Identification of a genetic marker of Helicobacter pylori strains involved in gastric extranodal marginal zone B cell lymphoma of the MALT-type

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Background and aims: Gastric extranodal marginal zone B cell lymphoma of the mucosa associated lymphoid tissue (MALT)-type (MZBL) is a rare complication of Helicobacter pylori infection. Currently, no bacterial factor has been associated with the development of this disease. Our aim was to identify genes associated with lymphoma development.

Methods: We used subtractive hybridisation as a tool for comparative genomics between H pylori strains isolated from a patient with gastric MZBL and from a patient with gastritis only.

Results: When gastric MZBL strains were compared with gastritis strains, two open reading frames (ORFs) were significantly associated with gastric MZBL: JHP950 (74.4% v 48.7%, respectively; p = 0.023) and JHP1462 (25.6% v 2.6%, respectively; p = 0.004). The prevalence of JHP950 was 48.8% (p = 0.024) in duodenal ulcer strains and 39.3% (p = 0.006) in gastric adenocarcinoma strains, which makes this ORF a specific marker for gastric MZBL strains. In contrast, the prevalence of JHP1462 was 16% (p = 0.545) and 35.7% (p = 0.429) in duodenal ulcer and adenocarcinoma strains, respectively. These ORFs were present in reference strain J99 but not in reference strain 26695. JHP950 is located in the plasticity zone whereas the other, JHP1462, is located outside. Both encode for H pylori putative proteins with unknown functions.

Conclusion: Despite its low prevalence, the ORF JHP1462 can be considered a candidate marker for H pylori strains involved in severe gastroduodenal diseases. In contrast, the ORF JHP950 has a high prevalence, and is the first candidate marker for strains giving rise to an increased risk of gastric MZBL strains. Further confirmation in other studies is needed.
Subtractive hybridisation

The PCR select bacterial genome subtraction kit (Clontech, Palo Alto, California, USA) was used with the “tester” (gastric MZBL) and “driver” (gastritis) strains. The manufacturer’s recommendations were followed with the exception of the choice of the restriction enzyme and the method of evaluation of the subtractive efficacy. The restriction enzyme AluI was chosen because of its ability to generate DNA fragments, from “tester” and “driver” strains, in the same molecular weight range (from 2.5 kb to 0.25 kb). The efficacy of subtractive hybridisation was evaluated using amplification of a fragment of H pylori 16S rDNA in a real time PCR experiment in a LightCycler thermocycler (Roche Diagnostics, Penzberg, Germany). The efficacy was determined by direct sequencing and dot blot hybridisation between “tester” (gastric MZBL) and “driver” (gastritis) DNAs, as described below.

Screening of the subtractive library

To determine if the subtractive clones were really “tester” specific, primers for each clone were designed using the conserved sequence between the sequence identified and the corresponding gene of H pylori in database sequences (http://www.probes.toulouse.inra.fr/multialin/multialin.html).26 The specificity of our primers was tested on both H pylori reference DNAs. For the sequences with no homology found in the databases, the specificity of the amplified fragment was determined by direct sequencing and dot blot hybridisation between “tester” (gastric MZBL) and “driver” (gastritis) DNAs, as described below.

If a PCR product was amplified from the “tester” DNA and not from the “driver” DNA, the sequence was considered to be “tester” specific. PCR amplifications were carried out in a 25 μl volume containing 2.5 μl of Taq buffer (Eurobio, Les Ulis, France), 1.5 mM MgCl2 (Eurobio), 200 μM (each) of the deoxynucleoside triphosphates (Eurobio), 2 U of Taq DNA polymerase (Eurobio), 1 μM (each) of the primers (Q Biogen, Strasbourg, France) and 10 ng of H pylori DNA. After four minutes of initial denaturation at 94°C, each reaction mixture was amplified for 35 cycles as follows: 30 seconds at 94°C, 30 seconds of annealing at 60°C, and extension at 72°C (the time depending on the length of the sequence to be amplified and the processivity of the Taq polymerase). After the last cycle, an extension was continued for another 7–10 minutes. PCR products were separated electrophoretically on agarose gels and stained with ethidium bromide.

