LETTER TO JMG

Interleukin 12 gene polymorphisms enhance gastric cancer risk in *H pylori* infected individuals

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**Key points**

- IL12, formed from p35 and p40 subunits encoded by IL12A and IL12B genes, favours T helper 1 (Th1) differentiation. Th1 lymphocytes prevail over Th2 in *H pylori* associated chronic gastritis, the first step in *H pylori* associated gastric carcinogenesis. In this study, 110 patients with non-cardia gastric cancer were compared with 251 patients with benign gastroduodenal diseases to see whether there was any correlation between IL12 gene polymorphisms and *H pylori* associated gastric adenocarcinoma.

- Two single nucleotide polymorphisms were identified on IL12A (−504 T/G and +6686 A/G) and one on IL12B (+15485 A/G). Eleven and six alleles were found for CT and TG-TA dinucleotide repeats (VNTR) of IL12A intron 6 and IL12B intron 4, respectively.

- The frequency of non-cardia gastric cancer was higher in patients with the IL12A −504 T/T (odds ratio (OR) = 2.38) or with the IL12B VNTR (TG-TA)9/(TG-TA)11 genotype (OR = 1.36).

- No IL12 gene polymorphisms were correlated with intestinal metaplasia.

- These findings suggest that IL12A and IL12B gene polymorphisms may affect the final steps in gastric carcinogenesis in *H pylori* infected subjects.

**METHODS**

We studied 251 unrelated Italian patients (112 male, 139 female; age range 27 to 88 years) who consecutively underwent upper gastrointestinal endoscopy (EGDS, oesophagogastroduodenoscopy) for dyspeptic symptoms. Diagnoses made on the basis of endoscopic findings were: absence of evident endoscopic lesions (10); antral gastritis (94); diffuse gastritis (57); duodenal ulcer (30); gastric ulcer (10); duodenitis (22); reflux gastritis (16); oesophagitis (12).

Three antral and two body biopsies were obtained at endoscopy from each patient for histological evaluation. Two antral and one body biopsy were also obtained for *H pylori* infection and its outcome.

Our aims in the present study were, first, to use denaturing high performance liquid chromatography (DHPLC) to screen the promoters and the coding sequences of IL12A and IL12B in order to identify any single nucleotide polymorphisms (SNPs); second, to analyse the SNPs identified, together with the number of CT and TG-TA dinucleotide repeats (variable number tandem repeats (VNTR)) of IL12A intron 6 and IL12B intron 4 in patients with or without *H pylori* infection; and third, to verify the association, if any, between the IL12 gene polymorphisms studied and the outcome of *H pylori* infection (gastric adenocarcinoma in particular).

**Abbreviations:** DHPLC, denaturing high performance liquid chromatography; EGDS, oesophagogastroduodenoscopy; IL, interleukin; SNP, single nucleotide polymorphism; Th, T helper; VNTR, variable number tandem repeats

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Bacterial, environmental, population related, and individual host factors are major determinants of the outcome of *H pylori* infection. Many bacterial virulence genes—including the pathogenicity island *cagA*, the s1m1 *vacA* alleles, *babA2*, *sabA*, and *oipA*—have been associated with a higher degree of gastric mucosal inflammation, intestinal metaplasia, gastric or duodenal ulcer, gastric adenocarcinoma, and MALToMA.**1** H pylori triggers and maintains gastric mucosal inflammation by different mechanisms, which are partly strain dependent and partly strain independent. T and B lymphocyte activation and infiltration of the gastric mucosa depend on *H pylori* antigen processing. The number of infiltrating polymorphonuclear cells varies depending on the virulence of the infecting strain, being much greater when infections are caused by *cagA* positive strains.**4**

The inflammatory cells infiltrating *H pylori* infected gastric mucosa produce a pattern of proinflammatory cytokines.**10** High mucosal levels of mononuclear cytokines (IL18, IL6, IL1B, tumour necrosis factor α (TNFα), and interferon γ (IFNγ)) and lymphocytic derived cytokines (IL2, IL2R) have been described in *H pylori* infected patients.**10–13** *H pylori* infection also induces the production of IL12, a heterodimeric proinflammatory cytokine, which triggers the production of IFNγ and favours the differentiation of T helper 1 (Th1) cells,**17** which, in *H pylori* infected mucosa, prevail over Th2 cells.**15** The ability of IL12 to induce Th1 is one of the biological bases of the importance of this cytokine in resisting most bacteria, including *H pylori*, and also intracellular protozoa and fungal pathogens.**10** The cellular sources of IL12 in response to infections are mainly dendritic cells and phagocytes.**15** The two subunits of IL12—p35 and p40—are encoded by different genes, named IL12A and IL12B respectively, which are unrelated and are located on separate chromosomes (3p12–q13.2 and 5q31–33).**14**

