Analysis of iceA genotypes in South African Helicobacter pylori strains and relationship to clinically significant disease

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Abstract

Background—South African Helicobacter pylori isolates are characterised by the universal presence of cagA but have differences in vacuolating cytotoxin gene (vacA) alleles which correlate with clinically significant disease. However, the candidate virulence marker gene iceA has not been investigated.

Aim—To characterise the genetic organisation and heterogeneity of iceA genotypes in different South African clinical isolates.

Patients and methods—We studied H pylori strains isolated from 86 dyspeptic patients (30 with peptic ulcer disease (PUD), 19 with distal gastric adenocarcinoma (GC), and 37 with non-erosive gastritis) for the presence of iceA1 or iceA2 genes, and for differences in the genetic organisation of iceA2 by polymerase chain reaction, Southern hybridisation analysis, and sequencing.

Results—Genetic analysis of iceA1 demonstrated significant homology (92–95%) with the USA type strain 26695 and probably functions as a transcriptional regulator, while a novel variant (iceA2D') of iceA2 and marked differences in predicted protein secondary structure of the iceA2 protein were defined. iceA1 was detected in 68% and iceA2 in 80% of all clinical isolates. Although approximately 40% of patients had both strains, a higher prevalence (p < 0.01) of GC patients were infected with iceA1 isolates which were invariably vacA s1/iceA1 (p < 0.005 v gastritis). Isolates from PUD patients were distinguished by the structurally altered iceA2D variant (53%; p < 0.03 v gastritis) while the iceA2C variant distinguished isolates from patients with gastritis alone (67%; p < 0.005 v PUD).

Conclusion—In this study, an association between iceA1 and GC was noted while differences in variants of iceA2 differentiated between PUD and gastritis alone. Combination analyses of iceA genotypes and vacA alleles supported these associations.

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Keywords: adenocarcinoma; gastritis; Helicobacter pylori; iceA; peptic ulceration; protein prediction; sequencing; South Africa

Helicobacter pylori induces gastric inflammation in virtually all colonised individuals and such gastritis increases the risk of peptic ulcer disease (PUD) and non-cardia/distal gastric adenocarcinoma (GC). However, only a minority of patients carrying H pylori develop clinical sequelae, suggesting that particular bacterial products may contribute to pathogenesis. Recently, a novel H pylori gene iceA was identified following transcriptional upregulation on contact with gastric epithelial cells. iceA exists as two distinct genotypes, iceA1 and iceA2, and only iceA1 RNA is induced following adherence in vitro. H pylori iceA1 demonstrates strong homology to a restriction endonuclease nlaIIIR in Neisseria lactamica, and in vivo carriage of H pylori iceA1 strains has been reported to be associated with peptic ulceration and enhanced acute neutrophil infiltration. However, linkage between the iceA1 genotype and ulcer disease is not universal, and thus may be population dependent.

In contrast with iceA1, iceA2 has no significant homology to known proteins and its structure reveals patterns of repeated protein cassettes. Recently, the genetic organisation and sequence heterogeneity of iceA2 has been studied, revealing five distinct iceA2 subtypes. While iceA2 strains are more prevalent among patients with asymptomatic gastritis and non-ulcer dyspepsia, a statistically significant relationship between iceA2 subtypes and disease has not yet been defined.

South African H pylori isolates are characterised by the universal presence of cagA but differences in the 3′ region of cagA and vacA alleles correlate significantly with clinical disease. Our hypothesis was that H pylori isolates from South African patients may also exhibit variability in their iceA alleles and that this variability may be related both to clinical outcome and to vacA allelic status.

Materials and methods

H pylori strains and DNA

H pylori isolates, cultured from single biopsies from 86 clinically defined patients (37 patients with gastritis alone, 30 patients with PUD, and 19 patients with GC), were examined. Fifty six of the resultant isolates had vacA and cagA determined in a previous study. Putative clonal isolates were obtained from eight of the parent strains by serially reculturing single colonies twice. Reference strains 26695 (iceA1) were used.

Abbreviations used in this paper: PUD, peptic ulcer disease; GC, gastric adenocarcinoma; PCR, polymerase chain reaction; SDS, sodium dodecyl sulphate; DAS, dense alignment surface; SSC, sodium chloride/sodium citrate.

