國家科學及技術委員會補助專題研究計畫報告

非酒精性脂肪肝的性別差異：聚焦於脂聯素的研究（第二年及第三年）

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計 畫 主 持 人 ： 張明鈴

計畫參與人員： 碩士級-專任助理：吳麗謹

本研究具有政策應用參考價值：■否 □是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：□否 □是

中 華 民 國 111 年 10 月 28 日

中 文 摘 要 ： 非酒精性脂肪肝（NAFLD）為非酒精造成的脂肪肝，是目前最全球常見的肝病（約23億人罹病）。與非酒精性脂肪肝相關的三個主要死亡原因包括心血管疾病，惡性腫瘤和肝臟相關疾病。目前並且沒有針對非酒精性脂肪肝的特殊藥物。幾項臨床研究證實，肝臟疾病具有明顯的性別差異。通常男性死於慢性肝病和肝硬化的可能性是女性的兩倍。有趣的是，非酒精性脂肪肝在絕經後女性中的發病率是絕經前女性的兩倍。性激素與脂肪分佈之間的相互作用或可以解釋肝病的性別差異。目前也仍無針對非酒精性脂肪肝性別差異的精確療法。

源自脂肪組織的分泌性蛋白統稱為脂肪激素。脂肪的積累，包括內臟肥胖和脂肪肝，被認為是脂肪組織功能障礙的結果，並導致脂肪激素分泌量改變。越來越多的證據顯示，脂肪激素可調節非酒精性脂肪肝的脂肪變性和發炎。男性和女性之間脂肪激素改變的模式是不同的。脂聯素(adiponectin)，一種30 kDa大小的脂肪激素，在脂肪細胞及肝細胞皆可表現。脂聯素及其受體可能經由增加β-氧化作用，減少脂肪酸的合成，促進攝取和抑制肝中葡萄糖的產生來保護肝細胞免受三酸甘油酯的累積。因此，非酒精性脂肪肝與低脂聯素有關。一些動物研究顯示脂聯素可助於免除肥胖相關的非酒精性脂肪肝的傷害。在人體研究中，脂聯素已被證明是非酒精性脂肪肝，非酒精性脂肪肝炎，肝臟纖維化和胰島素阻抗的生物標誌。並且，女性的脂聯素通常高於年齡相當的男性。

綜合以上，針對脂聯素相關途徑的研究有潛力釐清非酒精性脂肪肝的性別差異基轉。吾人已開發了在四環素反式激活因子控制下在肝臟具有高、中、低三種C型肝炎病毒核心蛋白不同表現量的轉基因小鼠，這些小鼠表現出非肥胖型的非酒精性脂肪肝。而成熟的商業化db / db小鼠則可作為肥胖型的非酒精性脂肪肝的動物模型。因此，基於吾人先前的研究結果，這些成果包涵了闡明脂聯素在脂肪肝的小鼠，非酒精性脂肪肝和慢性C型肝炎患者的作用，並闡明了各種慢性肝病中的脂肪激素和代謝體的變化，本計畫旨在經由對非酒精性脂肪肝進行前瞻性病例對照研究，並對病人的肝內和肝外併發症進行三年追蹤，以剖析性別對非酒精性脂肪肝的影響。而女性患者更會按月經期和更年期作分層分析，這一切皆會專注於脂聯素相關途徑的研究。此外，脂聯素相關途徑的治療將在具有與人非酒精性脂肪肝相似表現型的小鼠測試。此計畫有望提供針對關鍵的性別因素，以控制非酒精性脂肪肝及其相關的併發症發生。

