Evidence of clonal variants of *Helicobacter pylori* in three generations of a duodenal ulcer disease family

C U Nwokolo, J Bickley, A R Attard, R J Owen, M Costas, I A Fraser

Abstract

Nine members of a family with a high incidence of duodenal ulcer disease were studied by interview, examination of hospital records, endoscopy, and antral biopsy. *Helicobacter pylori* was confirmed by CLO test, histology and culture. DNA extraction from pure isolates of *H pylori* was possible in six family members and strain typing was performed by restriction fragment length polymorphism. DNA restriction digestion was followed by blotting and then DNA hybridisation, using a cDNA probe complimentary to *H pylori* rRNA cistrons. Eight of the nine family members were *H pylori* positive by CLO test and histology. Five had duodenal ulcer disease. Three family members (one from each generation) harboured clonal variants of a single parent strain of *H pylori* but only two had duodenal disease. The other three members harboured different strains. Intrafamilial clustering of clonal variants of *H pylori* occurs in some duodenal ulcer disease families. Family members however, may develop duodenal disease irrespective of the colonising strain.

(Gut 1992; 33: 1323–1327)

The microaerobic bacterium *Helicobacter pylori*, is widely accepted as an important cause of gastritis and infections are strongly associated with peptic ulcer disease and gastric cancer. The gastrin link and the 'leaking roof' are some of the hypotheses proposed to explain the pathogenic link between *H pylori* and duodenal ulcer disease. Pathogenic mechanisms of *H pylori* are poorly understood, but the existence of ulcerogenic strains of this bacterium may explain why only a minority of patients harbouring the organism develop duodenal ulcer disease. Certain virulence factors produced by *H pylori* have been identified and include a vacuolating, cytopathic agent and a protein that inhibits rabbit parietal cell acid secretion in vitro. *H pylori* strains positive for a 120-KDa protein have recently been described in duodenal ulcer patients.

Evidence is accumulating from DNA fingerprinting that each infected individual harbours a unique strain of *H pylori* although the reason for this diversity is unknown. As 20–50% of duodenal ulcer patients have a positive family history, it would be of interest to explore the hypothesis that familial peptic ulcer disease is the cluster effect of a virulent, ulcerogenic strain of *H pylori* transfecting family members.

Methods

PATIENTS

The extended family studied has been recognised in the Coventry area for up to 20 years. Scrutiny of hospital records revealed that during that period, the majority of family members had presented to the Walsgrave Hospital with duodenal ulceration or its complications.

The matriarchal head of the family (Fig 1, subject 1) provided a comprehensive family history. There were 25 family members aged over 10 years. Fourteen members lived in the Coventry area, the remainder living in Scotland or Australia. The 14 members living in the area were invited for interview and subsequent endo-

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**Table**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Cl test</th>
<th>Histology</th>
<th>Duodenal ulcer</th>
<th>Plasma gastrin (ng/litre)</th>
<th><em>H pylori</em> isolation</th>
<th>BamHI Total digest pattern</th>
<th>BamHI ribopattern</th>
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<tbody>
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</table>

CO refers to Coventry strain ribopattern; na = not available; nt = not typable.
to prevent transfer of *H pylori* between patients. Duodenal ulceration was recorded only when an ulcer crater of at least 5 mm diameter was observed. Pyloroduodenal scarring, antritis, duodenitis and gastric anatomical abnormalities from previous surgery were also recorded.

**BIOPSY**

Four antral biopsies were taken from each patient. One was used for a CLO test (Delta West limited, Bentley, Western Australia) and one was transferred into 10% w/v formaldehyde for histology. The other two were transferred separately into two bijoux containing *H pylori* selective enrichment (SE) medium and were number coded randomly to ensure that culture and DNA analysis were performed blind.

**CULTURE**

Antral biopsy specimens were placed in 5 ml of selective enrichment medium and incubated at 37°C on a gyratory platform (150 rpm) in a Variable Atmosphere Incubator (Don Whitley Scientific Ltd, Shipley, Yorks, UK) under microaerobic conditions (5% oxygen, 5% carbon dioxide, 2% hydrogen, 88% nitrogen). A sample from each flask was subcultured onto Oxoid brain heart infusion agar, supplemented with 5% horse blood and 1% Isovitalex after 48 hours. Positive growth was identified by Gram stain and production of urease, and cultures were preserved at −196°C on glass beads in Oxoid nutrient Broth No. 2 containing (v/v) glycerol.