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isolated from a patient in the UK with gastritis and J99 (iceA2) isolated from a USA patient with duodenal ulcer disease were used as iceA1 and iceA2 positive and negative controls, respectively, in this study.

**AMPLIFICATION OF iceA BY PCR**

Polymerase chain reactions (PCRs) were performed as described previously but a final [MgCl2] concentration of 3.5 mM was used for the iceA1 PCRs. For amplification of the iceA1 allele, forward primer IceA1F (5'-CGTTGGGTAGAAGGTCAAGAAATTTT) and reverse primer IceA1R (5'-TCATTGTATATCTATTTAT) yielded a fragment of 558 bp (fig 1). For iceA2, primers IceA2F2 (5'-GTGTTGGTCTTGGTATTTTAATGAA) and IceA2R (5'-GTTACTTTCGGACAGGTTAA) yielded a fragment of 120 bp. PCR was performed in a Sprint PCR (Hybaid, South Africa) under the following conditions: four minutes of preincubation at 94°C followed by 30 cycles of one minute at 94°C, one minute at 62°C (iceA1) or 53°C (iceA2), and one minute at 72°C. Final extension was performed for 10 minutes at 72°C.

Additional genotype specific PCR assays that used primers for iceA1 (IceA1F5 (5'-GTGTTTTTTAACCCAAGAT) and IceA1R4 (5'-CTATAACCTASTYTTCTTGTGA)) and primers flanking the iceA2 internal cassette were performed to confirm the presence of the genes and to identify the size and subtype of the iceA2 allele. Primers IceA2F6 (5'-GTGTTGGTCTTGGTATTTTAATGAA) and IceA2R3 (5'-TTRCCCTATTTTCTAGTGGT) yielded a fragment of 229, 334, or 439 bp according to the existence of repeated sequences of 105 nucleotides. PCR was performed under the following conditions: four minutes of preincubation at 94°C followed by 30 cycles of one minute at 94°C, one minute at 53°C (iceA1) and iceA2), and one minute at 72°C. Final extension was performed for 10 minutes at 72°C. iceA amplimers were examined by electrophoresis on 1% agarose gels according to standard procedures.

**SEQUENCING OF iceA1 AND iceA2**

The complete iceA1 gene, from the upstream cysE (HP1210) to the downstream hypIM (HP1208), was amplified using F1 (5'-GGG TGGATTTTTCGGTGCGCATGATG) and R10 (5'-GATCATGGCCTAACCAGCATGGA), as described previously, while iceA2 was amplified using primer sets IceA2F6/R5. PCR products were gel extracted (QIAEX II gel extraction kit; Qiagen, Cape Town, South Africa) and sequenced on an ABI PRISM 377 automated sequencer (ABI, Foster City, California, USA) using the ABI PRISM BigDye terminator cycle sequencing reagent kit with AmpliTaq DNA polymerase FS (PE Biosystems, Johannesburg, South Africa), as described previously. PCR and direct sequencing were performed at least twice to determine DNA sequences for each strain.

**SOUTHERN HYBRIDISATION ANALYSIS**

Southern hybridisation analysis was performed essentially as described previously. Chromosomal DNA digested with BglII was subjected to electrophoresis on a 0.8% Tris acetate/EDTA agarose gel, transferred to a Hybond-N membrane (AP Biotech, Piscataway, New Jersey, USA) by capillary transfer, and UV cross linked. Prehybridisation was performed using 6× sodium chloride/sodium citrate (SSC), 5× Denhardt’s, 0.5× sodium dodecyl sulphate (SDS), and 100 µg/ml of sheared salmon sperm DNA at 60°C for three hours. Buffer was replaced with fresh buffer containing denatured probe and hybridisation proceeded at 60°C for 18 hours. Membranes were washed for five minutes with 2× SSC, 0.5× SDS at room temperature, 15 minutes with 2× SSC, 0.1% SDS at room temperature, two hours with 0.2% SSC, 0.5% SDS at 60°C, and 30 minutes with 0.2% SSC, 0.5% SDS at 60°C. The membranes were then subjected to autoradiography for detection.