Dot blot hybridisation

Screening of the PCR sequences specific for the “tester” strain was performed using our collection of H pylori gastric MZBL and gastritis strains. Dot blot analyses were carried out using the same batch of membranes, prepared with the same dilution of DNA. Genomic DNA (100 ng) of each strain (MZBL, gastritis, J99, and 26695 reference strains) were spotted on a Hybond N+ membrane (Amersham Pharmacia Biotech) by means of a Bio-Rad dot blot 96 well filtration system apparatus (Bio-Rad, Ivry-sur-Seine, France). Hybridisation was performed using digoxigenin labelled probes of each ORF identified, as described previously.27 The probes were prepared by PCR after primer design in a conserved area between the “tester” DNA sequence and the J99 reference strain or between both the J99, 26695, and “tester” strain when the DNA sequence was present in all.

Reverse transcription-PCR

RNA was extracted from the “tester” strain and the reference strain J99 during the exponential growth phase using the RNeasy kit (Qiagen). RNA samples were treated with Rnase-Free Dnase (Promega, Madison, Wisconsin, USA) and quantified spectrometrically. Each reverse transcription PCR (RT-PCR) was carried out in a 10 μl volume containing 250 ng of RNA template, 0.6 μM of each oligonucleotide primer, 0.125 μl of RNAseIn (Promega), 2 μl of 5× Q buffer, and 2 μl of RT-Taq mix. Complementary DNA was synthesised at 45°C for 30 minutes following by 95°C heat inactivation of the RT Taq polymerase for 15 minutes, and then by the amplification program used for PCR amplification. Absence of DNA in RNA preparations was verified by omitting the RT amplification step. After 40 PCR cycles, products were separated electrophoretically on agarose gels.
Table 1 Summary of sequence analysis of “tester” specific DNA (gastric MZBL) fragments from Helicobacter pylori used for dot blot

<table>
<thead>
<tr>
<th>Clone</th>
<th>Strain J99</th>
<th>Strain 26695</th>
<th>Function</th>
<th>MZBL strains (n = 43)</th>
<th>Gastritis strains (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>A</td>
<td>HJP305</td>
<td>HP322</td>
<td>Putative poly E-rich protein†</td>
<td>9 20.9</td>
<td>10 25.6</td>
</tr>
<tr>
<td>B</td>
<td>HJP428</td>
<td>HP476</td>
<td>Predicted glutamyl-tRNA synthetase†</td>
<td>41 76.4</td>
<td>38 97.4</td>
</tr>
<tr>
<td>C</td>
<td>HJP1031</td>
<td>HP1105</td>
<td>Putative LPS biosynthesis protein</td>
<td>30 69.8</td>
<td>32 82.1</td>
</tr>
<tr>
<td>D</td>
<td>HJP1044</td>
<td>HP1116</td>
<td>Putative</td>
<td>17 39.5</td>
<td>11 28.2</td>
</tr>
<tr>
<td>E</td>
<td>HJP1285</td>
<td>HP1371</td>
<td>Putative</td>
<td>39 90.7</td>
<td>34 87.2</td>
</tr>
<tr>
<td>F</td>
<td>HJP1301</td>
<td>HP1409</td>
<td>Putative</td>
<td>16 37.2</td>
<td>14 35.9</td>
</tr>
<tr>
<td>G</td>
<td>HJP1424</td>
<td>HP1402</td>
<td>Type I RE [restriction subunit]†</td>
<td>40 93.0</td>
<td>33 84.6</td>
</tr>
<tr>
<td>H</td>
<td>HJP820</td>
<td>None</td>
<td>Predicted 1,2-glycosyltransferase†</td>
<td>16 37.0</td>
<td>22 56.4</td>
</tr>
<tr>
<td>I</td>
<td>HJP921†</td>
<td>None</td>
<td>Predicted DNA TC ComB8 homologue†</td>
<td>21 48.8</td>
<td>14 35.9</td>
</tr>
<tr>
<td>J</td>
<td>HJP922*</td>
<td>None</td>
<td>Predicted DNA TC ComB8 homologue†</td>
<td>25 58.1</td>
<td>16 41.0</td>
</tr>
<tr>
<td>K</td>
<td>HJP946*</td>
<td>None</td>
<td>Putative</td>
<td>24 55.8</td>
<td>25 64.1</td>
</tr>
<tr>
<td>L</td>
<td>HJP949*</td>
<td>None</td>
<td>Putative</td>
<td>17 39.5</td>
<td>14 35.9</td>
</tr>
<tr>
<td>M</td>
<td>HJP950*</td>
<td>None</td>
<td>Putative</td>
<td>32 74.4</td>
<td>19 48.7</td>
</tr>
<tr>
<td>N</td>
<td>HJP960*</td>
<td>None</td>
<td>Putative</td>
<td>23 53.5</td>
<td>20 51.3</td>
</tr>
<tr>
<td>O</td>
<td>HJP961*</td>
<td>None</td>
<td>Putative</td>
<td>23 53.5</td>
<td>19 48.7</td>
</tr>
<tr>
<td>P</td>
<td>HJP1297</td>
<td>None</td>
<td>Putative type III RE</td>
<td>43 100.0</td>
<td>36 92.3</td>
</tr>
<tr>
<td>Q</td>
<td>HJP1409</td>
<td>None</td>
<td>Putative type III DNA ME (methyltransferase)</td>
<td>33 76.7</td>
<td>33 84.6</td>
</tr>
<tr>
<td>R</td>
<td>HJP1462</td>
<td>None</td>
<td>Putative</td>
<td>11 25.6</td>
<td>2 6.2</td>
</tr>
</tbody>
</table>