**DNA DIGESTION AND ELECTROPHORESIS**

*H pylori* chromosomal DNA was isolated using the guanidium thiocyanate reagent method. The purified DNA was incubated with 11 endonucleases (HaeIII, HindIII, EcoRI, PstI, BamIII, SacI, Apal, SstI, HpaII, MspI) according to the conditions recommended by the manufacturer (Northumbria Biologicals Limited, UK). DNA samples (5 μg) were digested for four hours at 37°C. The digested DNA was electrophoresed at 30 V or 16 hours in a horizontal 0-8% (w/v) agarose gel in a buffer containing 89 mM Tris hydrochloride, 89 mM boric acid, and 2 mM disodium ethylene-diaminetetra-acetic acid (EDTA) (pH 8-3). After electrophoresis, the gels were stained with ethidium bromide and photographed.

**COMPARISON OF DIGEST PATTERNS BY DENSITOMETRY**

Patterns were scanned and analysed with a laser densitometer interfaced to a Compaq Deskpro 386 microcomputer. Profiles were compared by band matching, using the Dice correlation coefficient. Strains were then clustered and a dendrogram plotted (Fig 2).

**VACUOLLITING AND HYBRIDISATION**

A biotinylated cDNA probe was prepared from *H pylori* NCTC (National Collection of Type
PLASMA GASTRIN, ELECTROLYTES AND CALCIUM
Mildly raised plasma gastrin were observed in five subjects (Table). Subject 9 was taking omeprazole and subject 8 was taking an H₂ blocker. Two subjects (2 and 6) had undergone vagotomy. Serum electrolytes and calcium were normal.

DUODENAL ULCER DISEASE
A diagnosis of duodenal ulcer disease was accepted only if a family member was found to have an acute duodenal ulcer at the study endoscopy or had a history of gastric surgery performed for intractable duodenal ulceration. Duodenal ulcer disease was confirmed in five subjects (2, 3, 6, 8 and 9). Several other family members had a history of duodenal ulceration but were not available for inclusion in the study. All the subjects with duodenal ulcer disease had become symptomatic in the first or second decade of life. In all cases confirmatory evidence of active duodenal ulceration had been obtained by the end of the third decade, except for the 71 year old man (subject 2), in whom confirmation was obtained in the fifth decade.

H PYLORI STATUS
Eight of the nine (90%) family members were positive for H pylori by CLO test and histology (Table). The one negative subject (subject 4) was a woman (age 30) in the second generation. Although she was endoscopically normal, her 12 year old son (subject 8) had confirmed duodenal ulcer disease from the age of eight, requiring maintenance treatment with an H₂ blocker.

ISOLATION AND DNA FINGERPRINTING OF H PYLORI
Successful isolation of pure cultures of H pylori could be achieved in six of the nine subjects studied. One family member (subject 4) was negative for H pylori (confirmed by CLO test and histology). In one member (subject 5) unsuccessful isolation was caused by non-H pylori contaminants. Two biopsies were obtained from subject 8 (12 years old) for histology and CLO test before endoscopy was abandoned for technical reasons, and ethical considerations precluded a second endoscopy. Subjects 2, 7, and 9, from whom H pylori had been successfully isolated, harboured strains with similar but not completely identical DNA fingerprints (Figs 3, 4). The BamHI restriction digest patterns of these three strains differed only in one or two minor bands, whereas all the other strains in the study were very different (Fig 3). All isolates were designated a DNA type based of their BamHI total digest patterns. Subjects 2, 7, and 9 were designated as subtypes 2a, 2b, and 2c respectively, as their digest patterns were identical but for a few minor bands.

RESULTS
Nine family members representing three generations (I, II, III) participated in the study (Fig 1). Their median age was 42 years (range 12–71). There were three females and six males. All the males (except for the 12 and 18 year olds in generation III) had smoked, but none had smoked in the year preceding the study.
patterns were very similar (Table, Fig 3). The dendrogram in Figure 2 illustrates the similarities between the BamHI digest patterns obtained for all subjects. Similarities were obtained using band matching and calculation of the Dice coefficient. Isolates from subjects 2, 7, and 9 clustered at a similarity of 85%, with isolates from subjects 7 and 9 being most closely related at a similarity of 92%. All other subjects (1, 3, 6) clustered at similarities of less than 60%.

Less information was available from the BamHI ribopatterns obtained after hybridisation with the cDNA probe, because few bands were present. Different ribopatterns were obtained for subjects 1, 3, and 6, however, (Fig 4). Subjects 2 and 9 had the same ribopattern (Table, Fig 4), with a common band of molecular weight 8·8 kb. Subject 7 did not, however, have this band.

Results with other endonucleases showed that DNA from H pylori isolates from subjects 2, 7, and 9 shared the unusual characteristic of being undigested by enzymes which commonly digest DNA from H pylori strains, namely HaeII and HindIII.

