Le polymorphisme du récepteur de l'interleukine 4 (IL-4) −3223 C→T s'associe à une augmentation du risque d'adénocarcinome gastrique

HISTORIQUE : Le cancer gastrique demeure l'un des principaux types de cancer du monde, bien que son incidence et son taux de mortalité varient considérablement sur le plan géographique. Les polymorphismes des cytokines sont les polymorphismes de l'hôte les plus étudiés et s'associent à un risque accru de cancer de l'estomac dans de nombreuses régions, mais ils n'ont pas fait l'objet d'études approfondies dans les populations d'Europe de l'Est.

OBJECTIF : Examiner l'association potentielle entre cinq polymorphismes promoteurs des cytokines (interleukine [IL] 1β →IL-4R −3223C→T [rs1800896], IL-4 récepteur [IL-4R] →IL-4R −3223C→T [rs2057768], IL-8 →IL-8 −251TT→A [rs4073], TNF-α →TNF-α −308G→A [rs1800629] et facteur de nécrose tumorelle alpha →−308G→A [rs1800629]) et la susceptibilité à l'adénocarcinome gastrique dans une population roumaine.

MÉTHODOLOGIE : Au total, 347 sujets, soit 105 patients ayant un adénocarcinome gastrique et 242 sujets témoins, ont participé à l’étude. Les chercheurs ont procédé au génotypage de tous les polymorphismes des cytokines au moyen de sondes spécifiques à l'allèle offertes sur le marché. Ils ont analysé l'équilibre de Hardy-Weinberg dans les deux groupes au moyen de la méthode du khi-carré et ont évalué le lien entre les polymorphismes ciblés et le risque de cancer gastrique au moyen du RRR et de l'IC 95 %.

RÉSULTATS : Les chercheurs ont constaté une association significative entre le polymorphisme de l'IL-4R −3223C→T et le risque de cancer gastrique. Les porteurs du génotype de l'IL-4R −3223TT risquaient 2,5 fois plus de souffrir d'un cancer gastrique (RRR 2,51 [95 % CI 1,08 à 5,84]; P=0,041). De plus, la présence du génotype de l'IL-4R −3223TT s'associait à un risque plus élevé d'adénocarcinome gastrique ne touchant pas le cardia (RRR 3,08 [95 % CI 1,25 à 7,58]; P=0,023). Les chercheurs n'ont constaté aucun lien entre les autres polymorphismes.

CONCLUSION : D'après les résultats, le polymorphisme de l'IL-4R −3223C→T pourrait accréditer le risque d'adénocarcinome gastrique au sein de la population roumaine, notamment s'il ne touche pas le cardia.
countries and regions regarding the association of different cytokine gene polymorphisms have been published, but not from Eastern Europe, where gastric cancer incidence and mortality is the highest in the continent (1).

In Romania, gastric cancer incidence and mortality rates remain high. Gastric cancer is the fifth most common malignancy and the third-ranked cause of cancer death, despite the decreased mortality rates of gastric cancer in many European countries (8). The prevalence of *H pylori* generally ranges from 40% in developed countries to more than 80% in developing countries (9), and was estimated to be 68.5% in the adult population of Romania (10).

Accordingly, we investigated polymorphisms located in the promoter regions of five cytokine genes (IL-1β -511C→T [rs16944], IL-4R -3223C→T [rs2057768], IL-8 -251T [rs16944], IL-10 -1082A→G [rs1800689] and TNF-α -308G→A [rs1800629]) in a Romanian population (ie, Eastern European population) to determine whether these polymorphisms are associated with gastric adenocarcinoma susceptibility.

## METHODS

### Subjects

A total of 347 Romanian subjects were included in the present study: 105 unrelated gastric cancer patients from the Clinical Hospital of Craiova (Craiova, Romania), and 242 age- and sex-matched healthy controls. All subjects underwent upper endoscopy and diagnosis of gastric cancer was made by histological examination of biopsy specimens. Tumours were classified as intestinal or diffuse type according to the classification proposed by Laurén (11). Only *H pylori*-positive patients were selected. *H pylori* infection was evaluated by histological examination, rapid urease test and/or anti-*H pylori* immunoglobulin G quantification. Patients were considered to be infected when at least one of these diagnostic tests was positive. Both control and gastric cancer groups consisted of Romanian individuals of the same ethnic and geographical origins. Individuals with a positive family history of gastric and other types of cancer or inflammatory diseases were excluded.