Probes for iceA1 and iceA2 were generated by PCR using primer pairs IceA1F5/IceA1R4 and IceA2F6/IceA2R5, respectively, as described previously, with 26695 and J99 chromosomal DNA as respective templates. Products were purified using QiаQuick PCR purification columns (Qiagen, Valencia, California, USA) and labelled with [α-32P]dCTP using the PRIME-IT RmT labelling kit as recommended by the manufacturer (Stratagene, La Jolla, California, USA). Unincorporated nucleotides were removed using ProbeQuant G-50 microcolumns according to the manufacturer’s instructions (AP Biotech). Probe was denatured by boiling for five minutes and then placed on ice prior to hybridisation.

**COMPUTER ANALYSIS**

DNA sequences were analysed using the National Center for Biotechnology Information (NCBI) server (USA) and consensus secondary structure prediction for proteins was obtained using the META Predict Protein (which includes the SignalP, JPRED, TopPred, and DAS Servers (Columbia University, USA)). SignalP predicts the presence and location of signal peptide cleavage sites, while DAS predicts secondary structures, TopPred predicts secondary structures, and DAS Servers (Columbia University, USA). JPRED is useful for prediction of the location and orientation of transmembrane sequences through the use of hydrophobicity patterns and by applying the “positive inside” rule, while DAS predicts the location of transmembrane using hydrophobicity patterns. Internet based
searches were preformed at NCBI, the Institute for Genome Research (TIGR, Maryland, USA), and Astra-Zeneca (Boston, USA).

STATISTICS

Data were examined using the $\chi^2$ test or Fisher's exact test as appropriate. Probability levels of <0.05 were considered statistically significant.

Results

iceA1 SEQUENCE ANALYSIS

In a preliminary study, in order to establish the composition of iceA gene products in South African H pylori isolates, iceA1 (primers F1/R10: 952 bp) and iceA2 (primers F6/R5: 229–334 bp) were sequenced.

Five isolates with the iceA1 genotype (two from patients with gastritis alone, two from patients with peptic ulceration, and one from patients with gastric cancer) were sequenced and compared with the type strains 60190 (GenBank Accession No U43917) and 26695. Alignment of these sequences revealed the presence of a putative conserved initiation codon at position ATG919 in all five South African strains as well as conserved upstream transcription initiation sites, as previously reported (fig 2). Predicted proteins ranged in size from 128 to 136 amino acids, except for strain 54 (frameshift results in a protein of 53 amino acids). All isolates shared substantial protein homology (93–96%) with HP1209. Sequence gaps are indicated by dashes (–).

iceA2 SEQUENCE ANALYSIS

Seven isolates with the iceA2 genotype (two from patients with gastritis alone, two from patients with PUD, and three from patients with GC) were sequenced and compared with the predicted gene (JHP1132) present at the same locus in strain J99 (fig 3). Prior to our analysis, five different variants (A–E) based on size (24, 59, 94, or 129 amino acids) and/or sequence of iceA2 had been described. The iceA2 gene (JHP1132) from strain J99 which conforms to the structure of repeats (14–13–162B–6–10) demonstrated by Figueiredo and colleagues generates a predicted protein of 59 amino acids and is an example of the iceA2B subtype. None of the South African isolates sequenced in this series had either a 2A structure (14–13–16–6–10) or a
Table 1: Protein characteristics of iceA variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>JHP1132</th>
<th>HP27</th>
<th>Cai506</th>
<th>1715</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids (n)</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Cysteine (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proline (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Topology: N terminus</td>
<td>&quot;in&quot;</td>
<td>&quot;in&quot;</td>
<td>&quot;in&quot;</td>
<td>&quot;in&quot;</td>
</tr>
<tr>
<td>TopPred</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
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</tbody>
</table>

DAS

<table>
<thead>
<tr>
<th>Variant</th>
<th>JHP1132</th>
<th>HP27</th>
<th>Cai506</th>
<th>1715</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position (score)</td>
<td>58-78 (0.758)</td>
<td>23-41 (0.615)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: Amino acid alignment of iceA2 genotypes from seven South African isolates and homology with JHP1132. The different peptide domains are boxed and the amino acid number indicated. Asterisks (*) represent gaps in sequence homology.

