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

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Methods

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).

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 gastrointestinal 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.

Abbreviations:

DHPLC, denaturing high performance liquid chromatography; EGDS, (o)esophagogastroduodenoscopy; IL, interleukin; SNP, single nucleotide polymorphism; Th, T helper; VNTR, variable number tandem repeats.
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’gcccaagtgtctgcaacoa3’</td>
<td>–941/-922</td>
<td>Promoter</td>
<td>63, 64</td>
</tr>
<tr>
<td>5’tgagaggacgactgagc3’</td>
<td>–451/-469</td>
<td>Promoter</td>
<td>59, 6</td>
</tr>
<tr>
<td>5’gtgcctgtgcacagctc3’</td>
<td>–520/-501</td>
<td>Promoter</td>
<td>61, 8</td>
</tr>
<tr>
<td>5’ggaggacgagggacaacoa3’</td>
<td>–313/-294</td>
<td>Promoter</td>
<td>65, 5, 6, 7</td>
</tr>
<tr>
<td>5’gggctggttggatgcac3’</td>
<td>–71/-89</td>
<td>Promoter</td>
<td>69, 3</td>
</tr>
<tr>
<td>5’actgcacaggtctgcgac3’</td>
<td>–171/-152</td>
<td>Promoter</td>
<td>63, 8</td>
</tr>
<tr>
<td>5’ggaggagttctgaaagc3’</td>
<td>–22/-41</td>
<td>Promoter</td>
<td>69, 3</td>
</tr>
<tr>
<td>5’ggtgaggagtgagctg3’</td>
<td>–93/-75</td>
<td>Promoter and exon 1</td>
<td>63, 8</td>
</tr>
<tr>
<td>5’agtccgggaaagtcctg3’</td>
<td>+19/-36</td>
<td>Exon 1</td>
<td>65, 0; 67, 0</td>
</tr>
<tr>
<td>5’gagggcgcaacgagctg3’</td>
<td>+213/-119</td>
<td>Exon 2</td>
<td>63, 2</td>
</tr>
<tr>
<td>5’gagggcgacgactcaca3’</td>
<td>+1357/-1340</td>
<td>Exon 3</td>
<td>54, 9, 57, 9</td>
</tr>
<tr>
<td>5’ggtgagacctgccatccg3’</td>
<td>+369/-1398</td>
<td>Exon 4</td>
<td>56, 52; 57, 78</td>
</tr>
<tr>
<td>5’ccctggctgagggcaacoa3’</td>
<td>+4688/-4629</td>
<td>Exon 5</td>
<td>56, 52; 57, 78</td>
</tr>
<tr>
<td>5’tggagcttcccctcaaca3’</td>
<td>+4673/-4693</td>
<td>Exon 6</td>
<td>57, 7</td>
</tr>
<tr>
<td>5’gttacaggagaggctgc3’</td>
<td>+4888/-4869</td>
<td>Exon 7</td>
<td>56, 51; 57, 4</td>
</tr>
<tr>
<td>5’tgacaggagagaggagac3’</td>
<td>+6368/-6390</td>
<td>UTR</td>
<td>55, 0</td>
</tr>
<tr>
<td>5’gctagagttctgctagatcc3’</td>
<td>+6924/-6902</td>
<td>UTR</td>
<td>55, 0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Gene SNP</th>
<th>Genotype (frequency)</th>
<th>HWE</th>
</tr>
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<tbody>
<tr>
<td><strong>IL12A -504</strong></td>
<td>G/G (0.01)</td>
<td>G/T (0.22)</td>
</tr>
<tr>
<td><strong>IL12A -6686</strong></td>
<td>A/A (0.03)</td>
<td>A/G (0.22)</td>
</tr>
<tr>
<td><strong>IL12A -15485</strong></td>
<td>A/A (0.03)</td>
<td>A/C (0.23)</td>
</tr>
</tbody>
</table>

HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.
culture and genotyping. An EDTA-K$_3$ 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: TNM IA (4); TNM IB (7); TNM II (30); TNM IIIA (18); TNM IIIB (10); and TNM IV (41). According to the Lauren description of gastric 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 _H pylori_ 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), 1.5 mM MgCl$_2$, 200 µM each dNTPs, 500 nM primer URE-A F and URE-A R (5'gacatcactatacaggaag3' and 5'ngaaccacgcitctag3'), 160 nM primer Cag-A F and Cag-A R (5'ccaatcaccagcctec3' and 5'gaccttggtgggacaec3'), 600 nM primer S1/2-F and S1/2-R (5'aggaatcacaacaaaca3' and 5'tcggtaggtggcac3').

