

## Analysis of the relationship between oipA, babA2, babB genotypes and *helicobacter pylori*-related gastric precancerous lesions

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### Abstract

**Objective:** To investigate the distribution of *helicobacter pylori*-related genotypes of oipA, babA2, and babB in patients with gastrointestinal diseases.

**Method:** The retrospective study was conducted at the Jiamusi College, Heilongjiang University of Traditional Chinese Medicine, Harbin, China, and comprised data from February 2017 to May 2020 of patients of either gender 20-80 years who underwent gastroscopy. An instrument based on polymerase chain reaction was used to amplify oipA, babA2 and babB genes, and their distribution in terms of gender, age and pathological types was analysed.

**Results:** Among the 116 patients, 52(44.8%) had oipA genotype, 48(41.2%) babA2, and 72 (62.1%) babB, respectively, and the size of amplified products of 486bp, 219bp and 362bp, respectively. The infection rate of oipA and babB genotypes was highest [26(50.0%) and 31(43.1%)] in those aged 61-80 years, and lowest [9(17.3%) and 15(20.8%)] in those aged 20-40 years. The infection rate of babA2 genotype was highest [23(47.9%)] in those aged 41-60 years, and lowest [12(25.0%)] in those aged 61-80 years. Male patients were under a higher [28(53.9%) and 26(54.2%)] rate of infection with oipA and babA2, and female patients has a higher [40(55.6%)] rate of infection with babB. Among Hp-infected patients with digestive diseases, babB genotype was mainly found in patients with chronic superficial gastritis[17(58.6%)], duodenal ulcer[17(85.0%)], chronic atrophic gastritis[19(59.4%)] and gastric ulcer[16(72.7%)], while oipA genotype was mainly found in patients with gastric cancer[8(61.5%)].

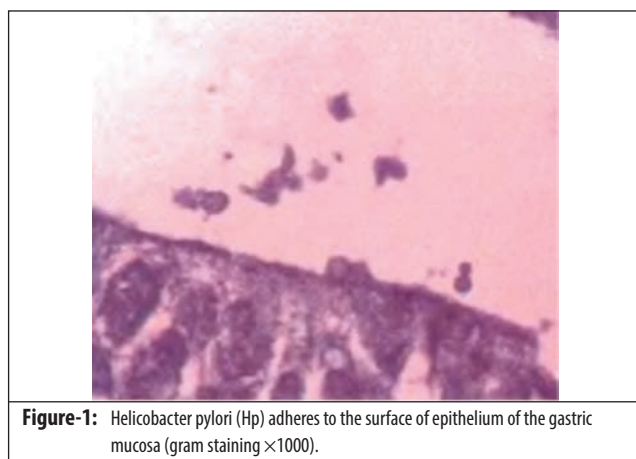
**Conclusion:** Chronic superficial gastritis, duodenal ulcer, chronic atrophic gastritis, and gastric ulcer may have a close bearing on babB genotype infection, while oipA genotype infection may be associated with gastric cancer.

**Keywords:** *Helicobacter pylori*, oipA, babA2, babB, Chronic superficial gastritis, Chronic atrophic gastritis, Duodenal ulcer, Gastric ulcer, Gastric cancer. (JPMA 72: 249; 2022) DOI: <https://doi.org/10.47391/JPMA.5137>

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### Introduction

*Helicobacter pylori* (Hp) may be colonised on the epithelium of the gastric mucosa (Figure-1), and most patients with Hp infection show no symptoms.<sup>1</sup> It has been shown in relevant studies that Hp infection is a vital pathogenic factor for gastrointestinal (GI) diseases, such as chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG), duodenal ulcer (DU), gastric ulcer (GU) and gastric cancer (GC), while host susceptibility, environmental factors and differences in virulence gene expression can affect the clinical outcome after Hp infection.<sup>2</sup> Differences in Hp virulence may be determined by Hp virulence gene polymorphism.<sup>3</sup> To date, Hp gene polymorphism has been studied mainly on the two genes of cytotoxin-related protein cagA and vacuolar toxin vacA, and Hp is divided into type I strains (cagA-positive and vacA-positive) and type II strains (cagA-negative and vacA-negative).<sup>4</sup> However, a few studies have been carried out on the



**Figure-1:** *Helicobacter pylori* (Hp) adheres to the surface of epithelium of the gastric mucosa (gram staining ×1000).

expression of virulence genes, such as oipA, babA2 and babB, in various types of gastrointestinal disorders (GIDs). The current study was planned to investigate the distribution of oipA, babA2 and babB genotypes in various GIDs.