p values were determined by Fisher’s exact test.
*Open reading frame (ORF) localised in the plasticity zone.
†Revised annotation according to Boneca and colleagues.**
MZBL, extranodal marginal zone B cell lymphoma of the MALT-type; LPS, lipopolysaccharide; RE, restriction enzyme; ME, modification enzyme; TC, transformation competence.

Statistical analysis

Statistical analysis was performed using STATA 7.0 statistical software (Stata Corporation, College Station, Texas, USA). A first comparison of the characteristics between MZBL and gastritis strains was performed using Fisher’s exact test.

In order to create a variable describing how the different strain characteristics were associated, a multiple correspondence analysis (MCA) was performed followed by a cluster analysis using the average aggregation method and Euclidian distance.28 Both methods used the Statbox 2.5 program (Grimmer Logiciels, Paris, France).

The odds of an association with MZBL and its 95% confidence interval (CI) were compared within groups of strains and between the different strata of the summary variables obtained by the MCA.

RESULTS

PCR analysis of the subtractive clones

We sequenced the inserts of 132 clones on both strands, of which 81 were non-redundant. PCR screening between “tester” (gastric MZBL) and “driver” (gastritis) DNAs of these 81 clones showed that 39 (48.2%) contained sequences which were specific to the “tester” strain used in the experiment. Homologies of these clones with the H pylori databases (http://www.tigrblast.tigr.org/cmr-blast) and the NCBI blast program (http://www.ncbi.nlm.nih.gov/BLAST) were analysed.

The following classification was made: 26 matched with sequences from the two reference strains (J99 and 26695), 10 matched with only J99 specific sequences, none matched with only strain 26695, and three presented no homology with H pylori reference strains.

For 10 of the 26 sequences which matched with the two reference strains, PCR products obtained from the “tester” DNA were different from the expected size when compared with the “driver” DNA and H pylori J99 and 26695 reference DNAs. Direct sequencing of these products showed significant variations, such as insertions or deletions, and therefore they were not included in the dot blot screening of our strain collections to avoid impairing the analysis. Nine other sequences where variation corresponded to intergenic regions were also not tested. The seven remaining sequences (clones

![Figure 1](http://gut.bmj.com) Expression of the open reading frames (ORFs) HJP950 and HJP1462 of Helicobacter pylori determined by reverse transcription-polymerase chain reaction (RT-PCR) in the “tester” strain. Lanes M1 and M2 correspond to 40X1.74 DNA/HaeIII markers (Eurobio) and to the 1 kb DNA ladder (Promega), respectively. Lanes 1, 4, and 7 correspond to PCR control on genomic DNA. Lanes 2, 5, and 8 correspond to RT-PCR product obtained on RNA extracted from the “tester” strain. Lanes 3, 6, and 9 correspond to PCR results on RNA preparations without the RT amplification step. Lanes 1–3, 4–6, and 7–9 correspond to templates amplified for HJP950, HJP1462, and vacA genes, respectively.

