財團法人明日醫學基金會專題研究計畫申請書

一、基本資料： 申請條碼：

本申請案所需經費(單選)	■A 類(執行計畫所需經費) ☐B 類(研究主持費，限人文處計畫，不須填寫表 C002 及 C004 至 C009)				
計畫類別(單選)	■一般型研究計畫 ☐特約研究計畫 ☐新進人員研究計畫 ☐其他				
研究型別	■個別型計畫 ☐整合型計畫				
申請機構/系所(單位)	長庚大學醫學院微生物及免疫學科				
本計畫主持人姓名	賴志河	職稱	教授	身分證號碼	
本計畫名稱 中文	探討降低膽固醇緩解幽門螺旋桿菌相關疾病的機制				
本計畫名稱 英文	Mechanism of lowering cholesterol ameliorates *Helicobacter pylori*-associated disease				
整合型總計畫名稱					
整合型總計畫主持人				身分證號碼	
全程執行期限	自民國 111 年 1 月 1 日起至民國 111 年 12 月 31 日				
研究學門(請參考本申請書所附之學門專長分類表填寫)	學門代碼	名稱(如為其他類，請自行填寫學門)			
研究性質	■純基礎研究 ☐導向性基礎研究 ☐應用研究 ☐技術發展				
本計畫是否為國際合作計畫	■否； ☐是，合作國家：_____，請加填表 I001～I003				
本計畫是否申請海洋研究船	■否； ☐是，請務必填寫表 C014。				
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三、主要研究人力：

（一）請依照「主持人」、「共同主持人」、「協同研究人員」及「博士後研究」等類別之順序分別填寫。

類 別	姓名	服務機構/系所	職稱	在本研究計畫內擔任之具體工作性質、項目及範圍	*每週平均投入工作時數比率(%)
主持人	賴志河	長庚大學/醫學院/微生物及免疫學科	教授	研究之規劃及推動、實驗設計、整理數據、撰寫研究成果與發表論文	75%

※註：每週平均投入工作時數比率係填寫每人每週平均投入本計畫工作時數佔其每週全部工作時間之比率，以百分比表示（例如：50%即表示該研究人員每週投入本計畫研究工作之時數佔其每週全部工時之百分五十）。

Abstract

The inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, known as statins, are widely prescribed for lowering serum cholesterol. Lipid rafts are membrane microdomains containing cholesterol and phospholipids that associated with *H. pylori*-induced pathogenesis. Treatment of cells with statins reduces bacterial infection which has been found can contribute to immune defense by degrading invading pathogens. Although the combined use of statins and antibiotics reportedly increases *H. pylori* eradication, the mechanisms of how statin mitigates *H. pylori*-associated gastrointestinal disorders remain unclear. This study will investigate the incidence of *H. pylori*-associated diseases in patients who were prescribed and unprescribed statins The results from this study will reveal that statin use may be a feasible method to attenuate *H. pylori*-associated gastrointestinal diseases.

Keywords: *Helicobacter pylori*; pathogenesis; cholesterol; statin

A. Background introduction

Helicobacter pylori infection and related diseases

H. pylori can penetrate the mucosal layer and survive intracellularly in the gastric epithelial cells, thereby escaping host immune response or antimicrobial therapy (1,2). Persistent *H. pylori* infection is associated with several gastroenterological illnesses including gastritis, peptic ulcer, and gastric adenocarcinoma (3).

Helicobacter pylori virulence factors

Several important virulence factors have been found to contribute to *H. pylori* pathogenesis, including vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA) (4). VacA is classified as a pore-forming toxin that possesses the capacity to stimulate intracellular acidic vacuole formation and disrupt cellular homeostasis, leading to apoptosis (5). CagA can be translocated by the *H. pylori* type IV secretion system (TFSS) upon attachment to cells, causing chronic inflammation and oncogenesis in gastric epithelial cells (6).