2-R (5'aggaatcacaacaaaca3' and 5'tcggtaggtggcac3') and 2.5 U AmpliTaq gold (Applied Biosystems). The thermocycling conditions were: 95°C for six minutes, then 40 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 40 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/s2 vacA_, respectively) were separated by electrophoresis on 2% NuSieve 1% Seakem 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 MgCl$_2$, 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

_II12A_ intron 6 and _II12B_ 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 MgCl$_2$, 200 µM each dNTP, 1.25 U AmpliTaq DNA polymerase (Applied Biosystems), and 500 nM of the following dye labelled primer pairs: HEX-5'tagggcgtaggtgataaa3' and 5'aagctttgtctcataac3' for _II12A_, and FAM-5'caggg gaaaggtgtgt3' and 5'tcggtgccagattttaa3' for _II12B_. 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 deionised formamide and 0.5 µl GeneScan−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×50 µm capillary. Results were analysed using GeneScan Analysis software 3.7. The expected size ranges of the two polymorphisms were: _II12A_ VNTR (149–161 bp), _II12B_ VNTR (237–245 bp).

Allelic ladders for each VNTR were constructed by allele sequencing carried out on DNAs from homozygous samples.
Weinberg equilibrium (Table 2). The tions were defined by sequencing. All SNPs were in Hardy (genotype was extremely rare (1% of the cases) and for this reason the four patients with this genotype were excluded from some of the statistical analyses. A significant association was found between IL12A +504 and IL12A +6686 SNPs ($\chi^2 = 16.52$, $p<0.001$). In particular, the IL12A +504 T/G genotype was correlated with the IL12A +6686 G/G genotype, while the IL12A +504 T/T genotype was correlated with IL12A +6686 A/A or A/G genotypes; no association was found between IL12A +504 and IL12B +15485 ($\chi^2 = 1.18$, NS) or between IL12A +6686 and IL12B +15485 ($\chi^2 = 4.05$, 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)$_{21}$, (GT)$_{20}$(GT)$_{20}$, and (GT)$_{20}$(GT)$_{21}$ were significantly correlated with the IL12A +504 T/T, whereas (GT)$_{18}$(GT)$_{18}$, (GT)$_{18}$(GT)$_{20}$, and (GT)$_{18}$(GT)$_{19}$ were correlated with the IL12A +504 T/G genotype ($\chi^2 = 68.59$, $p<0.001$). The IL12B VNTR genotypes ($GT_{18}$(GT)$_{18}$/ (GT)$_{18}$, (GT)$_{18}$(GT)$_{19}$, (GT)$_{20}$(GT)$_{18}$, and (GT)$_{20}$(GT)$_{19}$ were correlated with the IL12B +6686 G/G genotype, whereas (GT)$_{20}$(GT)$_{20}$, (GT)$_{20}$(GT)$_{21}$, and (GT)$_{21}$(GT)$_{21}$ were correlated with the IL12B +6686 A/G genotype ($\chi^2 = 244.51$, $p<0.001$) (fig 1). The IL12B VNTR genotype was correlated

