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Correlation of the *Helicobacter pylori* adherence factor BabA with duodenal ulcer disease in four European countries

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Abstract

Helicobacter pylori strains harboring the *vacAs1*, *cagA* and *babA2* have been associated with ulcer disease (UD). We compared the prevalence of these different genotypes and adhesive properties in *H. pylori* infected patients with UD in four European countries. Genomic DNA was isolated from 314 *H. pylori* strains: Germany (GER; $n = 92$), Sweden (SWE, $n = 74$), Portugal (POR, $n = 91$) and Finland (FIN, $n = 57$). The frequencies of *babA2* genotype varied from 35% to 60%. Triple-positive strains (*vacAs1*+, *cagA*+ and *babA2*+) were significantly associated with UD in GER and POR and were closely correlated with UD in FIN, but not in SWE. Classification as triple-positive strains had a higher specificity for detection of UD in GER, POR and FIN than *type1* or *cagA*+ strains. In vitro adhesion assays revealed that Swedish strains showed high adhesion properties and were thus correlated with the diagnosis of UD, although PCR detected the *babA2* gene at lower frequencies and failed to show a correlation with UD. This finding appears to reflect allelic variations of the *babA2* gene in SWE, although adhesive properties of the strains are retained.

Keywords: Adhesion; Ulcer disease; babA; Multicenter

1. Introduction

Infection with *Helicobacter pylori* is the major cause of gastroduodenal diseases, including gastritis, peptic ulcer [1], mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma [2]. The different outcomes are induced by complex interactions between environmental, host and bacterial virulence factors [3]. The importance of the classical *H. pylori* virulence factors, i.e. the vacuolating cytotoxin VacA and the cytotoxin associated gene product CagA, has been described in detail [4–6]. *H. pylori* strains have been divided into *type1* (VacA, CagA positive) and *type2* (VacA, CagA negative) strains. VacA is a secreted cytotoxin that has the capacity to form vacuoles in epithelial cells [7]. The P34 subunit of VacA acts on mitochondria,

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inducing the release of cytochrome *C* [8]. Atherton et al. [9] have identified the mosaic combination of signal regions (s1, s2) and middle regions (m1, m2). *vacAs1* strains produce a large amount of cytotoxin, whereas *s2* type strains secrete small amounts or no cytotoxin at all. CagA, which is known as a marker for presence of the *cag* PAI [10], is involved in cytoskeletal changes of the host cells. The increased production of IL-8, which is induced by the specific genes of *cag PAI*, may partially explain the relationship between *cag PAI* positive strains and more severe diseases induced by the infection.

Adherence factors of *H. pylori* are further considered to contribute to the pathogenicity of these bacteria. One of the major adhesins, BabA, encoded by the *babA2* gene, has been identified as the adhesin responsible for *H. pylori* binding to the fucosylated blood group antigens Lewis^b [11]. BabA is a member of a paralogous family of outer membrane proteins. Its capability of binding to Lewis^b facilitates *H. pylori* colonization of the stomach but may also play a direct role in pathogenesis. It has been demonstrated that bacterial binding to Lewis^b is mainly seen in *type1* isolates [12], indicating that no single factor can be considered solely responsible for determining outcome of *H. pylori* infection. An adherent strain however, might have more clinical impact than a strain, which lacks these properties [13].

Previous studies of our group have found that in a German population the presence of *babA2*, as well as the simultaneous presence of *vacAs1* and *cagA* (''triple-positive strains''), was significantly associated with duodenal ulcer [12], and adherence of *H. pylori* via BabA appeared to be important for efficient delivery of VacA and CagA. BabA may also play a role in the pathogenesis of severe histological changes, such as atrophy or intestinal metaplasia [14]. In contrast to our results, studies by other groups [15,16] did not reveal any direct association between *babA2* and the status of *cagA* or *vacA*, and also did not detect any correlation with the clinical outcome. The present study therefore aimed to compare these virulence factors of *H. pylori* isolated from patients from four European countries to further investigate the correlation of *babA2*, *cagA* and *vacAs1* genotypes with disease, as well as the effect of genetic diversity of *babA2* on its adhesive activity.

2. Materials and methods

2.1. Populations of the patients

Helicobacter pylori strains were isolated from a total of 314 patients suffering from peptic ulcer disease (UD) and gastritis (GA) from four European countries: 91 from Portugal (POR) (UD = 30, GA = 61, male 52%, median age = 67 years, range 27–87); 92 from Germany (GER) (UD = 25, GA = 67, 50% male, median age = 58, ranging from 19 to 91); 74 from Sweden (SWE) (UD = 30, GA = 44, male = 54%, median age = 62, range 28–85) and 57 from Finland (FIN) (UD = 31, GA = 26, 38% male, median age = 55, range 25–83), respectively. All of these patients presenting with abdominal pain or dyspeptic complaints were diagnosed endoscopically after informed consent was obtained. Only patients diagnosed with *H. pylori* positive duodenal ulcers were investigated that did not take aspirin or non-steroidal anti-inflammatory drugs (NSAID) medication. Patients using proton pump inhibitors (PPI) > double standard dose or patients with history of gastric cancer were excluded.

2.2. H. pylori *culture and isolation of genomic DNA*

Gastric biopsies were obtained from antrum of the patients for isolating *H. pylori*. The specimens were homogenized in 0.5 ml brucella broth with 10% (vol/vol) fetal calf serum (FCS) with a tissue homogenizer. The suspensions were inoculated onto Wilkins Chalgren agar (Oxoid, Basingstoke, UK) containing 10% horse blood, Dent supplement (Oxoid) and 0.4 g/L KNO_3. The plates were incubated up to 8 days in an CO_2 water jacketed incubator (Forma Scientific Inc.) with a microaerobic atmosphere of 10% CO_2, 85% N_2, 5% O_2 and 100% relative humidity at 37 °C. *H. pylori* were identified by colony morphology, Christensen urease (Bacto urea base, Difco), oxidase (Oxidase Dry Slide, Difco), catalase, and phase-contrast microscopy. Isolates were resuspened in brucella broth with 10% FCS and 20% glycerol and frozen at −80 °C for storage. Genomic DNA was isolated from harvested bacteria using the QIAamp Tissue Kit (Qiagen, Munich, Germany) according to the manufacturer's instructions. *H. pylori* isolates cultured for in vitro adherence assay were harvested within 24–48 h, and resuspended in PBS, then diluted to a density of $OD_{600} = 1$ for immediate use.

2.3. *Detection of* babA2, cagA *and* vacA *genotypes by PCR*

PCR amplifications were performed for detecting *babA2*, *cagA* and *vacAs1/2* genotypes of all isolated strains. Primer sequences for *babA2*, *cagA* and *vacA* have been published previously [9–12]. Amplification was carried out in a total volume of the 25 μl using Taq PCR Master Mix (Qiagen), containing 20 ng *H. pylori* genomic DNA and 0.5 μl of each primer (20 μM). Reaction was performed in a Primus PCR Cycler (MWG Biotech, Ebersberg, Germany) under the following conditions: 5 min initial denaturation at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C; and 10 min final extension at 72 °C. PCR prod-