Cellular cholesterol is responsible for *H. pylori* infection

Lipid rafts are membrane cholesterol-rich microdomains that provide entry portals for many bacterial pathogens or their virulence factors (7). Both CagA translocation and VacA delivery into host cells following *H. pylori* infection require lipid rafts (8-10). *H. pylori* exploits host externalized phosphatidylserine (PS) for CagA delivery *via* TFSS and it subsequently induces pathogenesis (11).

Statin use decrease the risk of bacterial infections

The inhibitors of HMG-CoA reductase, commonly known as statins, are widely prescribed for lowering serum cholesterol (12). In addition, statins are shown to reduce the risk of severe bacterial infections, including *Chlamydia pneumonia* (13), *Clostridium difficile* (14), *Streptococcus pneumonia* (15), and *Staphylococcus aureus* (16). However, the immunomodulatory properties of statins only partly explain the potential anti-infection mechanism (17).

Significance of the study

Cholesterol-rich rafts play a crucial role in *H. pylori*-induced pathogenesis and its progression to peptic ulcer diseases and gastric cancer (18,19). Given the evidence from our recent studies that statins, the rate-limiting enzyme in cholesterol biosynthesis, reduced *H. pylori*-induced pathogenesis of gastric epithelial cells and exhibited reduced risk of gastric cancer (20), we will examine the effect of statins on the risk of *H. pylori*-associated gastric disorders using the nationwide population-based case-control study. This study by using the clinical database analysis, we will validate that statin use attenuates the incidence of *H. pylori*-related gastroenterological illnesses.

B. Preliminary results

1. Statin use decrease the risk of gastric cancer

Our recent findings show that patients who used simvastatin exhibit a significantly reduced risk of gastric cancer (adjusted OR = 0.76, 95% CI = 0.70–0.83) (Table 1). A reduced risk of gastric cancer in patients prescribed lovastatin, compared with those who did not use lovastatin, is also observed (adjusted OR = 0.79, 95% CI = 0.72–0.87).

Table 1. Odds ratios and 95% confidence intervals of gastric cancer associated with statin use and covariates

	Crude		Adjusted†	
Variable	OR	(95% CI)	OR	(95% CI)
Medications				
Simvastatin	0.89	(0.82, 0.96)**	0.76	(0.70, 0.83)***
Lovastatin	0.97	(0.90, 1.04)	0.79	(0.72, 0.86)***
Baseline co-morbidities				
H. pylori-infection	9.38	(7.37, 11.9)***	5.09	(3.98, 6.51)***
Gastric diseases	4.49	(4.30, 4.69)***	4.00	(3.82, 4.19)***
Gastroesophageal reflux disease	3.24	(3.00, 3.49)***	2.13	(1.97, 2.31)***
Gastric polyp	7.32	(5.73, 9.36)***	5.14	(3.98, 6.62)***
Cirrhosis	1.29	(1.24, 1.35)***	0.95	(0.90, 1.00)
Gastritis	1.72	(1.65, 1.79)***	1.15	(1.10, 1.20)***

†Adjusted for *H. pylori*-infection, gastric diseases, gastroesophageal reflux disease, gastric polyp, and gastritis. **, $P < 0.01$; ***, $P < 0.001$.

Abbreviations: CI, confidence intervals; OR, odds ratios.

2. Analysis of defined daily dose

In the multivariate analysis, *H. pylori* infection (adjusted OR = 5.09, 95% CI = 3.98–6.51), gastric diseases (adjusted OR = 4.00, 95% CI = 3.82–4.19), gastroesophageal reflux disease (adjusted OR = 2.13, 95% CI = 1.97–2.31), and gastritis (adjusted OR = 1.15, 95% CI = 1.10–1.20) are associated with increased odds ratios of gastric cancer. We then perform an analysis of the dose-related response. Comparison with nonusers of statins, patients receiving both simvastatin and lovastatin exhibit a significant reduction in the risk of gastric cancer in all dose groups (Table 2). Although these results reveal that statin use reduces the risk of gastric cancer, the potential confounding variables such as *H. pylori* infection, concomitant use of other chemopreventive drugs, and other chronic gastrointestinal diseases, that are required to be clarified.

