Brief Report

Genotyping of Helicobacter pylori ureC Gene: Connection of Its Association with Gastric Cancer in Taiwan

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Posted Date: 30 August 2023

doi: 10.20944/preprints202308.2029.v1

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Genotyping of *Helicobacter pylori* ure C Gene: Connection of Its Association with Gastric Cancer in Taiwan

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Abstract: Introduction: *Helicobacter pylori* (*H. pylori*) is known to play a significant role in the development of peptic ulcer diseases and gastric cancer. Previous studies have reported the existence of polymorphism in the ureC gene of *H. pylori.* However, the relationship between genetic sequence variations in ureC and the pathogenesis of peptic ulcer diseases remains unclear. We aimed to investigate the associations between ureC sequence variations and the development of gastric ulcer and gastric cancer. PCR amplification and DNA sequencing to analyze ureC sequences, and conducted phylogenetic analysis using MEGA X software. **Methods:** Samples from Taiwanese patients with gastric ulcer and gastric cancer were included in this study. PCR was used to amplify the ureC gene from these samples, followed by DNA sequencing. The obtained sequences were then subjected to phylogenetic analysis using MEGA X software. In addition, ureC sequences from different geographical regions, including China and other countries, were included in the analysis for comparison. **Results:** Analysis of the ureC sequences from Taiwanese samples revealed that 6 out of 7 gastric ulcer samples clustered together in one group, while the sequences from 7 out of 8 gastric cancer samples were distributed across other groups. Phylogenetic analysis incorporating ureC sequences from different geographical regions showed that Taiwanese ureC sequences could be divided into two distinct groups, although the bootstrap values supporting this separation were low. Notably, the ureC sequences from China formed a distinct group with a high bootstrap value, separate from sequences from all other countries. **Discussion:** There are two genotypes of ureC sequences present in Taiwanese samples, with one genotype showing a closer association with gastric cancer. Additionally, the ureC sequences from China appear to be unique and separate from those obtained from other countries, indicating the presence of genetic diversity in ureC sequences among H. pylori strains from different regions, potentially contributing to differences in disease outcomes. Further research is needed to elucidate the specific mechanisms by which ureC sequence variations may influence the pathogenesis of peptic ulcer diseases and gastric cancer.

Keywords: *Helicobacter pylori;* UreC; phylogenetic tree; gastric cancer

Key Points

Question: What were the associations between genetic sequence variations of the *Helicobacter pylori* (*H. pylori*) ureC gene and the pathogenesis of peptic ulcer diseases?
Findings: The study found distinct genetic variations in the *H. pylori* ureC gene among Taiwanese samples. Gastric ulcer samples tended to cluster together, while gastric cancer samples were distributed among other groups. UreC sequences from China formed a separate and unique group.
Meaning: These findings suggest a potential correlation between genetic sequence variations of the ureC gene and the development of peptic ulcer diseases. The clustering patterns observed and the distinct group from China indicate the presence of different genotypes or strains of *H. pylori*. Further

 doi:10.20944/preprints202308.2029.v1

research is needed to explore the implications of these genetic variations and their role in the pathogenesis of peptic ulcer diseases.

1. Introduction

Helicobacter pylori (*H. pylori*) plays an important mediator role for development of peptic ulcer diseases, including gastric ulcer (GU) and duodenal ulcer (DU), and gastric cancer [1]. Numerous investigations reported that the prevalence of *H. pylori* infection worldwide was decreasing because of the improvement of living-style and sanitary environment, as well as availability of antibiotics and proton pump inhibitor for the treatment of *H. pylori* [2,3]. Nevertheless, the incidence of *H. pylori* infection-mediated gastric cancer remains high in Taiwan [4], and it is unclear what factors predispose the final development of gastric cancer in *H. pylori*-infected patients.