A–G (table 1) were distributed throughout the H pylori genome and were included in our dot blot experiments.

Among the 10 sequences matching with only strain J99, six were localised in the plasticity zone (clones I–N) and one of these corresponded to a deletion of two ORFs (that is, HJP960 and HJP961). These two ORFs were therefore PCR amplified using J99 DNA and also tested by dot blot. The four remaining sequences (clones H, O, P, and Q) were distributed throughout the H pylori J99 genome.

Analysis of the three original sequences allowed us to consider them as potentially coding but they did not present nucleic and protein homologies in databases to assign them a function (to be published elsewhere). These three sequences were included in our dot blot experiment.
Dot blot analysis of the selected clones (MALT v gastritis)
The distribution of the 24 selected sequences was tested by
dot blot using our collection of \textit{Helicobacter pylori} gastric MZBL and
gastritis strains. The specificity of the sequences found to be
“tester” specific by PCR was verified by (1) the absence of
hybridisation on the plot corresponding to the “driver” strain
and (2) the absence of hybridisation for the original sequences
on the plots corresponding to the “driver” DNA.

The 21 remaining sequences were then tested. The corres-
ponding genes and results obtained are summarised in table 1.
The proportion of each ORF was compared between strains
with gastric MZBL and with gastritis only. Two ORFs were signif-
cantly associated with gastric MZBL strains: JHP950 (74.4\% v
48.7\%, respectively; \(p = 0.023\)) and JHP1462 (25.6\% v
2.6\%, respectively; \(p = 0.004\)). JHP950 was located in
the plasticity region of strain J99 whereas JHP1462 was
located outside. These two ORFs encoded for two \textit{H pylori}
specific proteins of unknown function.

Study of the transcription of JHP950 and JHP1462
ORFs in gastric MZBL strain
RT-PCR was performed for JHP950 and JHP1462 using the
primers designed for PCR screening of our subtractive library
in order to verify if they were transcribed in our “tester”
strain. The \textit{vacA} gene was included as a positive control of RT-
PCR using primers previously published.\textsuperscript{27} The results showed
that both were expressed (fig 1).

Multiple correspondence analysis
Firstly, an MCA was performed for all variables obtained from
dot blot. We were unable to identify gene clusters which were
significantly associated with gastric MZBL or gastritis strains.
Thus the analysis was focused on the two ORFs found
associated with the gastric MZBL strains. Because the
prevalence of JHP1462 in gastric MZBL and gastritis strains
was low, it was impossible to keep it in our final analysis. In
contrast, the ORF JHP950 was included in the data from our
previous study in which we identified a gene cluster in gastric
MZBL strains, which is absent in gastritis strains, and
containing \textit{iceA1}, \textit{sabA} “on”, and \textit{hpZ} “off”.\textsuperscript{19} Interestingly,
the dendrogram obtained from the results of the cluster
analysis of the \textit{H pylori} gastritis MZBL strain population showed
that the ORF JHP950 was included in the formally identified
gene cluster (data not shown). The possible association
between the presence of gastric MZBL and the characteristics
of strains cultured from these patients was tested. On analysis
of the summary variables according to the MCA results, it was
remarkable that the odds of having gastric MZBL among
patients harbouring JHP950, \textit{iceA1}, and \textit{sabA} “on” strains were
10 times higher than for the other defined groups (odds ratio
10.3 (95\% CI 1.2–86.0)) (table 2); indeed among a total of 11
strains having these three markers, 10 (90.9\%) corresponded
to gastric MZBL strains (\(p = 0.018\)) (fig 2).