Table 2. Analysis of defined daily dose of statin prescription

	Case number/ control number	Crude odds ratio	(95% CI)	Adjusted odds ratio†	(95% CI)
Non-use of statins	17402/17291	1.00	(Reference)	1.00	(Reference)
Simvastatin					
<5 DDD	278/318	0.86	(0.74, 1.02)	0.70	(0.59, 0.83)***
5-25 DDD	399/440	0.90	(0.79, 1.03)	0.76	(0.65, 0.88)***
≥ 25 DDD	628/700	0.89	(0.80, 0.99)*	0.79	(0.70, 0.88)***
P for trend					< 0.001
Lovastatin					
<5 DDD	410/408	1.00	(0.87, 1.15)	0.84	(0.72, 0.98)*
5-15 DDD	392/389	1.00	(0.87, 1.15)	0.78	(0.67, 0.91)**
≥ 15 DDD	626/675	0.92	(0.83, 1.03)	0.80	(0.71, 0.90)***
P for trend					< 0.001

†Adjusted for *H. pylori* infection, gastric diseases, gastroesophageal reflux disease, gastric polyp, and gastritis.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
Abbreviations: CI, confidence intervals; DDD, defined daily dose.

C. Experimental designs

Aim 1. Data mining from National Health Insurance Research Database (NHIRD)

To further confirm our results that statin treatment reduces *H. pylori*-induced inflammation and extend these findings that may decrease the risks of gastrointestinal disorders, a clinical database from National Health Insurance (NHI) will be analyzed. The NHI program was implemented in 1995 and covers approximately 99% of the Taiwanese population, and has contracts with 97% of all medical providers (21). For research purposes, this case-control study will analyze the data from Taiwan's NHI electronic records system, which contains all medical claims from 1996 to 2014. The NHIRD contains all medical information, including data on inpatient and outpatient care facilities, drug prescriptions, and diagnoses coded in the format of the International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM). Patient consent is not required to access the NHIRD. By following the Act of Personal Information Protection in Taiwan, this analysis does not require to be approved by the Institutional Review Board (IRB).

Aim 2. Selecting the study subjects

The case group will include patients with hyperlipidemia (ICD-9-CM 272) who are newly diagnosed with peptic ulcer diseases (PUD) (ICD-9-CM 531-535) from 2005 to 2014. Patients aged < 20 years will be excluded. The date of the first diagnosis of PUD will be used as the index date. For each PUD patient, one hyperlipidemia patient without PUD from the same period will be selected using the same exclusion criteria, frequency-matched for sex and age (in 5-y groups). Fig. 11 shows the flowchart for selecting the study groups.