The Research Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania, approved the present study and written informed consent was obtained from all subjects.

### Genotyping assay

Blood samples were collected from all subjects and genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer’s protocol. Polymorphisms were selected on the basis of their previously published involvement in cancer risk and/or functional role. All cytokine polymorphisms were genotyped by allelic discrimination polymerase chain reaction assays (5’ nuclelease assay) using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, USA): IL-1β -511C→T (rs16944, assay C_1839943_10); IL-4R -3223C→T (rs2057768, assay C_2769607_10); IL-8 -251T→A (rs4073, assay C_1748816_10); IL-10 -1082A→G (rs1800689, assay C_1747360_10) and TNF-α -308G→A (rs1800629, assay C_7514879_10). The genotyping assay was performed using the RotorGene 6000 HRM-Corbett Real Time PCR system, and assays were validated and optimized as described on the SNP500 Cancer website (http://snp500cancer.nci.nih.gov). To ensure quality control, DNA samples from case patients and controls were randomly distributed and all samples were blindly genotyped. All samples that did not yield a reliable result in the first round were resubmitted for up to two additional rounds of genotyping. Also included were a negative control sample and three positive controls (homozygous for the wild-type allele, and heterozygous and homozygous for the mutant allele).

### Statistical data analysis

The χ² test was used to test the distribution of genotypes and allele frequencies for deviations from Hardy-Weinberg equilibrium. The linkage disequilibrium blocks were determined using D’ and r² values. Genotype frequencies of SNPs between patients with gastric cancer and the controls were compared using logistic regression, crude and adjusted ORs according to sex and age, and 95% CIs. The homozygous genotype of the common allele was used as the reference group. Recessive and dominant models were also used. All P values were two sided and P<0.05 was considered to be statistically significant. All data analysis was performed using SPSS version 17.0 (IBM Corporation, USA).

## RESULTS

A total of 105 gastric adenocarcinoma patients and 242 healthy controls were genotyped. Table 1 summarizes the characteristics of gastric cancer patients and controls; there were no differences in distribution with respect to age, sex or ethnicity. Among the gastric cancer cases,