Our previous study demonstrated that *ureC* genotypes was associated with patients with GU and gastric malignancy, by use of a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method [5]. However, the associations between the genetic information (DNA sequences) of *ureC* gene and pathogenesis of peptic ulcer diseases remain elusive. In the present study, *ureC* sequences derived from *H. pylori*-infected gastric tissues of different pathologies were amplified and subjected to DNA sequencing. Based on these sequences, phylogenetic analysis was conducted.

2. Materials and Methods

2.1. Patients and histopathological analysis

H. pylori-positive gastric specimens of different pathologies, including gastritis, gastric ulcer (GU), duodenal ulcer (DU) and gastric cancer, were obtained from Chang Gung Memorial Hospital. All tissues were retrieved from our tissue bank. The research had been approved by IRB committee (IRB: 202200046A3). Clinical parameters of patients in this study were shown in Table 1, including gender, age and pathology.

Table 1. Basic clinical information of patients included.

Patient ID	Gender	Age	Pathology
HP04	Female	49	GU[a]
HP09	Female	41	DU[b]
HP17	Female	37	Gastritis
HP38	Male	63	Gastritis
HP45	Male	63	DU
HP46	Female	41	Gastritis
HP48	Female	57	Gastritis
HP54	Female	76	GU
HP56	Female	32	Gastritis
HP60	Male	54	GU
HP62	Male	52	GU & DU
HP63	Male	44	Gastritis
HP72	Male	57	DU
HP77	Male	62	Gastritis
HP86	Male	53	Gastritis
HP91	Male	54	DU
C007	Female	38	Gastritis
C026	Female	43	Normal
C045	Female	62	Gastritis
C047	Male	74	GU
C062	Male	75	GU

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C066	Female	66	GU
C084	Male	40	Gastritis
C089	Female	39	Gastritis
C100	Female	53	Normal
GC02	Female	70	Gastric cancer
GC08	Male	71	Gastric cancer
GC14	Female	89	Gastric cancer
GC26	Female	69	Gastric cancer
GC32	Male	86	Gastric cancer
GC43	Male	88	Gastric cancer
GC52	Male	76	Gastric cancer
GC65	Female	68	Gastric cancer

[a]Gastric ulcer. [b]Duodenal ulcer.

2.2. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA extraction from frozen tissues was performed as previously described [5]. The nested PCR procedure was used: 95 °C for 10 min, 30 cycles of 95 °C for 30 s and 55 °C for 1 min, and 72 °C for 1 min using primers ureC-1 forward primer: 5′-TTTGGGACTGATGGCGTGAGGGGTAA-3′ and ureC-1 reverse primer: 5′-GGACATTCAAATTCACCAGGTTTTGAGG-3′ for the first round PCR and ureC-2 forward primer: 5′-TGGGACTGATGGCGTGAGGG-3′ and ureC-2 reverse primer: 5′-AAGGGCGTTTTTAGATTTTT-3′ for the second round PCR. The PCR amplicon was monitored by 1.2% agarose gel electrophoresis with DNA view (TOOLs). Subsequently, the *ureC* sequences in these samples were isolated and sent for DNA sequencing.

2.3. Data and statistical analysis

The *ureC* sequences in our samples and previous characterized sequences obtained from GenBank were aligned using CLUSTAL W mode. Moreover, phylogenetic tree analysis was conducted by MEGA X software (version 10.2.4) with Maximum-Likeihood method and bootstrap analysis (1000 replicates)[6].

3. Results

3.1. Identification and determination of ureC sequences in Taiwanese patients

To determine whether *H. pylori* infection based on *ureC* sequences was correlated the pathologies of gastric mucosal diseases, including gastritis, gastric ulcer (GU), duodenal ulcer (DU) and gastric cancer, 33 Taiwanese patients having received esophagogastroduodenoscopy examination and positive for urease test were enrolled for investigation (Table 1). *UreC* sequences in these samples were amplified by PCR (Figure 1) and subjected to DNA sequencing. Totally, 32 *ureC* PCR signals were detectable. Subsequently, a phylogenetic tree based on these *ureC* sequences was constructed by use of MEGA X software. The results indicated that *ureC* sequences in 6 of 7 GU samples (except for patient-HP60) were clustered together (Figure 2), implying that the *ureC* sequences in GU patients likely possessing common features. Interestingly, *ureC* sequences in only 1 of the 8 gastric cancer samples (GC65) was grouped to the GU-related group, whereas GC43, GC32, GC02 and GC08 were grouped in a separated cluster (Figure 2). The other sequences were randomly distributed in the phylogenetic tree (Figure 2).