Subjects 2 and 9 had duodenal ulcer disease; one had a vagotomy and drainage procedure, the other had persistent duodenal ulceration despite continuous omeprazole treatment. Subject 7 did not have duodenal ulcer disease according to our stringent criteria, but was a life long dyspeptic and was found to have significant antritis and duodenitis at endoscopy. Subject 6 had a completely different DNA fingerprint. This subject had undergone a vagotomy and pyloroplasty at age 26 years after many years of severe duodenal ulceration. Subjects 1 and 3 also had different strains of H pylori and these strains were different from the strains isolated from all other subjects. Subject 3 had a duodenal ulcer at endoscopy and subject 1 was endoscopically normal.

Discussion

The diversity of the strains found in this duodenal ulcer family suggests that cluster infection by a single putative ulcerogenic strain of H pylori does not completely explain familial peptic ulcer disease. Clonal variants however, that is, isolates with a high level of genomic relatedness, of the same strain of H pylori colonised three members of the family (subjects 2, 7, and 9). It is of interest that family members (subjects 7 and 9) who harboured the clonal variants with the highest similarity (92%) were only one generation apart. This adds to the evidence supporting a person to person mode of transmission. Alternatively, family members may become infected from a common source.

The reason for the minor genetic differences between these clonal variants is unknown. It may represent a tendency to spontaneous genomic rearrangement which may be in the nature of H pylori. Marked genomic heterogeneity is already a recognised characteristic of this bacterium. From our experience, in applying the technique of DNA fingerprinting to strain typing of over 500 isolates of H pylori,17 no two patients were found to harbour H pylori strains with such marked similarity as observed in subjects 2, 7, and 9. Indeed, the small amount of variation between the digest patterns of these three subjects was consistent with the observed variation within multiple isolates from single patients.17

Further evidence for the general similarities of these three isolates was obtained from results with other restriction enzymes. DNA from H pylori isolated from subjects 2, 7, and 9 were unusual in not being digested by HaeII or HindIII. These enzymes would normally digest DNA obtained from the majority of H pylori isolates.

Clustering of H pylori among relatives of infected individuals has been widely reported.18 Graham et al recently described the influence of age, sex, social class, and race on the prevalence of H pylori in a western population.19 One preliminary report has found a single strain of H pylori clustering in a duodenal ulcer family of eight members.20 This report was, however, based solely on total digest patterns obtained with a single restriction enzyme. Another report has described identical strains of H pylori colonising two pairs of mentally subnormal children living in close proximity.21

In this study we found eight of nine family members colonised by H pylori. Because the colonising strains were not all identical, this...
suggests that family members may be independently susceptible to *H pylori* infection.

The development of duodenal ulcer disease in family members seemed independent of the colonising strain. Even among the three family members colonised by clonal variants of the same strain, only two members had duodenal ulcer disease. One was apparently free of disease.

Some genetic subtypes of duodenal ulcer disease were proposed before the rediscovery of *H pylori*. A duodenal ulcer family with raised serum hyperpepsinogen 1 concentration inherited as an autosomal dominant trait has been described. A majority of duodenal ulcer patients, however, have raised serum pepsinogen 1. In addition there is some preliminary evidence to suggest that gastric colonisation with *H pylori* is associated with raised serum pepsinogen 1 and that the eradication of *H pylori* returns this towards normal.

A duodenal ulcer family with accelerated gastric emptying has also been described but recent data suggest that gastric colonisation with *H pylori* may modify antroduodenal motility. Data from the pre *H pylori* family studies of duodenal ulcer disease should now be reviewed and recent knowledge about *H pylori* factored into conclusions derived from those studies. The hypotheses derived from those studies should be reassessed.

Mildly raised fasting gastrin concentrations were observed in family members who were taking antisceretory drugs or who had had a vagotomy. There was no clinical or biochemical evidence to suggest that this family had multiple endocrine adenomatosis or other hypergastrinaemic state.

In general, only limited conclusions may be derived from single family studies. Reports of family studies in which *H pylori* strains have been characterised by molecular biology techniques, however, are increasingly frequent in the medical literature. This study supports the observations of Drumm et al. that *H pylori* clusters in some duodenal ulcer families. If gastric colonisation with *H pylori* precedes duodenal ulceration in all cases, then it may be that duodenal ulcer family members are simply more prone to *H pylori* infection than members of the general population. The colonising strain of *H pylori* did not seem to influence the development of duodenal ulcer disease in family members. Intrafamilial clustering of clonal variants arising from a common parent strain of *H pylori* may occur. An undefined tendency to duodenal ulceration may be inherited in some duodenal ulcer families. Subsequent colonisation with most strains of *H pylori* promotes this tendency, resulting in active duodenal ulceration.

Sister De Souza and the staff of the Wahgah Hospital endoscopy unit assisted in the endoscopic investigations. JR is indebted to the Procter and Gamble Company (Cincinnati, Ohio, USA) for financial support.