Host cytokine gene polymorphisms may be as important as exogenous stimuli in influencing the amount of cytokines produced and consequently the pattern and severity of inflammation.**10** IL12 gene polymorphisms in particular have been observed to affect autoimmune diabetes**22** and atopic and non-atopic asthma.**28** Both IL12A and IL12B have a polymorphic dinucleotide repeat region (CT for IL12A and TG-TA for IL12B) in introns 6 and 4, respectively (Gene bank accession numbers: AF404773 for IL12A and AT008847 for IL12B), which may affect the amount of the synthesised cytokine. No data have been reported on the possible influence of IL12A and IL12B polymorphisms on *H pylori* infection and its outcome.

Our aims in the present study were, first, to use DHPLC to screen the promoters and the coding sequences of IL12A and IL12B in order to identify any single nucleotide polymorphisms (SNPs); second, to analyse the SNPs identified, together with the number of CT and TG-TA dinucleotide repeats (variable number tandem repeats (VNTR)) of IL12A intron 6 and IL12B intron 4 in patients with or without *H pylori* infection; and third, to verify the association, if any, between the IL12 gene polymorphisms studied and the outcome of *H pylori* infection (gastric adenocarcinoma in particular).
Table 1  Primer sequences, primer spanning regions, amplified regions, and temperatures of denaturing high performance liquid chromatography analysis

<table>
<thead>
<tr>
<th>Primer sequences</th>
<th>Primer spanning region</th>
<th>Regions</th>
<th>Temp for DHPLC analysis (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL12A (p35)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'tccacactacagcaco3'</td>
<td>-941/-922</td>
<td>Promoter</td>
<td>63, 64</td>
</tr>
<tr>
<td>5'ctagattataacacagctc3'</td>
<td>-598/-621</td>
<td>Promoter</td>
<td>59.6</td>
</tr>
<tr>
<td>5'ggagatttaagaggacac3'</td>
<td>-693/-675</td>
<td>Promoter</td>
<td>61.8</td>
</tr>
<tr>
<td>5'ggacacccaggagacac3'</td>
<td>-451/-469</td>
<td>Promoter</td>
<td>65.5, 67</td>
</tr>
<tr>
<td>5'tgctccacacacacacag3'</td>
<td>-520/-501</td>
<td>Promoter</td>
<td>69.3</td>
</tr>
<tr>
<td>5'gacctgctctacacac3'</td>
<td>-241/-259</td>
<td>Promoter</td>
<td>63.8</td>
</tr>
<tr>
<td>5'ggagaggagtacacacacac3'</td>
<td>-313/-294</td>
<td>Promoter</td>
<td></td>
</tr>
<tr>
<td>5'ggacagagacacac3'</td>
<td>-71/-89</td>
<td>Promoter</td>
<td></td>
</tr>
<tr>
<td>5'gtggtcagacatccgctt3'</td>
<td>-171/-152</td>
<td>Promoter</td>
<td></td>
</tr>
<tr>
<td>5'gacgatccgctctgct3'</td>
<td>-22/-41</td>
<td>Promoter</td>
<td></td>
</tr>
<tr>
<td>5'gaggagattagagagac3'</td>
<td>-93/-75</td>
<td>Promoter</td>
<td></td>
</tr>
<tr>
<td>5'ctggtcttgccagcgtg3'</td>
<td>+129/+112</td>
<td>Promoter and exon 1</td>
<td>63.8</td>
</tr>
<tr>
<td>5'aggccgaaaagagctctg3'</td>
<td>+19/+36</td>
<td>Exon 1</td>
<td>65.0, 67.0</td>
</tr>
<tr>
<td>5'gagccgagctgaagacg3'</td>
<td>+213/-196</td>
<td>Exon 2</td>
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<tr>
<td>5'atcggtgcagctgaacg3'</td>
<td>+1130/-1148</td>
<td>Exon 3</td>
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<tr>
<td>5'agggggaaggacagcaco3'</td>
<td>+1357/-1340</td>
<td>Exon 4</td>
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<tr>
<td>5'gggccagaggatacat3'</td>
<td>+396/-3980</td>
<td>Exon 5</td>
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<tr>
<td>5'ttttaacacagctgctg3'</td>
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<td>Exon 6</td>
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<tr>
<td>5'ttgagggagtacagagcagga3'</td>
<td>+4385/-4408</td>
<td>Exon 7</td>
<td>56.1, 57.4</td>
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<tr>
<td>5'cctccagcttgagagac3'</td>
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<td>UTR</td>
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<td>5'tgatattccacctgctg3'</td>
<td>+4673/-4693</td>
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<td></td>
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<td>5'gctgaagagcagctcaca3'</td>
<td>+4888/-4869</td>
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<td></td>
</tr>
<tr>
<td>5'tgagctacacagtacagat3'</td>
<td>+6368/-6390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'tgacagaggtataaggtg3'</td>
<td>+6699/-6677</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'gacatagaggagggagagac3'</td>
<td>+6642/-6661</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'gacagaggtccattgac3'</td>
<td>+6924/-6902</td>
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</tbody>
</table>