Verification of the specificity of the two potential
markers
The specificity of the association of the ORFs JHP950 and
JHP1462 with gastric MZBL strains was evaluated by
determining their prevalence in a collection of duodenal
ulcer strains \((n = 41)\) by dot blot analysis, as described above.
The prevalence in duodenal ulcer strains of ORFs JHP950 and
JHP1462 was 48.8\% (\(p = 0.024\)) and 16\% (\(p = 0.545\)),
respectively.

The prevalence of ORFs JHP950 and JHP1462 on strains
isolated from 28 gastric adenocarcinoma was also deter-
mimed. As DNA obtained from these strains was not
concentrated enough to perform dot blot analysis, PCR
screening was performed with the primers used for dot blot
probe synthesis. A prevalence of 39.3\% (\(p = 0.006\)) for
JHP950 and of 35.7\% (\(p = 0.429\)) for JHP1462 ORFs was
found (table 3).

These results confirm that JHP950 is a specific marker for
\textit{H pylori} strains isolated from gastric MZBL (table 3).

DISCUSSION
Following sequencing of the whole genome of two strains of
\textit{H pylori}, the possibility arose to study the genetic diversity of

\begin{table}
\centering
\caption{Strength of the association of \textit{Helicobacter pylori} genotypes and extranodal marginal zone B cell lymphoma of the MALT-type (gastric MZBL) status versus gastritis status}
\begin{tabular}{|c|c|c|c|}
\hline
ORF & Total No of patients & Patients with MZBL & Patients with gastritis & OR (95\% CI) \\
\hline
JHP950 & 82 & 43 & 39 & \\
Absent & 31 & 11 & 20 & \\
Present & 51 & 32 & 19 & 3.1 (1.2–7.8) \\
MCA summary variables & 70 & 39 & 31 & \\
Others & 59 & 29 & 30 & \\
\textit{iceA1}, \textit{sabA} “on”, and JHP950+ & 11 & 10 & 1 & 10.3 (1.2–86.0) \\
\hline
\end{tabular}
\end{table}
Subtractive hybridisation was later validated by H pylori. Our hypothesis was that H pylori strains using DNA arrays. Indeed, this approach is tempting but limits the possibilities to the genes already known. Our hypothesis was that H pylori pathogenesis in gastric MZBL could be linked to currently unidentified bacterial factors, and therefore that subtractive hybridisation represented the most accurate approach.

Subtractive hybridisation is now considered an important tool for comparative prokaryotic genomics. Most of the studies performed previously compared bacterial strains with apparent enhanced capacity for human infection and/or interpatient transmission and colonisation. For example, genomic differences have been identified in a virulent strain of Klebsiella pneumoniae as well as in the uropathogenic Escherichia coli strain 536 compared with the non-pathogenic strain MG1655, and more recently a genomic locus was identified and characterised in the Brazilian pupuric fever strain MG1655, and more recently a genomic locus was represented the most accurate approach.

The first category of sequences identified presented apparent enhanced capacity for human infection and/or infection begins in the stomach. Indeed, following the discovery of the CagA protein with the epithelial cell, it is now considered to be a true virulence factor associated with severe clinical outcome (that is, duodenal ulceration and gastric adenocarcinoma). Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, the CagA protein with the epithelial cell, it is now considered to be a true virulence factor associated with severe clinical outcome (that is, duodenal ulceration and gastric adenocarcinoma). Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains.

Table 3: Prevalence of JHP950 and JHP1462 open reading frames (ORFs) in Helicobacter pylori strains isolated from patients with gastritis, duodenal ulcer, and gastric adenocarcinoma in comparison with strains isolated from patients with gastric MZBL

<table>
<thead>
<tr>
<th>Gastritis</th>
<th>Duodenal ulcer</th>
<th>Gastric adenocarcinoma</th>
<th>Gastric MZBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>JHP950</td>
<td>48.7 (p = 0.023)*</td>
<td>48.8 (p = 0.024)*</td>
<td>39.3 (p = 0.006)*</td>
</tr>
<tr>
<td>JHP1462</td>
<td>2.6 (p = 0.004)*</td>
<td>16.0 (p = 0.545)</td>
<td>35.7 (p = 0.429)</td>
</tr>
</tbody>
</table>

*Significant values versus gastric MZBL strains.