### Table 3 Variable number tandem repeat (VNTR) allele frequencies of IL12A intron 6 and of IL12B 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)$_{m}$ repeats</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT)$_{18}$</td>
<td>18/18</td>
<td>12.4</td>
<td>(TG-TA)$_{9}$</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)$<em>{9}$(TG-TA)$</em>{11}$</td>
<td>9/11</td>
<td>31.5</td>
</tr>
<tr>
<td>(GT)$<em>{18}$/ (GT)$</em>{21}$</td>
<td>18/21</td>
<td>12.7</td>
<td>(TG-TA)$<em>{9}$(TG-TA)$</em>{others}$</td>
<td>9/others</td>
<td>4.1</td>
</tr>
<tr>
<td>(GT)$<em>{20}$(GT)$</em>{18}$</td>
<td>20/20</td>
<td>4.1</td>
<td>(TG-TA)$<em>{9}$(TG-TA)$</em>{others}$</td>
<td>11/others</td>
<td>30.3</td>
</tr>
<tr>
<td>(GT)$<em>{20}$(GT)$</em>{21}$</td>
<td>20/21</td>
<td>4.8</td>
<td>(TG-TA)$<em>{9}$(TG-TA)$</em>{others}$</td>
<td>11/others</td>
<td>3.8</td>
</tr>
<tr>
<td>(GT)$<em>{20}$(GT)$</em>{18}$</td>
<td>20/others</td>
<td>11.5</td>
<td>(TG-TA)$<em>{9}$(TG-TA)$</em>{others}$</td>
<td>11/others</td>
<td>4.1</td>
</tr>
<tr>
<td>(GT)$_{20}$</td>
<td>21/11</td>
<td>13.3</td>
<td>(GT)$<em>{9}$(GT)$</em>{18}$</td>
<td>11/11</td>
<td>1.3</td>
</tr>
<tr>
<td>(GT)$<em>{20}$/ (GT)$</em>{21}$</td>
<td>21/others</td>
<td>7.3</td>
<td>(GT)$<em>{9}$(GT)$</em>{18}$</td>
<td>11/others</td>
<td>4.1</td>
</tr>
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<td>(GT)$_{20}$</td>
<td>21/others</td>
<td>8.6</td>
<td>(GT)$<em>{9}$(GT)$</em>{20}$</td>
<td>11/others</td>
<td>4.1</td>
</tr>
</tbody>
</table>

The most common alleles were considered singly, while the remaining were considered overall and classified as "others."
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)₉/(TG-TA)others 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% vs 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)₀/(TG-TA)₁₁ genotype (χ² = 12.40, p < 0.05) (Fig 3).

Table 5 shows the association between IL12B 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)₀/(TG-TA)₁₁ genotype was confirmed to increase the risk of gastric cancer, while (TG-TA)₀/others 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 m 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 two genes coding for the two IL12 subunits were also analysed for the number of dinucleotide repeats located in intron 6 and intron 4 for IL12A and IL12B, respectively. These VNTRs were studied bearing in mind that the amount of secreted protein might vary not only in relation to differences in the nucleotide sequence of the promoter or of the coding regions, but also in relation to intron VNTR, as already described for IL1RN,30 although the exact mechanism linking intron VNTR and protein synthesis has not yet been completely defined. The number of GT repeats of IL12A VNTR varied in our patients from a minimum of 11 to a maximum of 23, while TG-TA of IL12B ranged from eight to 13.

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).32 This classification is feasible for IL1RN intron 2 VNTR, as each repeat corresponds to an oligonucleotide sequence of 86 bp, but it is difficult to apply to the present VNTRs as the maximum difference between the shorter and the longer alleles corresponds to only 24 bp. Therefore, in agreement with Cai et al.,30 who studied 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.33 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.21 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 reported15: 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 (GCCG12GGG), with the penultimate base in our sequence being an A instead of a G.34 Using the program MATRIX SEARCH 1.0,35 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 IL6,36 potently stimulated by *H pylori*.11–13 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.37

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.15–16 Taking into account the above hypotheses concerning the involvement of IL12A –504 SNP in the regulation of gene transcription, in T/H homoygote subjects the transcription factor AP2 should have a limited transcriptional effect on IL12A, 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 tumours38 and that treatment with IL12 has a marked anti-tumour effect on mouse carcinomas.39 It is more difficult to interpret the association between IL12B VNTR (TG-TA)9/(TG-TA)11 and (TG-TA)short/(TG-TA)short genotypes and gastric cancer risk. Increasing numbers of intrinsic 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.24 In addition, the VNTR polymorphism may be in linkage disequilibrium with exon alterations that may affect protein function.
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. 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. 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 subtypes, which are thought to be associated (intestinal type) or not (diffuse type) with the precancerous intestinal metaplasia.

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. IL12A→504 SNP and IL12B VNTR may modulate IL12 production in response to H pylori infection.

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