**Table 2**  Genotype frequencies and Hardy-Weinberg equilibrium analysis of the three single nucleotide polymorphisms studied

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype frequency (+)</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL12A</strong></td>
<td>-504</td>
<td>G/G (0.01) G/T (0.22) T/T (0.77)</td>
<td>$\chi^2 = 2.46$, NS</td>
</tr>
<tr>
<td><strong>IL12A</strong></td>
<td>+6686</td>
<td>A/A (0.03) A/G (0.22) G/G (0.75)</td>
<td>$\chi^2 = 3.40$, NS</td>
</tr>
<tr>
<td><strong>IL12B</strong></td>
<td>+15485</td>
<td>A/A (0.74) A/C (0.23) C/C (0.03)</td>
<td>$\chi^2 = 3.33$, NS</td>
</tr>
</tbody>
</table>

HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.
culture and genotyping. An EDTA-K3 treated blood sample was obtained from all patients for host genomic DNA isolation.

A second series of 110 unrelated Italian patients (66 male, 44 female; age range 34 to 90 years) who underwent surgery for non-cardia gastric cancer was also studied. TNM stages were: T1 (7); T1 T2 (3) T2 T4 (5); T3 (6); T4 (4); T1 N1 (15); T1 N1 N2 (6); T2 N1 (2); T2 N1 N2 (2); T4 N1 (2); T4 N1 N2 (2); T4 N2 (1). According to the criteria for ulcerating cancers, tumours were classified as “intestinal-type” in 82 patients and “diffuse” in 28. Two tissue samples for H pylori genotyping and genomic DNA analysis were obtained from 50 non-cardia gastric cancer patients: one from the neoplastic area and another from the adjacent (but at least 3 cm distant) non-neoplastic mucosa. The tissue samples were stored at -80°C until DNA extraction was carried out. Whole blood from all patients with non-cardia gastric cancer was used to obtain genomic DNA. Sera were also obtained for measurement of anti-H pylori antibodies. 

**Histological evaluation**

In mucosal biopsies from patients who underwent EGDS, H pylori colonisation density, chronic inflammation, polymorphonuclear cell infiltration (activity), and intestinal metaplasia were evaluated and graded according to the updated Sydney system. Non-cardia gastric cancer diagnosis was always confirmed histologically on samples taken intraoperatively.

**H pylori culture and genotyping**

In the series of 251 patients who underwent EGDS, H pylori was cultured as described elsewhere. DNA extracted from positive colonies was used to amplify ureA, cagA, and vacA under conditions specified by us elsewhere. In the subgroup of 50 non-cardia gastric cancer patients for whom tissue samples were available, H pylori infection and strain virulence gene characterisation were assessed in DNA extracted from tissue. Urea, cagA, and s1/s2 vacA were multiplex polymerase chain reaction (PCR) amplified in a 25 µl final reaction volume containing: 150 ng DNA, 1×PCR gold buffer (Applied Biosystems, Foster City, California, USA), 1.5 mM MgCl2, 200 µM each dNTPs, 500 nM primer URE-A F and URE-A R (5′gacatcactatacaggaag3′ and 5′tgaaaaccacgctctttag3′), 160 nM primer Cag-A F and Cag-A R (5′caatacatacagagtctc3′ and 5′agcttgcttggggacaatc3′), 600 nM primer S1/2-F and S1/2-R (5′atggaataacacaacacac3′ and 5′ctctgtaagggeccaaa3′), and 2.5 U AmpliTaq gold (Applied Biosystems). The thermocycling conditions were: 95°C for six minutes, then 42 cycles at 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, and finally 72°C for seven minutes. The fragments (323 base pairs (bp) for ureA, 194 bp for cagA, and 259 or 286 bp for s1 and s2 vacA, respectively) were separated by electrophoresis on 2% NuSieve agarose gel (BMA, Rockland, Maine, USA) and stained with ethidium bromide. The PCR amplification conditions used for m1/m2 vacA are described by us elsewhere. Past or actual H pylori infection in the remaining 60 non-cardia gastric cancer patients was established on the basis of positive findings from serum anti-H pylori antibodies (Inova Diagnostics, San Diego, California, USA).