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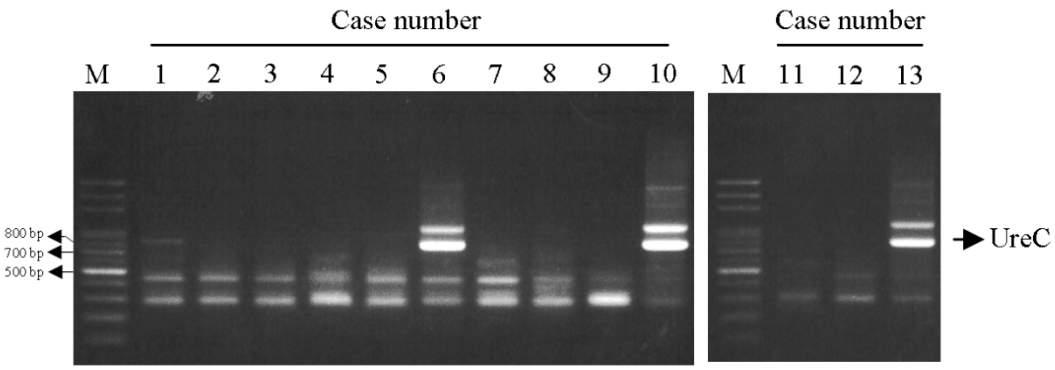

Figure 1

Figure 1. Determination of *ureC* sequence.

Partial *ureC* DNA sequences were amplified by PCR and subject to DNA sequencing. The arrowheads indicated *ureC* amplicon after sequence verification.

A phylogenetic tree was constructed based on 32 partial *ureC* sequences by Mega X software with Maximum-Likelihood method and bootstrap analysis (1000 replicates). The status of gastritis, GU, DU and gastric cancer was displayed by green, blue, brown and red color.