2B structure. Two isolates (Hp27 and 215) however had a 14-13-16-6-10 motif and were therefore classified as iceA2C subtypes. Four isolates had the pattern 14-13-16-6-13-16-6-10 and are examples of the ice A2D subtype. These subtypes would be predicted to generate proteins of 94 amino acids but one isolate (Ca102) had an inframe three nucleotide deletion within cassette 16β which resulted in a putative protein truncation of 93 amino acids. Sequence analysis of a fifth isolate (1715) demonstrated a novel variant of the iceA2D subtype that was typified by the sequence 14-13-16-6-13-16-6-10 (sequence identity 71% and shared residues 80%) with iceA2D.

Analysis of the protein signal sequence, secondary structures, and topology for the South African iceA2 variants is provided in table 1. Of note are the observations that these proteins do not have a typical prokaryote signal sequence (from the SignalP server), and the N terminus appears to be embedded within the cell membrane. The TopPred topology program demonstrated that variants 2B and 2C had no predicted transmembrane regions while 2D and the novel 2D variant (2D') had a single putative transmembrane region with scores of 0.758 and 0.615, respectively (fig 4). The dense alignment surface (DAS) method confirmed that variant 2B had no transmembrane region but suggested a possible transmembrane region in 2C and confirmed two putative transmembrane regions in 2D and the 2D' variant. Variant 2B can be classified as a mixed class protein but 2C, 2D, and 2D' are all all beta proteins.

CLINICAL RELEVANCE OF iceA GENOTYPES

After validating the PCR protocol and examining the structure of iceA1 and iceA2, PCR was performed in clinical isolates and products were analysed in terms of the distribution of genotypes. Fifty four isolates were positive for either iceA1 or iceA2 using previously described primers IceA1F/1R and IceA2F/2R. 54 isolates were positive for all genotypes and one isolate did not yield any PCR product for either of the iceA genotypes. Amplicons of the respective sizes were obtained from reference strains 26695 and J99 as expected. Twenty one of the 54 iceA1+/iceA2+ isolates were re-examined with different iceA1 primer sets (F5/R4 and F6/R5 (iceA2)) to determine whether this “mixed genotype” was a consistent finding. All 21 (100%) were positive for both genotypes, indicating that these primer sets are concordant. These isolates each had a single vacA allele, a single cagA amplicon, which suggested the presence of multiple iceA genotypes in these strains.

We then examined the presence of these putative “mixed genotypes” further by performing repeat PCR (using two primer sets) and Southern hybridisation in multiply recultured single colonies from eight iceA1+/iceA2+ parent strains. Seven of the eight isolates had a single iceA allele by both PCR and Southern analysis (fig 5). No hybridisation was seen in one isolate. A single PCR band using primer sets IceA2F/2R/IceA2R5 (which identifies iceA2 sizes and subtypes) and a single Southern band was seen in the three clonal strains that were iceA2+. These data signify the presence of multiple iceA alleles rather than mixed genotypes within a single strain in these cultures.

The finding that the 54 iceA1+/iceA2+ strains were mixed cultures suggested identification of both iceA1+ and iceA2+ strains from a single gastric biopsy. Fifty four (63%) of the 86 patients were therefore infected with a single iceA strain. Examination of the distribution of iceA alleles in patients with single strains demonstrated that the distribution differed between the clinical groups (χ² 7.13, p<0.03). Specifically, significantly more patients with GC (10 (67%) of 15) were infected with iceA1 strains compared with five (23%) of 22 with gastritis alone (p<0.01) (fig 6). This was not different to patients with PUD (seven (41%) of 17; p>0.1).
Analysis of South African iceA genotypes

The type strain JHP1132 (iceA2B variant) is indicated in the top panel and South African strains are included with the iceA2 variants signified (2C–2D'). Arrows indicate putative transmembrane regions.

Figure 4  Topology profiles of four iceA2 variants. Left: protein predictions from the TopPred program; right: predictions from the dense alignment sequence (DAS) program. The type strain JHP1132 (iceA2B variant) is indicated in the top panel and South African strains are included with the iceA2 variants signified (2C–2D'). Arrows indicate putative transmembrane regions.

Figure 5  Southern analysis of putatively clonal isolates derived from eight parent strains (lanes 1–8) that were originally iceA1’/2’. polymarker chain reaction. Four were iceA1’, three iceA2’, and one had no hybridisation. Positive controls (lanes 9 and 10) were 26695 (iceA1’) and J99 (iceA2’).