In a subtractive hybridisation experiment, the choice of strains to be compared is particularly important. Considering the fact that the natural evolution of H pylori infection begins with gastritis and moreover that host susceptibility factors of developing gastric MZBL are currently unknown, this choice is especially difficult. We based our choice on the pathology (gastric MZBL versus gastritis) and age of the patients from whom the strains were isolated (29 year old patient with gastric MZBL, 74 year old patient suffering from gastritis). The advanced age of the “driver” strain patient was chosen to minimise the possibility that gastritis would evolve to more severe gastrointestinal disease. As the mean age of patients suffering from gastric MZBL is close to 50 years, we could anticipate a particular pathogenic property in the tester strain issued from a much younger patient. Furthermore, the two strains were chosen because of their common profile for six known H pylori virulence genes—that is, cagA+, cagE+, vacA S1m1, babA2, icaA1, and mipA “on”, and one adhesin sabA “on”.

MCA analysis, initially carried out on all of our dot blot data, failed to identify a gene cluster associated with gastric MZBL strains. However, despite the fact that our collection of gastric MZBL strains is the largest of those published, the number of strains included in this study may not be sufficient to efficiently perform MCA.

The first category of sequences identified presented significant homologies with genes present in strains J99 and JHP1462 but with important deletions or insertions. Such variations can modify the functional status of a gene or the functionality of the deduced protein. Moreover, it has been suggested that mixtures of matching and divergent sequences can facilitate quite dramatic changes in bacterial phenotype. We originally expected to find new H pylori genes and indeed we identified three sequences of unknown genomic localisation, corresponding to new putative coding regions. Nevertheless, none of these sequences was significantly associated with gastric MZBL. Such results could not have been found using a standard approach for detecting genetic diversity, and thus our findings provide more information for the future in investigating the particular properties of these strains.

The results of the dot blot experiments (MZBL versus gastritis) highlighted two ORFs (JHP950 and JHP1462). Globally, repartition of both ORFs (JHP950 and JHP1462) was statistically different (p = 0.016 and p = 0.002, respectively) when comparing the four H pylori strain collections studied.

The prevalence of ORF JHP1462 (table 3) in MZBL strains was indeed low (25.6%) but when compared with gastritis strains the difference was highly significant (p = 0.004). However, no significant difference was found between gastric MZBL strains and duodenal ulcer and gastric adenocarcinoma strains (table 3), indicating that JHP1462 is not a specific genetic marker for H pylori gastric MZBL strains but could be considered more generally as a candidate marker for H pylori strains associated with severe gastroduodenal diseases. It is located outside the plasticity zone, in common with two thirds of H pylori strain specific genes.

In contrast, the prevalence of the JHP950 ORF in gastric MZBL strains was significantly higher than in strains isolated from duodenal ulcer and gastric adenocarcinoma. The difference in prevalence for this marker was in the same range as the difference between the prevalence of cagA in duodenal ulcer and gastric adenocarcinoma strains compared with gastritis strains. Indeed, in the past we looked for the cagA gene in relation to clinical presentation in a collection of 167 H pylori strains. The cagA gene was found in 82.6% of duodenal ulcer strains versus 63.5% of gastritis strains. These results were confirmed later in several European studies. The cagA gene was first considered as a marker of virulence for H pylori. Indeed, following the discovery of the cag pathogenicity island and of the interaction of the CagA protein with the epithelial cell, it is now considered to be a true virulence factor associated with severe clinical outcome (that is, duodenal ulceration and gastric adenocarcinoma). Similarly, the ORF JHP950 is part of the plasticity zone of strain J99. This region is approximately 45 kb long in strain J99 and 68 kb long in strain 26695, and contains one third of the strain specific genes. Occhialini et al studied several ORFs in the plasticity region of strain J99 by hybridisation and found that most were functional. We verified by RT-PCR that JHP950 and JHP1462 were transcribed. They were originally annotated as H pylori specific proteins with unknown functions (that is, they have no orthologs in other bacterial sequences). This classification is