### Materials and Methods

The retrospective study was conducted at the Jiamusi College, Heilongjiang University of Traditional Chinese Medicine, Harbin, China, and comprised data from

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February 2017 to May 2020 of patients who underwent gastroscopy. Data related to patients of either gender aged 20-80 years who met the diagnostic criteria of various GIDs and the diagnoses were confirmed through gastroscopic biopsy<sup>5-7</sup> Patients gave voluntary consent for their data to be used for research purposes provided a study was approved by the institutional ethics review committee, which the current study obtained. Data was excluded for patients with missing information, those who had taken antibiotics, non-steroidal antacids, gastric mucosal protective agents, and anti-inflammatory drugs within 2 weeks prior to specimen collection, patients with immune system diseases, and those with gastroesophageal reflux.

Deoxyribonucleic acid (DNA) samples were generated through an extraction kit (Article No.: K368-50RXN; Qingdao Jieshang Biotechnology Co., Ltd.), amphotericin B (Article No.: K0018; Shanghai Baoman Biotechnology Co., Ltd.), vancomycin (Article No.: V76062; Shanghai Acme Biochemical Co., Ltd.), trimethoprim (Article No.: SND-1171; Chuzhou Shinuoda Biological Technology Co., Ltd.), polymyxin (Article No.: P8350; Shanghai Zhenyu Biological Technology Co., Ltd.), agar (Article No.: A111; Shanghai Yanhui Biological Technology Co., Ltd.), electrophoresis instrument (Model: 81-0478-01; Shanghai Fuze Trading Co., Ltd.), low-temperature high-speed centrifuge (Model: TG-16; Thermo Fisher Scientific, United States), polymerase chain reaction (PCR\_ instrument (Model: Veriti 96-Well Thermal Cycler; Thermo Fisher Scientific, USA), and a gel imaging system (Model: Quick Gel 6200; Monad [Suzhou] Biotech Co., Ltd).

For sample collection, the gastroscope was moved into the patient's digestive tract to search for the location of the lesion. The oesophagus, stomach and duodenum were explored. After finding the suspected lesion, gastric mucosa specimens were taken and stored at -80°C for testing and genotyping.

All the specimens were inoculated in prepared Hp medium (10% glucose, 7% defibrinated sheep blood, amphotericin B 10mg/L, vancomycin 10mg/L, trimethoprim 5mg/L, polymyxin 2500U/L, 2% agar) for 4d under micro-oxygen conditions (85% nitrogen gas [N<sub>2</sub>], 37°C, 10% carbon dioxide [CO<sub>2</sub>], 5% oxygen [O<sub>2</sub>]). Subsequently, biochemical reactions (urease, thiasse, oxidase) and colony morphology were observed. Hp strains were confirmed by gram stain smears and stored at -80°C for later use.

The preserved Hp strains were resuscitated and the bacteria were scraped after 48h of culture, and then washed with phosphate-buffered saline (PBS) 1-2 times. Hp nucleic acid DNA was extracted with the extraction kit and stored at -80°C for later use.

**Table-1:** Primer sequences of helicobacter pylori (Hp) babA2, oipA and babB genotypes.

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
oipA	GGTCCGCCACCTAGGTTAAC	GAGTTCGGAAAGTAAGG
babA2	GGTGTGTGTACTCAGGTGAGT	CGTTTCAGCCTGGAGGATCC
babB	GTTTATGCCTGTGAGGGAT	CCGTGTCTCAGGAGAATC'

A PCR instrument was utilised to amplify oipA, babA2, and babB genes, and the primer sequences were noted (Table-1). PCR was performed in 25µL of the reaction system, containing 1µL of Hp template (50 ng/µL), 2µL each of forward and reverse primers (10µM), 2µL of deoxynucleoside triphosphate [dNTP], 2.5µL of 10×Buffer, 1µL of *Thermus aquaticus* DNA [TaqDNA], plus double-distilled water [ddH<sub>2</sub>O] 25µL. Each sample was repeated 3 times. After amplification, agarose gel electrophoresis was performed. A gel imager was used for observation, and ddH<sub>2</sub>O was used as the negative control.

Data was analysed using SPSS 25. Data was expressed as mean±standard deviation and frequencies and percentages, as appropriate. To compare the differences, t test and chi-square test were used, as appropriate. P<0.05 was taken as statistically significant.