**Genomic DNA extraction and PCR amplification**

Genomic DNA was extracted from 3 ml blood samples using the QIAamp DNA blood midi kit (Qiagen, Hilden, Germany). Promoters, coding sequences, splicing sites, and UTR regions of IL12A and IL12B were PCR amplified using the primers listed in table 1. Briefly, 100 ng of extracted DNA were amplified in a 50 µl final reaction volume under the following conditions: 1×DNA polymerase gold buffer (Applied Biosystems), 1.5 mM MgCl2, 200 µM each dNTPs, 500 nM each primer, and 2.5 U AmpliTaq gold (Applied Biosystems). PCR conditions were: 94°C for seven minutes, then 40 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds, and finally 72°C for 20 minutes.

**DHPLC analyses**

PCR amplicons were analysed using a DHPLC screening strategy to detect any sequence polymorphisms (Transgenomic, Omaha, Nebraska, USA). This system uses the principles of reverse phase ion pair high performance liquid chromatography (RP-IP-HPLC) to carry out analytical separations of heteroduplexes. Table 1 reports temperatures for DHPLC analysis. The types of DNA polymorphism and their positions were defined by fluorescence dye terminator cycle sequencing on an ABI PRISM 310 genetic analyser (Applied Biosystems). Data were analysed with Sequencing Analysis 3.3 Software.

**Capillary electrophoresis**

IL12A intron 6 and IL12B intron 4 VNTRs polymorphisms were studied by capillary electrophoresis. Samples (100 ng) of genomic DNA were PCR amplified in a total reaction volume of 20 µl containing 1×PCR buffer with 1.5 mM MgCl2, 200 µM each dNTPs, 1.25 U AmpliTaq DNA polymerase (Applied Biosystems), and 500 nM of the following dye labelled primer pairs: HEX-5′agcttgcttggggacaatc3′ and 5′taagcagacctgctttag3′, 500 nM primer S1/2-F and S1/2-R (5′gacatcactatacaggaag3′ and 5′tgaaaaccacgctctttag3′), and 600 nM primers S1/2-F and S1/2-R (5′atggaataacacaacacac3′ and 5′ctctgtaagggeccaaa3′) for IL12A, and FAM-5′caatacatacagagtctc3′ and 5′agcttgcttggggacaatc3′, and 5′ctctgtaagggeccaaa3′ for IL12B. The temperature profile was: pre-PCR denaturation of seven minutes at 94°C; 30 seconds at 94°C; 30 seconds at 55°C; and one minute at 72°C for 30 cycles, and a final extension of 30 minutes at 72°C. Samples for capillary electrophoresis were prepared using 2 µl of amplified products, added to 20 µl distilled formamide, 500 ROX size standard (Applied Biosystems). The mixtures were heated at 95°C for three minutes and snap cooled at 4°C for three minutes. The samples were detected by using an ABI PRISM 310 genetic analyser with a 47 cm capillary. Results were analysed using GeneScan Analysis software 3.7. The expected size ranges of the two polymorphisms were: IL12A VNTR (149–161 bp), IL12B VNTR (237–245 bp).

Allelic ladders for each VNTR were constructed by allele sequencing carried out on DNAs from homozygous samples.

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**Figure 1** Association between IL12A variable number tandem repeats (VNTR) and IL12A *+6686* genotypes. We genotyped IL12A VNTRs by considering separately the most common alleles ([GT]18, [GT]20, and [GT]21) and combining all the remaining alleles as “others” (OTH).
### Table 3  Variable number tandem repeat (VNTR) allele frequencies of IL12A intron 6 and of IL12 B intron 4 found in the present series