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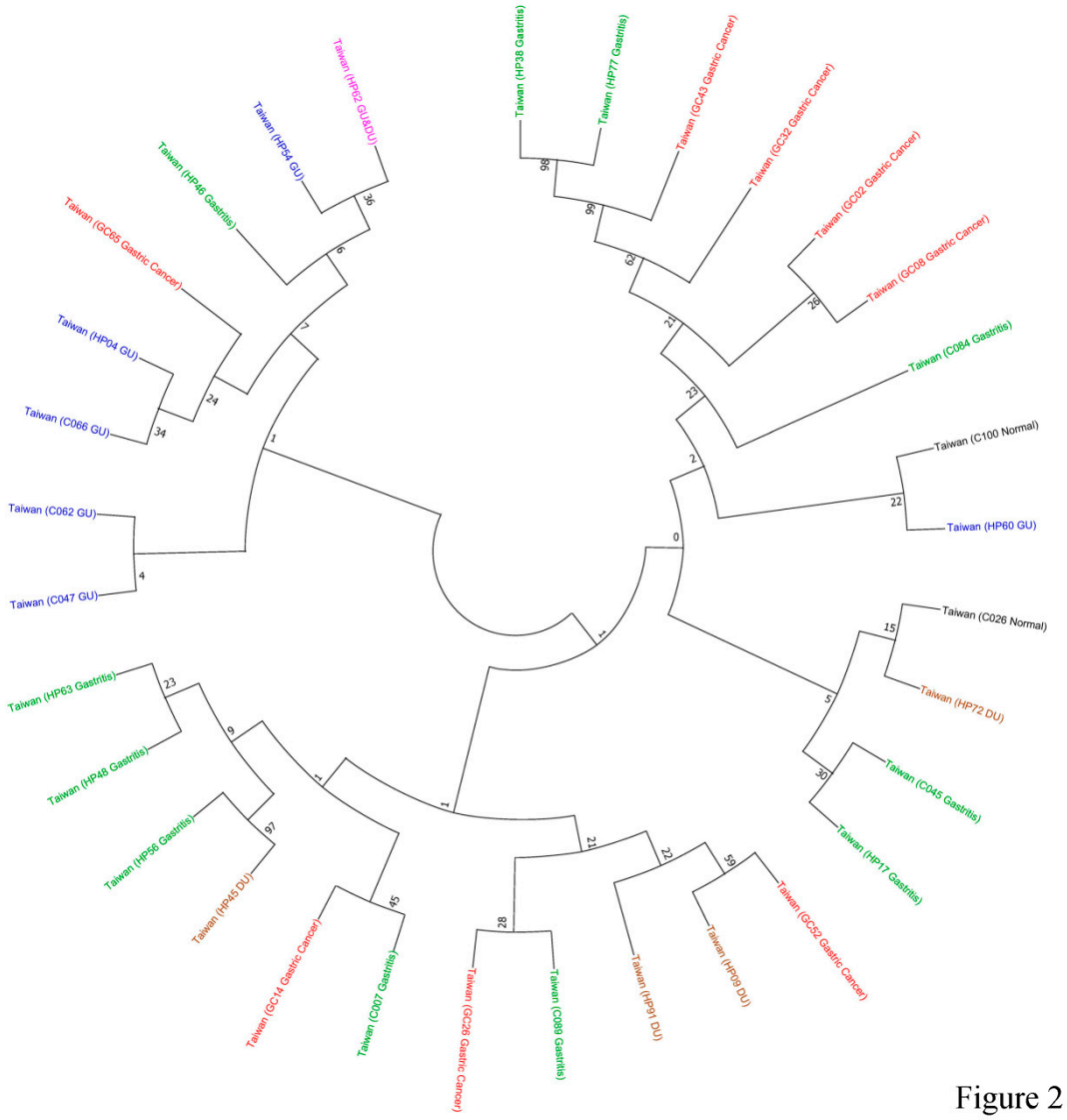

Figure 2

Figure 2. Phylogenetic tree of *ureC* sequences in Taiwanese *H. pylori*-positive samples with different pathologies.

3.2. *Comparison of ureC sequences from Taiwan and other countries*

Previous studies reported that the geographical factor was associated with diversity of *H. pylori* strains [7,8]. To understand the sequence variations between *ureC* sequences from Taiwan (our cohort) and those from 20 different countries (Kuwait, South Africa, Mexico, France, Angola, Malaysia, Australia, Papua New Guniea, India, USA, Vietnam, Japan, Canada, China, Peru, Colombia, Germany, Switerland, South Korea and Singapore) downloadable from GenBank, a phylogenetical tree analysis was performed. The results indicated that two groups of Taiwanese *ureC* sequences (group 1: HP56, HP45, HP63, HP48, HP62, HP54, HP46, GC26 and C089; group 2: GC32, GC43, HP77, HP38, C084, GC08, HP60, C100, HP17, C045, HP72, C026, C007, GC02 and GC14) were separately clustered, but in low bootstrap values (Figure 3). Notably, the 5 of 8 *ureC* sequences from gastric cancer were clustered in group 2.