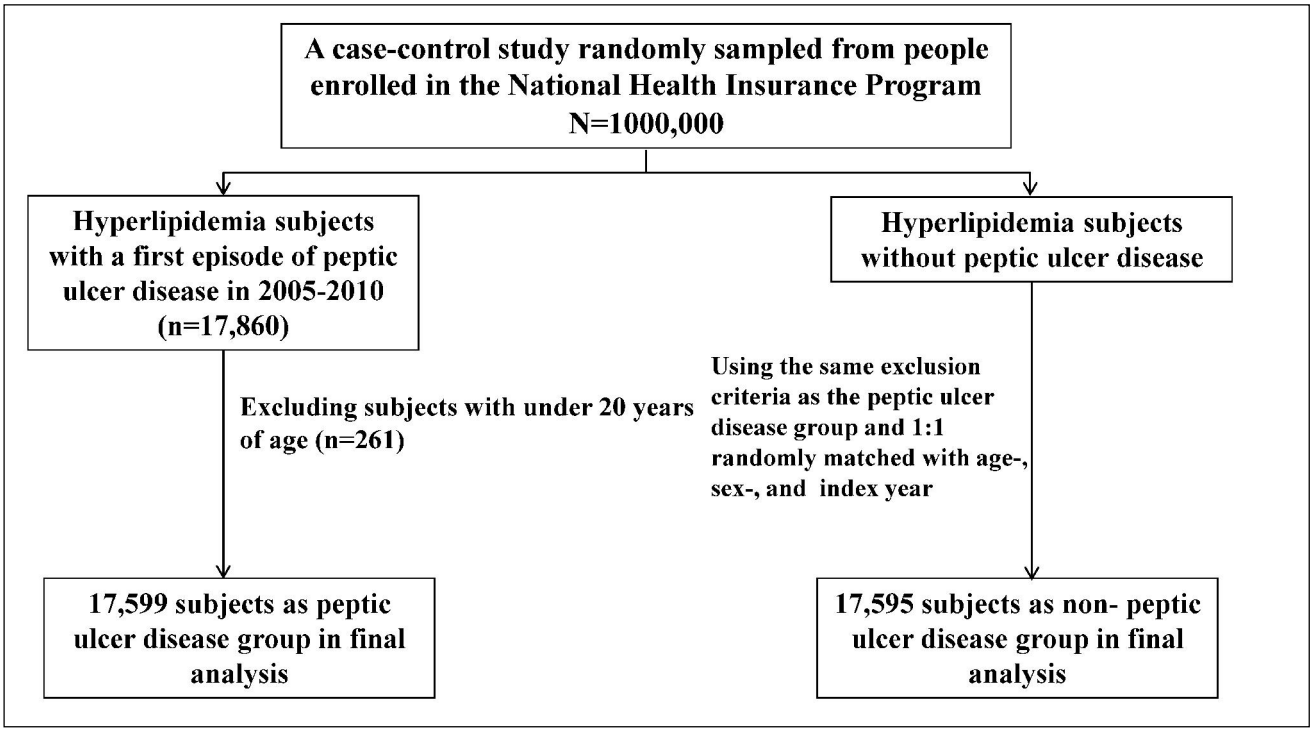

Fig. 1. Flowchart of the database analysis process.

Aim 3. Analysing co-morbidities and measuring statin prescription

The co-morbidities of chronic diseases and the association of statin prescription will be further analyzed. Major co-morbidities considered as covariates are diabetes (ICD-9-CM code 250), stroke (ICD-9-CM codes 430–438), coronary artery disease (CAD) (ICD-9-CM codes 410–414), gastroesophageal reflux disease (ICD-9-CM code 530.81 and 530.11), gastric polyp (ICD-9-CM code 211.1), and *H. pylori* infection (ICD-9-CM code 041.86) at the baseline. Statin usage records are retrieved from the ambulatory and inpatient claims data. According to the total supply in days and the quantity of statin, the cumulative defined daily dose (DDD) of each type of statin, including simvastatin, lovastatin, pravastatin, fluvastatin, atorvastatin, and rosuvastatin, will be analyzed. For each type of statin, the cumulative DDD will be divided into 2 levels according to the median dose. For NHI database analysis, the baseline characteristics of the PUD and non-PUD groups will be compared using a chi-square test. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for factors associated with the risk of PUD will be estimated using univariable and multivariable logistic regression models. All statistical analyses will be performed using SAS statistical software (SAS Institute, Inc).

D. Anticipated results

The main goal of this proposal is to reveal how statins inhibit the *H. pylori*-associated diseases and analyze the Taiwan's National Health Insurance Research Database (NHIRD) in clinical aspects. We will integrate the scientist and physician who are major in infectious diseases, cell biology, and microbiology to form a research team for fundamental scientific problems on pathogen-host clinical issues. Studies from this proposal will demonstrate the use of statins may be able to attenuate *H. pylori*-induced pathogenesis in host stomachs and reduce the risk of gastrointestinal diseases as well.

1. In this study, a nationwide case-control study based on data from the NHIRD will be analyzed to compare the incidence of gastric disorders in patients who prescribed statins.
2. This project is proposing to collaborate with Professor. Hwai-Jeng Lin (林懷正教授). Standing on the novel technology, high quality, and clinical collaborations, we believe that the results from this study would have a great impact on the research of clinical aspects.
3. It is expected that we will have 1-2 high-quality publications based on this proposal. Moreover, we will train skillful biologists who will be the future human resource for the biomedical and clinical researches in Taiwan.

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