<table>
<thead>
<tr>
<th>IL12A VNTR</th>
<th>No of (GT)$_n$ repeats</th>
<th>Allele frequency (%)</th>
<th>IL12B VNTR</th>
<th>No of (TG-TA)$_n$ repeats</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT)$_1$</td>
<td>11</td>
<td>0.1</td>
<td>(TG-TA)$_9$</td>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>(GT)$_2$</td>
<td>12</td>
<td>0.2</td>
<td>(TG-TA)$_9$</td>
<td>9</td>
<td>24.1</td>
</tr>
<tr>
<td>(GT)$_5$</td>
<td>15</td>
<td>0.5</td>
<td>(TG-TA)$_{10}$</td>
<td>10</td>
<td>3.3</td>
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<tr>
<td>(GT)$_6$</td>
<td>16</td>
<td>5.6</td>
<td>(TG-TA)$_{11}$</td>
<td>11</td>
<td>67.6</td>
</tr>
<tr>
<td>(GT)$_7$</td>
<td>17</td>
<td>5.9</td>
<td>(TG-TA)$_{12}$</td>
<td>12</td>
<td>3.9</td>
</tr>
<tr>
<td>(GT)$_8$</td>
<td>18</td>
<td>37.2</td>
<td>(TG-TA)$_{13}$</td>
<td>13</td>
<td>0.2</td>
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<tr>
<td>(GT)$_9$</td>
<td>19</td>
<td>9.7</td>
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<tr>
<td>(GT)$_{20}$</td>
<td>20</td>
<td>21.3</td>
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<td>(GT)$_{21}$</td>
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<td>(GT)$_{22}$</td>
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<tr>
<td>(GT)$_{23}$</td>
<td>23</td>
<td>0.8</td>
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</table>

### Table 4  Variable number tandem repeat (VNTR) genotype frequencies of IL12A intron 6 and of IL12 B intron 4 found in the present series

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No of (GT)$_n$ repeats</th>
<th>Genotype frequency (%)</th>
<th>Genotype</th>
<th>No of (TG-TA)$_n$ repeats</th>
<th>Genotype frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{18}$</td>
<td>18/18</td>
<td>6.4</td>
<td>(TG-TA)$<em>{19}$/ (TG-TA)$</em>{19}$</td>
<td>9/9</td>
<td>6.1</td>
</tr>
<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{20}$</td>
<td>18/20</td>
<td>17.8</td>
<td>(TG-TA)$<em>{19}$/ (TG-TA)$</em>{11}$</td>
<td>9/11</td>
<td>31.5</td>
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<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{21}$</td>
<td>18/21</td>
<td>12.7</td>
<td>(TG-TA)$<em>{19}$/ (TG-TA)$</em>{others}$</td>
<td>9/others</td>
<td>4.1</td>
</tr>
<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{20}$/ (GT)$_{20}$</td>
<td>20/20</td>
<td>4.1</td>
<td>(TG-TA)$<em>{19}$/ (TG-TA)$</em>{others}$</td>
<td>11/others</td>
<td>3.8</td>
</tr>
<tr>
<td>(GT)$<em>{20}$/ (GT)$</em>{21}$</td>
<td>20/21</td>
<td>4.8</td>
<td>(TG-TA)$<em>{19}$/ (TG-TA)$</em>{others}$</td>
<td>Others/others</td>
<td>4.1</td>
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<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{others}$</td>
<td>20/others</td>
<td>11.5</td>
<td>(GT)$<em>{20}$/ (GT)$</em>{21}$</td>
<td>21/13</td>
<td>1.3</td>
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<td>(GT)$<em>{18}$/ (GT)$</em>{others}$</td>
<td>21/others</td>
<td>7.3</td>
<td>(GT)$<em>{20}$/ (GT)$</em>{21}$</td>
<td>21/others</td>
<td>7.3</td>
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<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{others}$</td>
<td>Others/others</td>
<td>8.6</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The most common alleles were considered singly, while the remaining were considered overall and classified as "others".

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**Statistical analysis**

Statistical analysis of data involved the χ² test, logistic regression analysis, and the non-parametric Mann–Whitney U test.

