). In populations of Asian origin, however, a different picture emerges from the analysis of published data. All five studies on record report significant associations between the *IL8*-251*T/*A polymorphism and risk for development of GC. The results are quite reproducible with four of them reporting an association between the *IL8*-251*A/*A or *T/*A genotypes and increased risk of GC (Savage *et al.*, 2004; Lu *et al.*, 2005; Ohyauchi *et al.*, 2005; Taguchi *et al.*, 2005) (Table 2). To reinforce these findings, Taguchi *et al.* (2005) also described a significant association between the *IL8*-251*A/*A genotype and increased risk for developing chronic atrophic gastritis (Table 2). In contrast, the study by Lee *et al.* (2005), conducted in Taiwan, describes an association between the *IL8*-251*T/*A and T/T genotypes and increased risk of GC.

Conceptually, the discrepant results between Asian and white populations may be explained by differences in the genetic background of the populations under study. In Asian populations, the *IL8*-251*A allele may be in linkage disequilibrium with an as yet unidentified sequence variant, which is responsible for the association with risk of GC. This hypothesis is supported by data on other disease models, such as the association between the *IL8*-251*T/*A polymorphism and risk for development of bronchiolitis (Hull *et al.*, 2000) or prostate cancer (Yang *et al.*, 2006). In the former model, Hull *et al.* (2000) have demonstrated that the *IL8*-251*A allele resides on two different haplotypes, only one of which is associated with disease. In this context, finding associations with different polymorphisms in different populations would not be unexpected as haplotype structure may vary considerably between distinct populations. Thus, haplotype-based approaches involving genotyping of several genetic markers simultaneously, will constitute a more

Table 2 Summary of published studies on the association between the *IL8*-251*T/*A polymorphism and risk of gastric carcinoma (GC) in Asian and white populations

Study	Population	Genotype (%)			Allele (%)	
		*T*T	*T*A	*A*A	*T	*A
Asian						
Savage *et al.* (2004)	Linxian, China					
	Controls ($n=429$)	147 (34.3)	207 (48.3)	75 (17.5)	58	42
	Cardia GC ($n=88$)	26 (29.6)	39 (44.3)	23 (26.1)	52	48
	OR (95% CI)	1 (Referent)	1.1 (0.7–1.9)	2.0 (1.0–3.8)		
Ohyauchi *et al.* (2005)	Sendai, Japan					
	Controls ($n=244$)	149 (61,1)	84 (34.4)	11 (4.5)	78	22
	GC ($n=212$)	93 (43.9)	106 (50.0)	13 (6.1)	69	31
	OR (95% CI)	1 (Referent)	2.0 (1.4–3.0)	1.9 (0.8–2.9)		
Lu *et al.* (2005)	Shangdong and Beijing, China					
	Controls ($n=300$)	119 (39.7)	144 (48.0)	37 (12.3)	64	36
	GC ($n=250$)	94 (37.6)	102 (40.8)	54 (21.6)	58	42
	OR (95% CI)	1 (Referent)	0.9 (0.6–1.3)	1.9 (1.2–3.2)		
Taguchi *et al.* (2005)	Aichi, Japan					
	Controls ($n=252$)	125 (49.6)	105 (41.7)	22 (8.7)	70	30
	Atrophic gastritis ($n=215$)	90 (41.9)	99 (46.0)	26 (12.1)	65	35
	OR (95% CI)	1 (Referent)	1.4 (0.9–2.1)	2.4 (1.1–4.9)		
	GC ($n=396$)	161 (40.7)	191 (48.2)	44 (11.1)	65	35
	OR (95% CI)	1 (Referent)	1.4 (0.9–2.1)	2.2 (1.1–4.6)		
Lee *et al.* (2005)	Taipei, Taiwan					
	Controls ($n=308$)	108 (35.1)	138 (44.8)	62 (20.1)	57	43
	GC ($n=470$)	198 (42.1)	213 (45.3)	59 (12.6)	65	35
	OR (95% CI)	1.9 (1.3–3.0)	1.6 (1.1–2.5)	1 (Referent)		
White						
Kamangar *et al.* (2006)	Southern Finland					
	Controls ($n=207$)	72 (34.8)	111 (53.6)	24 (11.6)	62	38
	GC ($n=112$)	42 (37.5)	56 (50.0)	14 (12.5)	63	37
	OR (95% CI)	1 (Referent)	0.9 (0.5–1.4)	1.0 (0.4–2.7)		
Savage *et al.* (2006)	Warsaw, Poland					
	Controls ($n=428$)	106 (24.8)	205 (47.9)	117 (27.3)	49	51
	GC ($n=287$)	71 (24.7)	140 (48.8)	76 (26.5)	49	51
	OR (95% CI)	1 (Referent)	1.0 (0.7–1.5)[a]	1.0 (0.6–1.5)[a]		

CI, confidence interval; OR, odds ratio.
[a]ORs calculated using raw data provided in the original publication.

efficient way of capturing the genetic diversity present in a given genomic region and thus help clarify the association between the *IL8*-251*T/*A polymorphisms and GC risk in different populations. Obviously, gene–environment interactions may also contribute to the conflicting findings on this topic and add enormously to the complexity of this issue.

Despite the lack of association between the *IL8*-251*T/*A polymorphism and risk of GC in our series, we could confirm that the *IL8*-251*A allele is indeed associated with increased transcriptional activity of the *IL8* promoter in an in-vitro assay. Our results are in agreement with several other studies showing an increased effect of the *IL8*-251*A allele over transcription and protein production (Hull *et al.*, 2000; Ohyauchi *et al.*, 2005; Taguchi *et al.*, 2005). These results show that even when a functional effect of the candidate polymorphism has been well established, the detection of statistically significant disease associations is likely to be influenced by the genetic background and haplotype structure of the population under study.

In summary, our results do not support the existence of an association between the *IL8*-251*T/*A polymorphism and risk of GC in white populations. In contrast, studies conducted in Asian populations show that the association between the *IL8*-251*T/*A polymorphism and increased risk of GC is likely to be ethnic-specific. In the latter populations, carriage of the *IL8*-251*A allele may help determine why some individuals infected with *H. pylori* develop GC whereas others do not.

Acknowledgements

This work was supported by funding under Fundação para a Ciência e Tecnologia (POCTI/CBO/41550/2001, REEQ/218/SAU/2005, POCTI/ESP/35769/99, POCI/SAU-ESP/56126/2004 and POCI/SAU-ESP/61685/2004), Programa Operacional Ciência, Tecnologia e Inovação (POCTI), Fundo Comunitário Europeu (FEDER), Programa Operacional de Saúde/SAUDE XXI, Associação Portuguesa da Industria Farmacêutica (APIFARMA) and Sixth Research Framework Programme of the European Union, Project INCA (LSHC-CT-2005-018704).

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