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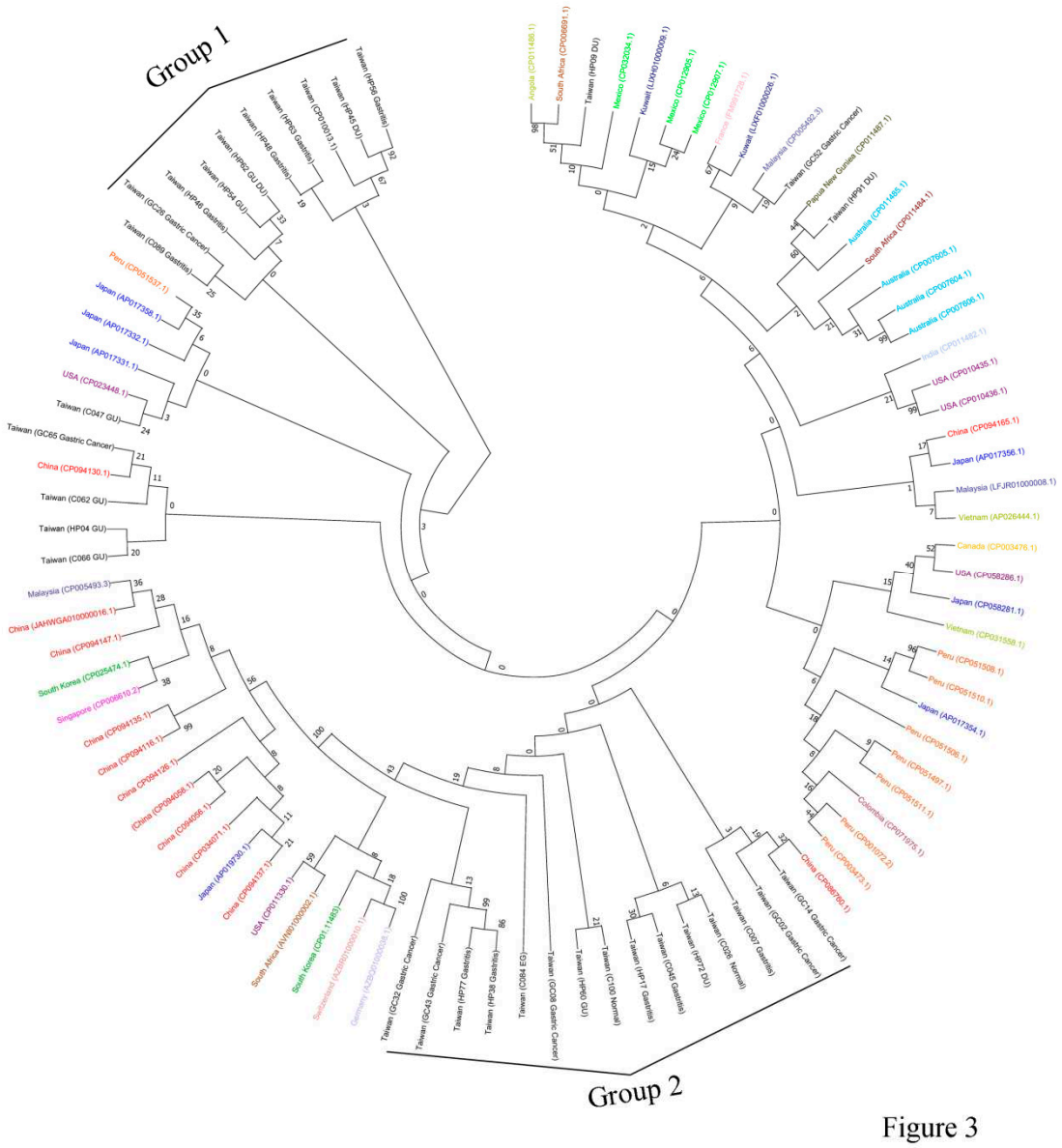

Figure 3

Figure 3. Phylogenetic tree of *ureC* sequences in Taiwan and other country.