**RESULTS**

*H. pylori* infection was diagnosed histologically in 36.4% of patients with non-evident endoscopic lesions, 46.3% with antral gastritis, 43.6% with diffuse gastritis, 72.4% with duodenal ulcer, 77.8% with gastric ulcer, 36.4% with duodenitis, 60% with reflux gastritis, and 36.4% with oesophagitis. In patients with non-cardia gastric cancer, *H. pylori* infection was established in 69.4% of the non-cardia gastric cancer patients for whom tissue samples were available; in the remaining 60 non-cardia gastric cancer patients, *H. pylori* infection was established in 69.4% of the non-cardia gastric cancer patients. With DHPLC screening we identified three SNPs: two located in *IL12A* and one in *IL12B* gene polymorphisms. While the *IL12A* −504 T/G genotype was correlated with the *IL12A* −6686 G/G genotype, the *IL12A* −504 T/G genotype was correlated with the *IL12A* −6686 A/A or A/G genotypes; no association was found between *IL12A* −504 and *IL12B* +15485 (χ² = 1.18, NS) or between *IL12A* −6686 and *IL12B* +15485 (χ² = 4.05, NS). The number of GT repeats in intron 6 of *IL12A* (IL12A VNTR) and the number of TG-TA repeats in intron 4 of *IL12B* (IL12B VNTR) varied widely when patients were considered overall. Table 3 shows the VNTR allele frequencies of *IL12A* and *IL12B* found in the present series. We genotyped *IL12A* and *IL12B* VNTRs by selecting the most common alleles ((GT)$_{18}$/ (GT)$_{20}$, and (GT)$_{21}$ for *IL12A* VNTR; (TG-TA)$_{9}$ and (TG-TA)$_{11}$ for *IL12B* VNTR) and combining them, as reported in table 4. The *IL12A* VNTR genotypes (GT)$_{18}$/ (GT)$_{20}$, (GT)$_{18}$/ (GT)$_{21}$, (GT)$_{20}$/ (GT)$_{20}$, and (GT)$_{20}$/ (GT)$_{21}$ were significantly correlated with *IL12A* −504 T/T, whereas (GT)$_{18}$/ (GT)$_{others}$, (GT)$_{20}$/ (GT)$_{others}$, and (GT)$_{others}$/ (GT)$_{others}$ were correlated with the *IL12A* −504 T/G genotype (χ² = 68.59, p < 0.001). The *IL12B* VNTR genotypes (GT)$_{18}$/ (GT)$_{18}$, (GT)$_{18}$/ (GT)$_{others}$, (GT)$_{20}$/ (GT)$_{others}$, and (GT)$_{others}$/ (GT)$_{others}$ were correlated with the *IL12A* −6686 G/G genotype, whereas (GT)$_{18}$/ (GT)$_{18}$, (GT)$_{20}$/ (GT)$_{20}$, and (GT)$_{20}$/ (GT)$_{21}$/ (GT)$_{others}$ were correlated with the *IL12A* −6686 A/G genotype (χ² = 244.51, p < 0.001) (fig 1). The *IL12B* VNTR genotype was correlated...
IL12 and gastric cancer

![Graph](http://jmg.bmj.com/)

**Figure 2.** Associations between IL12A – 504 SNP and cagA or antral activity in H pylori positive patients.

with IL12B +15485 (χ² = 24.04, p<0.01); in particular, the genotypes (TG-TA)₉/(TG-TA)₉, (TG-TA)₉/(TG-TA)₁₁, and (TG-TA)₁₁/(TG-TA)₁₁, were correlated with IL12B +15485 A/A, whereas the (TG-TA)₁₁/(TG-TA)₁₁ genotype was correlated with IL12B +15485 A/G and G/G (χ² = 24.04, p<0.01).

**IL12A and IL12B polymorphisms and H pylori infection**

IL12A and IL12B SNPs or VNTRs were not correlated with H pylori infection, cagA, s or m vacA, antral or body inflammation, and antral activity. Body activity was correlated with IL12B VNTR: mild to moderate body activity was more commonly recorded in patients with the (TG-TA)₁₁/ (TG-TA)₁₁ genotype (χ² = 47.75, p<0.001).

In relation to H pylori positive patients, the only associations we found were those between IL12A – 504 SNP and cagA (Fisher’s exact test, p<0.05; odds ratio = 2.34, 95% confidence interval (CI), 0.91 to 5.98) or IL12A – 504 SNP and antral activity (χ² = 8.83, p<0.05) (Fig 2).

**IL12A and IL12B polymorphisms and H pylori associated diseases**

Among the five IL12 genetic polymorphisms studied, and when considering the patients overall, no statistically significant association was found with the disease diagnosis. The patients were then subdivided into two main groups: those with non-cardia gastric cancer and those with benign diseases. H pylori infection was more often recorded in non-cardia gastric cancer patients (69.4% v 49.0%) (Fisher’s exact test: p<0.01). On considering H pylori infected patients only, non-cardia gastric cancer was correlated with cagA (Fisher’s exact test, p<0.05) and s1 vacA (Fisher’s exact test, p<0.05).

The frequency of non-cardia gastric cancer was higher in patients with the IL12A – 504 T/T genotype (Fisher’s exact test, p<0.05) and in those with IL12B VNTR (TG-TA)₉/(TG-TA)₁₁ genotype (χ² = 12.40, p<0.05) (Fig 3).