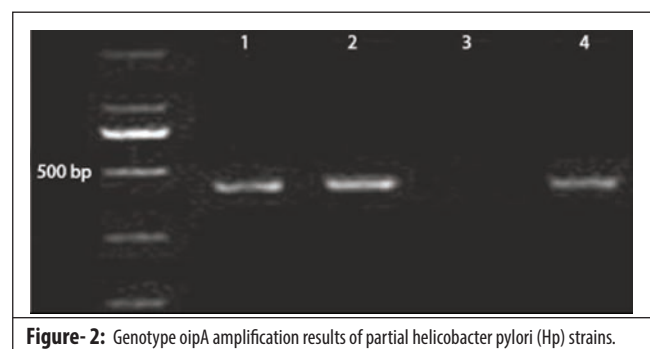
## Results

Among the 116 patients, no significant difference was observed related to age or gender among the five disease groups (Table-2).

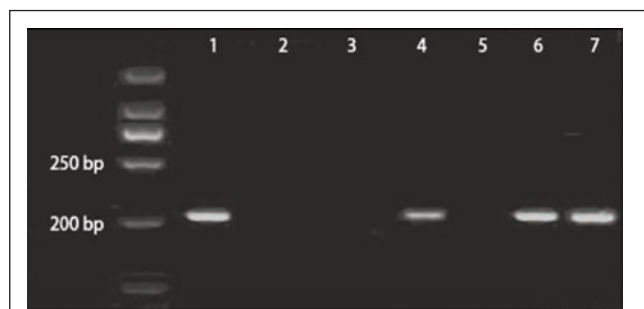
There were 52 (44.8%) cases of oipA genotype and the size of amplified product 486 bp (Figure-2). There were 48 (41.2%) cases of babA2 and the size of amplified product 219bp (Figure-3). There were 72 (62.1%) cases with babB

**Table-2:** Gastrointestinal (GI) disease distribution in relation to age and gender groups.

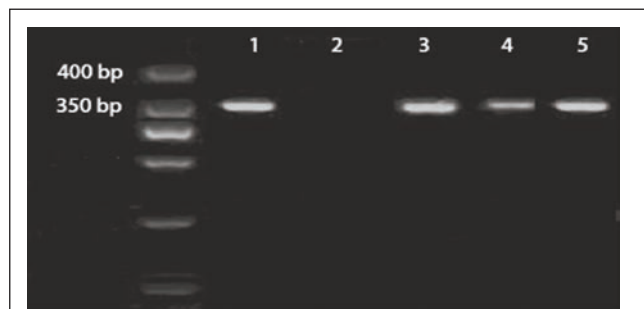
GI disease type	n	Mean Age (years)	Gender	
			Male	Female
Chronic Superficial gastritis	29	50.26±13.36	13(44.83)	16(55.17)
Chronic atrophic gastritis	32	50.72±12.96	15(46.87)	17(53.13)
Duodenal ulcer	20	51.12±13.89	8(40.00)	12(60.00)
Gastric ulcer	22	50.88±13.14	9(40.91)	13(50.09)
Gastric cancer	13	51.23±12.81	5(38.46)	8(61.54)



**Figure- 2:** Genotype oipA amplification results of partial helicobacter pylori (Hp) strains.



**Figure-3:** Genotype babA2 amplification results of partial helicobacter pylori (Hp) strains.



**Figure-4:** Genotype babB amplification results of partial helicobacter pylori (Hp) strains.

**Table-3:** Distribution of oipA, babA2 and babB genotypes.

Genotype	n	Age (years)			Gender	
		20-40 (n=37)	41-60 (n=39)	61-80 (n=40)	Male (n=50)	Female (n=66)
oipA genotype	52	9 (17.3) *	17(32.7) *	26(50.0) *	28 (53.9) #	24 (46.2) #
babA2 genotype	48	13 (27.1) *	23(47.9) *	12(25.0) *	26 (54.2) #	22 (45.8) #
babB genotype	72	15 (20.8) *	26(36.1) *	31(43.1) *	32 (44.4) #	40 (55.6) #

\*:  $p > 0.05$ , #:  $p > 0.05$ .

**Table-4:** Distribution of oipA, babA2 and babB genotypes in relation to different pathological types.

Genotype	n	oipA genotype (n=52)	babA2 genotype (n=48)	babB genotype (n=72)
Chronic superficial gastritis	29	11 (37.9) *	8 (27.6) *	17 (58.6) *
Chronic atrophic gastritis	32	16 (50.0) *	8 (25.0) *	19 (59.4) *
Duodenal ulcer	20	10 (50.0) *	14 (70.0) *	17 (85.0) *
Gastric ulcer	22	7 (31.8) *	12 (54.6) *	16 (72.7) *
Gastric cancer	13	8 (61.5) *	6 (46.2) *	3 (23.1) *

\*:  $p > 0.05$

and the size of amplified product 362bp (Figure-4).