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Gastric cancer (n=105)</th>
<th>Control (n=242)</th>
<th>OR (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β -511C→T</td>
<td>52 (49.5)</td>
<td>110 (45.45)</td>
<td>Reference</td>
<td>–</td>
</tr>
<tr>
<td>CC</td>
<td>42 (40.0)</td>
<td>102 (42.15)</td>
<td>0.87 (0.54–1.42)</td>
<td>0.63</td>
</tr>
<tr>
<td>CT</td>
<td>11 (10.5)</td>
<td>30 (12.40)</td>
<td>0.78 (0.36–1.67)</td>
<td>0.56</td>
</tr>
<tr>
<td>TT</td>
<td>53 (50.5)</td>
<td>132 (54.55)</td>
<td>0.89 (0.54–1.34)</td>
<td>0.54</td>
</tr>
<tr>
<td>IL-4R -3223C→T</td>
<td>53 (50.5)</td>
<td>144 (59.5)</td>
<td>Reference</td>
<td>–</td>
</tr>
<tr>
<td>CC</td>
<td>40 (38.1)</td>
<td>85 (35.1)</td>
<td>1.28 (0.78–2.09)</td>
<td>0.43</td>
</tr>
<tr>
<td>CT</td>
<td>12 (11.4)</td>
<td>13 (5.4)</td>
<td>2.51 (1.08–5.84)</td>
<td>0.041</td>
</tr>
<tr>
<td>TT</td>
<td>52 (49.5)</td>
<td>98 (40.5)</td>
<td>1.44 (0.91–2.28)</td>
<td>0.17</td>
</tr>
<tr>
<td>IL-8 -251T→A</td>
<td>31 (29.5)</td>
<td>82 (33.9)</td>
<td>Reference</td>
<td>–</td>
</tr>
<tr>
<td>TT</td>
<td>54 (51.4)</td>
<td>112 (46.3)</td>
<td>1.27 (0.75–2.16)</td>
<td>0.22</td>
</tr>
<tr>
<td>AA</td>
<td>20 (19.1)</td>
<td>48 (19.8)</td>
<td>1.10 (0.57–2.14)</td>
<td>0.4</td>
</tr>
<tr>
<td>A carriers</td>
<td>74 (70.5)</td>
<td>160 (66.1)</td>
<td>1.22 (0.74–2.01)</td>
<td>0.82</td>
</tr>
<tr>
<td>IL-10 -1082A→G</td>
<td>62 (59.1)</td>
<td>153 (63.2)</td>
<td>0.84 (0.53–1.34)</td>
<td>0.51</td>
</tr>
<tr>
<td>GG</td>
<td>13 (12.4)</td>
<td>35 (14.4)</td>
<td>0.77 (0.37–1.60)</td>
<td>0.52</td>
</tr>
<tr>
<td>G carriers</td>
<td>8 (7.6)</td>
<td>16 (6.6)</td>
<td>Reference</td>
<td>–</td>
</tr>
<tr>
<td>NN</td>
<td>43 (40.9)</td>
<td>89 (36.8)</td>
<td>Reference</td>
<td>–</td>
</tr>
<tr>
<td>GA</td>
<td>49 (46.7)</td>
<td>118 (48.8)</td>
<td>0.86 (0.53–1.41)</td>
<td>0.6</td>
</tr>
<tr>
<td>GG</td>
<td>12 (11.4)</td>
<td>35 (14.4)</td>
<td>0.77 (0.37–1.60)</td>
<td>0.52</td>
</tr>
<tr>
<td>GG78 (74)</td>
<td>196 (81.0)</td>
<td>Reference</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>26 (24.8)</td>
<td>44 (18.2)</td>
<td>1.49 (0.86–2.58)</td>
<td>0.28</td>
</tr>
<tr>
<td>AA</td>
<td>1 (0.9)</td>
<td>2 (0.8)</td>
<td>1.26 (0.11–14.05)</td>
<td>0.9</td>
</tr>
<tr>
<td>A carriers</td>
<td>27 (25.7)</td>
<td>46 (19.0)</td>
<td>1.47 (0.86–2.54)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data presented n (%) unless otherwise indicated. IL Interleukin; TNF Tumour necrosis factor
Cytokine polymorphisms are the most studied host genetic variants for association with gastric cancer in many regions, but not in Eastern European populations. Because such a list of candidate genes would be prohibitively extensive, our initial search focused on SNPs located in promoter regions that are most relevant to gastric physiology. We assessed whether genetic variations in five promoter region polymorphisms (three in proinflammatory and two in anti-inflammatory cytokine genes) are associated with gastric cancer risk in the Romanian population.

We found that the IL-4R −3223TT polymorphism increased gastric cancer susceptibility, mainly for the noncardia type (OR 3.08 [95% CI 1.25 to 7.58]; P=0.023). The IL-4R gene encodes the IL-4 receptor, the specific cell surface receptor for the anti-inflammatory cytokine IL-4. In contrast with other cytokines, little data are available in the literature on the role of IL-4 and IL-4R genetic polymorphisms in H pylori-induced diseases. The promoter −3223C>T polymorphism has been demonstrated to gradually decrease circulating IL-4R levels (12) and, thus, the presence of IL-4R −3223T allele contributes to impaired cytokine balance by decreasing the anti-inflammatory response. No association was found between IL-4R −3223C>T and any stages of premalignant lesions (intestinal metaplasia and dysplasia) in a gastric precancerous study in South America (13), whereas a European study conducted in Western countries reported a positive association for IL-4R −3223T allele only for noncardia (OR 1.74 [95% CI 1.15 to 2.63]) in a dominant model, but not for all gastric cancer cases (14).