Table 5 shows the association between IL12 VNTR polymorphism and the risk of gastric cancer. We selected subjects homozygous for the most common allele (TG-TA)₁₁ as the reference group in the initial odds ratio estimations. The IL12B VNTR (TG-TA)₉/(TG-TA)₁₁ genotype was confirmed to increase the risk of gastric cancer, while (TG-TA)₁₁/other/other, was associated with a decreased risk of gastric cancer. When performing the logistic regression analysis considering the diagnosis of gastric adenocarcinoma as a dependent variable and the gene polymorphisms of IL12A – 504, IL12B VNTR, and cagA as predictors, the odds ratios were 2.38 (95% CI, 0.96 to 5.88), 1.36 (1.05 to 1.76), and 1.68 (0.84 to 3.35), respectively. None of the IL12 gene polymorphisms studied was correlated with Lauren’s non-cardia gastric cancer classification.

In relation to patients with benign diseases, we analysed the association between the precancerous intestinal metaplasia and H pylori infection, H pylori virulence genes, and IL12 gene polymorphisms. Intestinal metaplasia was significantly correlated with H pylori infection (χ² = 10.05, p<0.01), cagA (χ² = 28.70, p<0.001), s1 vacA (χ² = 26.18, p<0.001), and with m1 vacA (χ² = 6.64, p<0.01), but not with IL12A or IL12B gene polymorphisms.

**DISCUSSION**

Using the DHPLC screening strategy followed by direct sequencing we identified the following polymorphisms: IL12A – 504 T/G, IL12A +6686 A/G, and IL12B +15485 A/C. The first SNP was located in the promoter region of the IL12A gene, while the latter two were localised in the UTR regions of the two genes. These three polymorphisms were in Hardy-Weinberg equilibrium.
The wide range of the dinucleotide repeats for both VNTRs implies an excessive data dispersion whenever a statistical analysis is to be carried out. To overcome this limitation other investigators have classified the VNTR alleles of IL1RN on the basis of their length (long/short). This classification is feasible for IL1RN intron 2 VNTR, as each repeat corresponds to only 24 bp, and therefore, in agreement with Cai et al, who described the GT dinucleotide repeat polymorphism of the oestrogen receptor α gene, we classified the VNTR genotypes by considering the combinations of the most frequent alleles (three for IL12A and two for IL12B) and by grouping the less frequent alleles as "others". Interestingly, significant associations were found between the VNTR genotypes and the SNPs of the corresponding gene. These associations might be the expression of linkage or of a selective advantage for some combinations over others.

None of the IL12A or IL12B polymorphisms and VNTRs studied was correlated with the presence or absence of H pylori infection. We therefore suggest that the establishment of H pylori infection depends on bacterial characteristics (urease production, expression of adhesins) more than on host cytokine gene polymorphisms, including IL12—although a role has suggested for TNFα –308 G to A transition. By contrast, cytokine gene polymorphisms may correlate with H pylori virulence genes in H pylori infected subjects. It has already been reported that TNFα polymorphisms are associated with infections from the more virulent cagA positive strains. In the present paper, another association was recorded: between cagA and the IL12A –504 T/G genotype. This finding may be explained on the basis that an association between cag PAI and IL12 has already been reported: in infections caused by cagA positive strains, enhanced gastric mucosal transcription of IL12B is recorded, and enhanced release of IL12 by dendritic cells in vitro is found after exposure to cagE positive strains. IL12A –504 polymorphism might be involved in the regulation of gene expression and, consequently, of IL12 production. This nucleotide is contained within a site possibly recognised by the transcription factor AP2 (GCCT^G,GGG), with the penultimate base in our sequence being an A instead of a G. Using the program MATRIX SEARCH 1.0, which allows a search for potential transcription factor binding sites, the sequence with the G allele was recognised for AP2 binding with a match ratio of 0.74, the range which denotes lack of exact match being 0.0 to 1.0. With the T allele, no match was found. The AP2 transcription factor can, in turn, be induced by IL6, potentely stimulated by H pylori. The IL12A –504 T/G genotype was also correlated with gastritis activity, which is scored on the basis of the degree of infiltrating polymorphonuclear cells. This finding might either be spurious and consequent on the prevalence of cagA positive infecting strains recorded in patients with this genotype, or be the result of an enhanced release of IL12, which is known to stimulate the production by T cells and natural killer cells of GM-CSF.

