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 vacu- 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.

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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 I) 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-

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CLO 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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</thead>
<tbody>
<tr>
<td>Generation I</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>34</td>
<td>+</td>
<td>1</td>
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<td></td>
<td>2</td>
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<td>+</td>
<td>+</td>
<td>181</td>
<td>+</td>
<td>2a</td>
</tr>
<tr>
<td>Generation II</td>
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<td>+</td>
<td>+</td>
<td>35</td>
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<td>3</td>
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<td></td>
<td>4</td>
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<td>-</td>
<td>-</td>
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<td></td>
<td>6</td>
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<td>-</td>
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<td>177</td>
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<td>+</td>
<td>+</td>
<td>131</td>
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<tr>
<td></td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>199</td>
<td>+</td>
<td>2c</td>
</tr>
</tbody>
</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 guanidinium thiocyanate reagent method. The purified DNA was incubated with 11 endonucleases (HaeIII, HindIII, EcoRI, PstI, BamIII, SacI, ApaI, SnuI, 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 DENISITOMETRY**

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).

**VACUBLOTTING AND HYBRIDISATION**

A biotinylated cDNA probe was prepared from *H pylori* NCTC (National Collection of Type...
Cultures) 11638 16S and 23S rRNA using Moloney mouse leukaemia virus reverse transcriptase (Gibco-BRL). Biotinylation was achieved by the incorporation of biotin-16-dUTP. After electrophoresis and photography, the gels were transferred to nylon membranes (Hybond-N, Amersham International) by means of vacu blotting (Vacu-Gene XL, Pharmacia LKB Biotechnology). The membranes were then hybridised by standard procedures for 16 hours at 42°C using the hybridised probe detected colorimetrically using a nonradioactive detection kit – BluGENE (Gibco-BRL).

ETHICS
Ethical approval was obtained from the Coventry and Warwickshire Postgraduate Medical Ethics Committee as part of a larger epidemiological study. Informed consent was obtained from all family members except for the 12 year old. In his case an endoscopy was indicated for the investigation of abdominal pain. His mother provided consent.

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.

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 P Y L O R I 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 P Y L O R I
Successful isolation of pure cultures of H pylori was possible 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 indicated.
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,91 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.92

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 HaeIII 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.93 Graham et al recently described the influence of age, sex, social class, and race on the prevalence of H. pylori in a western population.94 One preliminary report has found a single strain of H. pylori clustering in a duodenal ulcer family of eight members.95 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.96

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 hypergastrinemia 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 antr duodenal 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 antisyecretory 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 hypergastrinemic 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 infrequent 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.

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