The anti-inflammatory cytokine IL-10 gene contains three confirmed bi-allelic promoter polymorphisms (−1082A→G, −819C→T and −592C→A) reported to produce mainly three haplotypes: GCC, ACC and ATA (15). The presence of the −1082A allele is associated with lower IL-10 production both in vitro and in vivo (15,16). We did not detect significant differences for the IL-10 −1082A→G polymorphism between gastric cancer and control groups. Published findings on the IL-10 −1082A→G polymorphism are inconsistent. While some studies revealed a positive association between −1082G (the high producer allele) and gastric cancer risk (17,18), others showed a
positive association for −1082A (the low producer allele) (19,20). There are also studies with no detected relationship between IL-10 polymorphisms and gastric cancer (21,22).

We did not observe associations for either IL-1β −511T→C or TNF-α 308G→A polymorphisms. IL-β and TNF-α are crucial cytokines in initiating and amplifying the inflammatory responses to H pylori infection. In addition, IL-β and TNF-α are potent inhibitors of gastric acid secretion (23). Therefore, increased production of IL-1β and TNF-α in the gastric mucosa would theoretically lead to an enhanced suppression of gastric acid secretion as well as inflammation and, finally, to an increased risk of gastric cancer. Two major IL-1β gene polymorphisms, IL-1β −511T→C and IL-1β −31T→C, in the promoter region are in complete linkage disequilibrium (7) and have been reported to be associated with interindividual differences in IL-1β production. Individuals homozygous for the IL-1β −511T/−31C haplotype were found to produce between two and three times more IL-1β than others haplotypes (24). Controversial results related to the IL-1β −511T→C polymorphism and gastric cancer risk have been reported: some studies found a positive association (7,25,26) whereas others were not able to reproduce it (27,28). The frequency of the IL-1β −511TT genotype in our control group was 12.40%, similar to other Caucasian populations (13% in Scottish and Polish populations, and 14.2% in the Portuguese population) (7,25) and were lower than the Hispanic and Asian populations (approximately 24%) (21,26,28).

The proinflammatory cytokine TNF-α shares many biological properties with IL-1β. Most studies have focused on the TNF-α −308G→A polymorphism, although some other polymorphic positions in the promoter region have already been described (29). The A allele of TNF-α −308G→A polymorphism increases gene expression, with a resulting alteration of the immune response due to the higher production of TNF-α (30). Several studies have examined the association of the TNF-α −308G→A polymorphism and gastric cancer risk. Positive reports in Caucasian populations have been published (19,31), but these findings are in contrast with others studies (27,32).

IL-8 is involved in the recruitment and activation of immune cells in the gastric mucosa, and the presence of the −251A allele has been found to increase its expression (33). Almost all studies reporting a positive association were conducted in populations of Asian origin (17,34,35), whereas negative findings were found in Caucasian populations (36,37). Moreover, the IL8 −251A allele was associated with a significantly reduced risk of noncardia gastric cancer in an H pylori-positive group and intestinal type in a European study (14). We did not find any association between the IL-8 −251T→A polymorphism and gastric cancer susceptibility. These results are in accordance with previous studies on Caucasian populations.

Our findings show a consistent association between the IL-4R −3223TT genotype and gastric cancer susceptibility in an Eastern European population, with some differences to associations found in studies conducted in Western European populations. One possible explanation for those differences is that a cytokine allele may be functional only in a specific haplotype context that varies among different ethnic groups and regions. Our results indicate that this association is mainly for the noncardia type, but the small size of these subgroups precludes drawing reliable conclusions.

**CONCLUSION**

The IL-4R −3223C→T polymorphism increases gastric cancer susceptibility in the Romanian population. Further genome-wide association and generic functional studies may help to identify the potential role of this polymorphism in human gastric carcinogenesis.

**ACKNOWLEDGEMENTS/FUNDING:** FB was supported by research grant POSDRU/61/1.5/S/64109. MI was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

**REFERENCES**