The 32 partial *ureC* sequences obtained in the current study were indicated by blue line. *UreC* sequences from various geographic regions were retrieved from GenBank and were shown in different color. These included sequences from Kuwait, South Africa, Mexico, France, Angola, Malaysia, Australia, Papua New Guniea, India, USA, Vietnam, Japan, Canada, China, Peru, Colombia, Germany, Switerland, South Korea, Singapore and Taiwan. The Genbank accession number was shown in brackets. Phylogenetic tree of *ureC* DNA sequence was analyzed using the Maximum-Likelihood method with General Time Reversible model and bootstrap analysis (1000 replicates).

When examining sequences from different geographical regions, it was found that *ureC* sequences from China formed a distinct group with a very high bootstrap value (bootstrap value = 100). Only two Chinese *ureC* sequences, with Genbank accession number CP094130.1 and CP086760.1 were grouped with Taiwanese group 1 and group 2 (gastric cancer group) sequences, respectively, whereas all other 9 Chinese *ureC* sequences clustered to a separated group. Sequences from Peru also formed a separated group, but with low bootstrap values.

 doi:10.20944/preprints202308.2029.v1

4. Discussion

H. pylori urease C gene encodes an open reading frame close to those of the urease AB genes, from which a phosphoglucosamine mutase is derived [9]. This gene is an important regulator to modulate bacterial growth. Comparison of sequences between H. pylori-encoded urease C and Escherichia coli-encoded GlmM, a similarity of 43% is found. Biochemically, GlmM was responsible for converting glucosamine-6-phosphate to glucosamine-1-phosphate, which is an important component of cell wall as well as a building unit of lipopolysaccharides. A meta-analysis reported that the prevalence of *H. pylori* infection in Taiwan was decreasing in the past decades [10]. Concordantly, the incidence of gastric cancer in Taiwan also reduced overtime [11]. It is generally believed that *H. pylori* infection is a risk factor for gastric cancer [12]. However, the cellular and/or molecular mechanisms for gastric cancer oncogenesis have not been completely illustrated. In *H. pylori* positive patients, the associations between urease C (or *ureC* sequences) and pathogenesis of gastric cancer have never been raised. In the present study, by performing phylogenetic analysis, it was found that Taiwanese *ureC* sequences could be separated into two genotypes, with one of them associated with gastric cancer.

On the other hand, it was found that the *ureC* sequences from China formed a robust group with high bootstrap value, separating them from the *ureC* sequences derived from other parts of the world. It implies that Chinese *H. pylori,* based on the *ureC* sequence analysis, have a unique evolutional path, comparing to *H. pylori* from other countries.

Previous study reported that the prevalence of *H. pylori* infection in China ranged from 41.5% to 72.3% [13]. According to our analysis, only two Chinese *ureC* sequences, with Genbank accession number CP094130.1 and CP086760.1 were grouped with Taiwanese group 1 and group 2 (gastric cancer group) sequences, respectively, while all other Chinese *ureC* sequences formed a separated group. This finding is consistent with the long-standing separation between Taiwanese and Chinese people, albeit geologically close between the two areas.

The limitations in this study are: (1) This is a retrospective study. With the availability of anti-*H. pylori* treatment and increasing contact between people over the world, the genetic differences may change. (2) The sample size is small, owing to the decreased number of *H. pylori*-positive gastric samples in Taiwan. (3) Only *ureC* sequences, but not the whole genomes, were used for analysis. (4) The functional association between urease C and gastric cancer remains undetermined.

In conclusion, by analyzing *H. pylori ureC* sequences obtained from Taiwan and the world, we discovered a subgroup of *ureC*, which was associated with gastric cancer. The Chinese *ureC* sequences formed a separated genetic group, distinguishable from sequences from Taiwan and other parts of the world.

Funding: This work was supported by grants from Chang Gung Memorial Hospital, Taoyuan, Taiwan (CMRPG3L1811 to THC).

Conflicts of Interest: The authors have no conflicts to disclose.

List of Abbreviations

H. pylori, Helicobacter pylori; GU, gastric ulcer; DU, duodenal ulcer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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