The distribution of oipA, babA2 and babB genotypes was noted in relation to age and gender (Table-3), and with respect to different pathologies (Table-4).

### Discussion

Gastric ailments and chronic infections of the duodenum are common diseases of the digestive tract. Gastric cancer is a common malignant tumour of the digestive tract, and is also among the top three tumours with a low 5-year survival rate.<sup>8</sup> The aetiology of gastric cancer has remained unclear to date. However, in recent years, many studies

have proposed the development model of “chronic gastritis-atrophic gastritis-intestinal metaplasia-gastric cancer”, and studies have shown that Hp infection affects gastric mucosal inflammation and immunity of patients, which will increase the incidence of gastric cancer.<sup>9,10</sup> It is currently believed that the principal pathogenic factors of GIDs caused by Hp are adhesion, flagella, reactive oxygen species (ROS), heat shock protein, urease, cytotoxin, etc., all of which are involved in gastric mucosal damage.<sup>11</sup>

Epidemiological investigations have revealed that only a small proportion of Hp-infected people will develop GIDs, such as CSG and CAG, which may be mainly caused by differences in Hp strains. On the other hand, differences in Hp strains were in turn associated with virulence gene polymorphism.<sup>12</sup> In recent years, babA has likewise gradually become a research hotspot among Hp virulence genes. Gene babA, as one of the outer membrane proteins of Hp, can mediate the adhesion of Hp and has two genotypes, babA1 and babA2. Both are sequentially homologous, but only babA2 is biologically active because it has a 10bp insertion sequence that produces a translation starting point.<sup>13</sup> As shown in the current study,

among 116 Hp-infected patients with GIDs, the positive rate of babA2 was 41.4%, which was higher than the detection rate of babA2 in an earlier study.<sup>14</sup> The positive rate of babA2 gene in Brazil was 44%, which was related to peptic ulcer and gastric cancer.<sup>15</sup> The positive rate of babA2 in Japan was 84.9%, but it had nothing to do with the clinical outcome.<sup>16</sup>

Moreover, in the current study, the infection rate of babA2 genotype was highest in patients aged 41-60 years, and lowest in patients aged 61-80 years; The detection rate of babA2 in patients with GU, DU and GC was higher than that in patients with CAG and CSG, though not statistically significant, which is basically consistent with earlier results,<sup>17</sup> suggesting that babA2 genotype may be associated with various GIDs. Genotype oipA is a pro-inflammatory outer membrane protein, which has a close bearing on neutrophil infiltration, clinical symptoms of patients and bacterial colonisation density after Hp infection, contributing to the colonisation of Hp in gastric mucosa.<sup>18</sup> A study confirmed that the oipA gene is closely related to inflammation.<sup>19</sup> As shown in the current study, the positivity rate of oipA was 44.83%, and Hp in GC patients was dominated by oipA genotype, while patients with DU and GU had a lower infection rate of oipA genotype, which was basically consistent with earlier findings,<sup>20</sup> suggesting that oipA infection may cause more severe gastric mucosal

damage and is more likely to induce GC compared to babA2 and babB genotypes. Also babB itself acts as a lectin that binds to an unknown receptor on host epithelial cells or modulates the binding of other adhesins.<sup>18</sup> As shown in the current study, the positivity rate of babB was 62.07%, which was consistent with earlier reports.<sup>21</sup> Furthermore, the infection rate of babB genotype was the highest in patients aged 61-80 years, and the lowest in patients aged 20-40 years. Patients with CSG, DU, CAG, and GU are mainly infected with babB genotype Hp, while patients with GC have a low babB infection rate,<sup>21</sup> suggesting that babB genotype may be highly correlated with elderly patients and patients with CSG, DU, CAG and GU, but may have relatively little relationship with GC.

The current study has certain limitations as patients from only one region were enrolled. The mechanism of oipA, babA2 and babB genotypes involved in GC needs to be further studied via cell model experiments or animal model experiments, and other studies need to be combined to further explore the polymorphism and diversity of Hp virulence genes.

## Conclusion

CSG, DU, CAG and GC were found to be mainly babB genotype infections, and oipA genotype infections were found to be associated with GC.

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**Conflict of Interest:** None.

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