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still accurate after publication of a revised annotation and comparative analysis of \(H\) pylori genomes.\(^{46}\) Our results are in line with the study of Salama et al who found that the most important class of strain specific genes was more frequent among strain specific genes (58\%) than in the core of the genome (21\%) which contains genes encoding, for example, metabolic functions.\(^{47}\)

A limit of our study was the use of PCR instead of hybridisation for detection of the ORFs JHP950 and JHP1462 in adenocarcinoma strains. Indeed, there are very few collections of \(H\) pylori strains obtained from patients with gastric adenocarcinoma in Europe. The DNA material obtained did not allow us to perform dot blot hybridisation but only detection by PCR. The latter technique can have false negative results because of a lack of primer binding due to limited differences in sequences. Therefore, we tested the accuracy of our method on our strain collections and found a sensitivity of 90\% (JHP950) and 95\% (JHP1462) versus dot blot hybridisation, which confirms the relevance of our approach.

MCA analysis allowed us to show that the JHP950 marker is part of a gene cluster significantly associated with gastric MZBL strains. This gene cluster, which is comprised of JHP950, icaA1 alleles, and a sab\(A\) “on” status, partially validates our initial findings.\(^{31}\) The sab\(A\) gene encodes for an adhesin. The association of JHP950 with icaA1, a gene activated by contact with the gastric epithelium, and an adhesin seems to be in line with the function of these genes.\(^{32} 40\) This underlines the fact that JHP950 might have functional importance during interaction of the bacterium with cells. We can hypothesise that if JHP950 corresponds to a new \(H\) pylori virulence factor, \(H\) pylori strains harbouring this triple association of genes could have a pathogenic advantage. Indeed, the odds of having gastric MZBL among patients harbouring JHP950, icaA1, and sab\(A\) “on” strains were 10 times higher than for the other defined groups. Koehler et al recently reported that the vacA m2 allele was the predominant subtype in MALT lymphoma.\(^{20}\) We also previously reported a slight association between m2 genotype and MALT strains (when considering the m genotype only) but we found no association between vacA s1m2 alleles and icaA1 genotypes.\(^{46}\) However, we share the similar conclusion (that is, certain \(H\) pylori subtype combinations have a predictive value for the development of gastric MZBL).

In conclusion, if JHP1462 cannot be considered a specific genetic marker of gastric MZBL strains but rather as a new candidate marker of \(H\) pylori virulent strains in general, in contrast JHP950 can be considered as the first candidate marker of gastric MZBL \(H\) pylori strains. The association of JHP950 with gastric MZBL strains is similar to that between cagA and duodenal ulcer strains. Moreover, JHP950 forms a gene cluster with icaA1 and sab\(A\) “on” which is associated with gastric MZBL strains. Future studies are needed to confirm this association and to understand the biological function of this ORF in order to gain insight into the pathogenesis of \(H\) pylori and the development of gastric MZBL.

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REFERENCES


A 62 year old woman was admitted to Elche University General Hospital for further evaluation of an intermittent right upper quadrant pain unrelated to meals or posture. She had previously been well with no history of liver disease, alcohol abuse, or abdominal trauma. On physical examination, there were no signs of chronic liver disease or portal hypertension. Results of laboratory studies, liver function tests, and ultrasound guided liver biopsy were also normal. Real time and colour Doppler ultrasonography showed an anechoic mass-like lesion at the porta hepatitis with continuous non-pulsatile flow within the lesion. The gallbladder was normal with no gallstones visualized. Then, a helical computed tomography (CT) scan was performed.

**Question**

What does this investigation show (fig 1).

**See page 943 for answer**

This case is submitted by:

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**Figure 1** Contrast computed tomography showing a high signal intensity mass over the hilum of the liver, suggesting an aneurysmal dilatation of the portal vein.
Identification of a genetic marker of *Helicobacter pylori* strains involved in gastric extranodal marginal zone B cell lymphoma of the MALT-type

P Lehours, S Dupouy, B Bergey, A Ruskoné-Foumestraux, J C Delchier, R Rad, F Richy, J Tankovic, F Žerbib, F Mégraud and A Ménard

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**Notes**