Examination of the distribution of iceA genotypes in all 86 patients demonstrated a significant relationship (p<0.05) between infection with iceA2 strains and patients with gastritis alone (86%) and PUD (77%) compared with patients with GC (47%).

**DISTRIBUTION OF iceA2 SUBTYPES**

The size and subtype of the iceA2 alleles were next investigated using primer set IceA2F6/IceA2R5. As previously reported,

**COMBINATION OF iceA, vacA, AND cagA GENOTYPES**

All strains were cagA’ and gave a single 3’ amplicon. By using the method of van Doorn and colleagues,

**Figure 6**  Distribution of iceA alleles in all isolates. **p<0.01 versus gastritis alone; fp<0.04, ffp<0.003 versus gastric cancer.

**Figure 7**  Distribution of iceA2 subtypes in patients with gastritis alone or peptic ulcer disease.

to the presence of 105 nucleotide repeat sequences which resulted in PCR products of either ~229 bp (iceA2B or iceA2C) or ~334 bp (iceA2D). A number of isolates had either both 229 and 334 bp products or a combination of 229 bp and/or 334 bp and ~449 bp (iceA2E) products. Subsequent analysis of three clonally derived isolates from the latter group demonstrated only single PCR and Southern bands (fig 5), which suggested the existence of multiple iceA2 strains, rather than multiple iceA2 gene copies in these isolates.

Sixty five (75%) of 87 patients were infected with a single iceA2 strain. The distribution of the iceA2 subtypes was significantly different between the different clinical groups (χ² 9.4, p=0.009). Specifically, the iceA2B or iceA2C subtype (229 bp) was predominant in gastritis cases (26 of 35 (76%); p<0.02 v PUD) (fig 7) while the iceA2D subtype (334 bp) was predominant in PUD cases (14 of 21 (67%)). No differences in distribution were noted among the 22 patients with multiple iceA2 subtypes (data not shown).
Gastric adenocarcinoma.

Figure 8 Combination analysis of vacA/iceA alleles and disease outcome in all clinical isolates. **p<0.01, ***p<0.005 versus gastritis alone; †p<0.05 versus peptic ulceration or gastric adenocarcinoma.

Table 2 Distribution of vacA/iceA alleles in single genotype isolates (n (%))

<table>
<thead>
<tr>
<th>Allele</th>
<th>G gastritis (n=22)</th>
<th>PUD (n=17)</th>
<th>GC (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vacAs1/iceA1</td>
<td>2 (9)</td>
<td>8 (47)*</td>
<td>10 (67)**</td>
</tr>
<tr>
<td>vacAs1/iceA2BC</td>
<td>3 (14)</td>
<td>3 (18)</td>
<td>4 (26)</td>
</tr>
<tr>
<td>vacAs1/iceA1D</td>
<td>3 (14)</td>
<td>6 (35)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>vacAs2/iceA1</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>vacAs2/iceA2BC</td>
<td>10 (45)</td>
<td>0 (0)**</td>
<td>0 (0)**</td>
</tr>
<tr>
<td>vacAs2/iceA2D</td>
<td>3 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

PUD, peptic ulcer disease; GC, gastric adenocarcinoma.
*p<0.01, **p<0.02, ***p<0.0005 versus gastritis.

8). Significant associations between genotypes and clinical outcome were noted (χ² 57.8, p<10−5). Significantly more PUD isolates (69%; p<0.005 v gastritis alone) and GC isolates (81%; p<0.0005 GC v gastritis) were vacAs1/iceA2D. In addition, significantly more PUD isolates were vacAs1/iceA2D (53%; p<0.006 v gastritis alone). In contrast, isolates from patients with gastritis alone were identified as either vacAs2/iceA1 (21%; p<1×10−7 v PUD; p<0.0001 v GC) or vacAs2/iceA2 (22%; p<1×10−7 v PUD; p<3×10−5 v GC). Additionally, a similar analysis was undertaken in patients with a single iceA strain, with concordant results (table 2). Significant associations between genotypes and clinical outcome were noted (χ² 30.4, p<0.0008). Significantly more PUD isolates (47%; p<0.01 v gastritis) and GC isolates (67%; p<0.0004 v gastritis) were vacAs1/iceA1. In contrast, significantly more (p<0.009) isolates from patients with gastritis alone were vacAs2/iceA2BC.