We also investigated whether there was any association between IL12A or IL12B polymorphisms and H pylori associated diseases. After the patients had been subdivided into two groups—one consisting of those with non-cardia gastric cancer and the other of those with benign gastroduodenal diseases—non-cardia gastric cancer was found to be correlated with the IL12A –504 T/T genotype and with the IL12B VNTR (TG-TA)9/(TG-TA)11 genotype, but also with H pylori infection and its virulence determinants, cagA and s1 vacA. For a better definition of the contribution of IL12 gene polymorphisms in enhancing non-cardia gastric cancer risk in H pylori infected subjects, logistic regression analysis was undertaken and both cagA, IL12A –504 and IL12B VNTR polymorphisms were confirmed as risk factors for non-cardia gastric cancer. The onset of non-cardia gastric cancer can be considered the result of a process in which the complex interplay between H pylori infection, host genetic background, and environmental factors creates conditions favouring or counteracting carcinogenesis. Taking into account the above hypotheses concerning the involvement of IL12A –504 SNP in the regulation of gene transcription, in T/T homozygote subjects the transcription factor AP2 should have a limited transcriptional effect on IL12, and this might lead to reduced IL12 production. It is known that lack of IL12 production results in a reduction in host resistance to infections and tumours and that treatment with IL12 has a marked anti-tumour effect on mouse carcinomas. It is more difficult to interpret the association between IL12B VNTR (TG-TA)9/(TG-TA)11 and (TG-TA)9 others/(TG-TA)11 others genotypes and gastric cancer risk. Increasing numbers of intronic VNTRs have been found to interfere with transcription processes, either by their effect on secondary DNA structure, by their action as protein binding sites, or by their influence on the transcription or stability of mRNA. In addition, the VNTR polymorphism may be in linkage disequilibrium with exon alterations that may affect protein function.

**Table 5 Association between IL12B VNTR polymorphism and gastric cancer risk**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case (gastric adenocarcinoma)</th>
<th>Control (benign gastroduodenal diseases)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TG-TA)11/(TG-TA)11</td>
<td>47</td>
<td>111</td>
<td>1.00</td>
<td>Reference</td>
</tr>
<tr>
<td>(TG-TA)9/(TG-TA)11</td>
<td>8</td>
<td>11</td>
<td>1.42</td>
<td>0.79 to 2.53</td>
</tr>
<tr>
<td>(TG-TA)9/(TG-TA)11</td>
<td>42</td>
<td>57</td>
<td>1.43</td>
<td>1.02 to 1.99</td>
</tr>
<tr>
<td>(TG-TA)9/(TG-TA)9</td>
<td>3</td>
<td>10</td>
<td>0.77</td>
<td>0.28 to 2.15</td>
</tr>
<tr>
<td>(TG-TA)11/(TG-TA)9</td>
<td>4</td>
<td>8</td>
<td>0.89</td>
<td>0.39 to 2.06</td>
</tr>
<tr>
<td>(TG-TA)11/(TG-TA)11</td>
<td>47</td>
<td>111</td>
<td>1.00</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Subjects homozygous for the most common allele (TG-TA)11 formed the reference group in the initial odds ratio estimations. *p<0.05,
C, confidence interval; OR, odds ratio.
Non-cardia gastric cancer, “intestinal-type” in particular, is the end stage of a series of lesions which apparently represent a continuum of changes from normal to carcinoma.39 These include, in order of increasing severity, chronic gastritis, chronic atrophic gastritis, intestinal metaplasia, and dysplasia. In agreement with the multistep process hypothesis, patients with intestinal metaplasia are at increased risk of non-cardia gastric cancer.40 We therefore looked for an association between the presence or absence of intestinal metaplasia and IL12 gene polymorphisms on the one hand, and H pylori infection and its virulent determinants on the other. A strong correlation was found between intestinal metaplasia and H pylori infection, cagA, and vacA. None of the host genetic IL12 gene polymorphisms studied was correlated with intestinal metaplasia. This indicates that they do not play a role in determining this histopathological alteration. The association between IL12 polymorphisms and gastric cancer risk, but not with intestinal metaplasia, suggests that this cytokine probably plays an important role in the progression of intestinal metaplasia to dysplasia and cancer. IL12 gene polymorphisms may act by allowing dysplastic cells, originated from a normal mucosa or from intestinal metaplasia, to escape immune surveillance. This concept is in agreement with the lack of any association between IL12 gene polymorphisms and non-cardia gastric cancer histological subtype, which are thought to be associated (intestinal type) or not (diffuse type) with the precancerous intestinal metaplasia.39

Conclusions

IL12A and IL12B gene polymorphisms may affect the latest steps of gastric carcinogenesis in H pylori infected subjects. The exact mechanism underlying this phenomenon has yet to be defined, although it is reasonable to suggest that IL12A –504 SNP and IL12B VNTR may modulate IL12 production in response to H pylori infection.

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Competing interests: none declared

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