Discussion

These findings support the hypothesis that strain differences in iceA genotypes may partially explain the differences in disease outcome associated with H pylori infection in South African populations.

Almost 40% of South African patients were distinguished by a high prevalence of mixed iceA strains while examination of cultures demonstrated that ∼50% were comprised of strains that contained different iceA types (either iceA1/2 or 1> iceA2 subtypes). The reason for the high incidence of infection with mixed iceA strains in African patients may simply reflect multiple colonisation but mixed strains do appear to be commonly found and range from 4% in the USA to 15% in the Netherlands, 17% in Japan, 9 and 22% in Colombia.7

In the present study, analysis of iceA genotypes in South African patients demonstrated that although iceA1 was more prevalent in PUD patients than in gastritis alone patients, this was not statistically significant. Previous studies in the USA9 and the Netherlands7 but not in Southeast Asia10 have demonstrated a strong association between this allele and PUD. It is possible that the high prevalence (∼50%) of mixed iceA strains in PUD patients may obscure any potential relationship between the allele and the disease. There was however a strong relationship between the combination of vacA signal sequence subtype and iceA alleles, and PUD. Significantly more PUD isolates were vacA s1/iceA1 compared with 40% of isolates from patients with gastritis alone.

The function of iceA2 is unknown. In this study, amplicon size of this gene appeared to discriminate between PUD and gastritis alone. iceA2C (229 bp) occurred in ∼62% of all isolates from South African patients with no clinically significant disease while iceA2D (334 bp) was more prevalent in isolates from patients with PUD. Combination vacA/iceA analysis demonstrated that the vacAs1/iceA2D was more prevalent in PUD patients (53%) than in patients with gastritis alone (23%). Consistent with these findings we have recently reported a significant relationship between the cassette structure of iceA2 and expression in vivo; iceA2B/C mucosal transcript levels were higher than iceA2D levels.21 This suggests that downregulation of iceA2 expression, in addition to induction of iceA1, may also contribute to disease outcome. It is possible therefore that South African strains from patients with clinically significant disease may not readily express iceA2 as the majority of these isolates encode the iceA2D variant. Alternatively, alterations in the number of repeat cassettes may result in potential changes in protein secondary structure. For example, variants 2A and 2B may exist as a single globular entity outside the cell while variants 2C and 2D are predicted to display one and two surface exposed regions, respectively. These changes may translate into differential binding and/or function of the protein.

There is a paucity of investigations of iceA alleles in isolates from patients with GC. In the one small study, gastric cancer isolates from Japan and Korea were distinguished by the prevalence of iceA1 (67%) while 75% of cancer isolates from the USA were iceA2.9 The numbers in this study were relatively small which limited statistical analysis. In a second Japanese gastric cancer study, iceA1 was associated with enhanced gastric inflammation but not adenocarcinoma per se.9 South African H
Analysis of South African iceA genotypes in infection require a wild-type mouse and monkey models of experimental infection. The functional role of IceA1 in gastric cancer is not compared with patients without disease. The putative role of IceA1 in gastric cancer is not defined but experimental studies have demonstrated that long term colonization in both mouse and monkey models of experimental H. pylori infection require a wild-type iceA1 strain. In the present study, iceA1 does not encode a restriction enzyme and probably, as has been previously suggested, acts as a transcriptional regulator for downstream genes. It is however possible that the higher percentage of iceA1 strains in South African gastric cancer patients may simply reflect the observation that these patients have been potentially infected for a longer time period. Alternatively, analysis of differences in iceA1 gene transcription and therefore protein production may reveal a functional role for this protein in this condition.

In summary, the sequences and prevalence of iceA1 and iceA2 in South African H. pylori isolates has been investigated, the observation that ~40% of patients are infected with mixed strains made, and a novel iceA2D subtype identified. The potential association between iceA1 and GC, iceA2D and PUD, and iceA2C and gastritis alone has been demonstrated in our study populations. No isolate from patients with clinically significant disease exhibited either the vacA s2/iceA1 or vacA s2/iceA2 allelic type.

Overall, these data support the hypothesis that there is a difference between organisms associated with and without disease. Absolute separation cannot be made which suggests that other factors must play a role in disease pathogenesis. Analysis of iceA allelic types however is useful in South Africa and certain combinations of virulence factors may provide excellent negative markers for disease.

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