中 文 關 鍵 詞 ： 非酒精性脂肪肝; 性別差異; 脂聯素; C型肝炎病毒核心蛋白轉基因小鼠; db / db小鼠

英 文 摘 要 ： Nonalcoholic fatty liver disease (NAFLD) is defined by a non-alcoholic nature in the presence of fatty liver, and is currently the most common liver disease with increasing importance globally (2300 million individuals worldwide). The 3 major causes of NAFLD-related mortality include cardiovascular disease, all-cause malignancy, and liver-related death. Currently, diet and exercise are the mainstay treatment for the majority of patients with NAFLD and no specific medication for NAFLD is available. Several

clinical studies show a profound gender dimorphism in liver diseases. In general, men are 2-fold more likely to die from chronic liver disease and cirrhosis than are women. Interestingly, NAFLD is twice as common in postmenopausal women as in premenopausal women. Substantially, interactions between sex hormones and adipose distribution may explain the differences in the sex-specific liver diseases. However, the precise therapy to probe the gender dimorphism in NAFLD remained unidentified.

Secretory proteins derived from adipose tissue are collectively called adipokines. Ectopic fat accumulation, including visceral obesity and fatty liver, can be considered a consequence of adipose tissue dysfunction and results in altered adipokine levels. Increasing evidence indicates that adipkines regulate steatosis and inflammation in NAFLD. The pattern of adipokine alterations are distinct between the men and women. Adiponectin, a 30-kDa adipokine, is highly expressed in adipocytes and is also expressed in hepatocytes. Adiponectin and its receptors might protect hepatocytes from triglyceride accumulation by increasing β-oxidation, decreasing the de novo synthesis of fatty acids, and promoting the uptake and inhibiting the production of glucose in the liver. Thus, hepatitis steatosis is usually associated with low levels of adiponectin. Some animal studies had showed the beneficial effect of adiponectin in protecting obesity-related NAFLD. In human studies, adiponectin had been documented as biomarkers for NAFLD, nonalcoholic steatohepatitis, fibrosis, and insulin resistance. Higher adiponectin levels are usually noted in females than age-matched males.

Taken together, targeting the adiponectin-associated pathway has the potentiality to dissect the gender-dimorphism basis in NAFLD. By using conditional transgenic mice that over-express the hepatitis C virus (HCV) core in the liver, we had developed three transgenic mouse lines with core expression under the control of the tetracycline transactivator, those mice exhibited non-obese NAFLD. While well-established commercialized db/db mutant mice may serve as a suitable animal model of obese NAFLD. Thus, based on the results our previous studies investigating the roles of adiponectin in mice with hepatic steatosis, in human with NAFLD and chronic HCV infection; and surveying the adipokine and metabolic profiles in chronic liver diseases, the present proposal is designed to dissect the impact of gender on NAFLD by conducting a prospective case-control cohort of NAFLD, with 3-year follow-up of hepatic and extra-hepatic manifestations, stratified by the

menstruation period and the presence of menopause in women, and focused on the adiponectin-associated pathway. In parallel, the associated basis will be probed by using the aforementioned mice with equivalent phenotypes for human NAFLD. The current proposal holds promise to provide therapeutic interventions targeting crucial gender factors to control NAFLD-associated complications in Taiwan.

英文關鍵詞：NAFLD; gender dimorphism; adiponectin, HCV core transgenic mice; db/db mutant mice

報告內容：

Background

Nonalcoholic fatty liver disease (NAFLD) is defined by a non-alcoholic nature in the presence of fatty liver, a reversible condition wherein triglyceride fat accumulates in more than 5% of the hepatocytes [1]. The prevalence of NAFLD is increasing as a result of increasingly sedentary lifestyles, globalization of the Western diet, and improving food supplies in previously famine-stricken areas [2]. According to World Health Organization estimates, approximately 2300 million individuals likely have NAFLD worldwide, while 500 million are living with chronic hepatitis B or C [3]. The estimated local prevalence of NAFLD is up to 66.5% in Taiwan [4-6]. Given that the overwhelming estimated prevalence rate of NAFLD, the presence of effective nucleot(s)ide analogues and vaccines for treating and preventing hepatitis B virus infection [7], and the potent direct-acting anti-viral agents for eliminating hepatitis C virus infection [8], NAFLD is currently the most common liver disease with increasing importance globally [9]. The term NAFLD covers a pathologic spectrum from lipid accumulation alone (simple steatosis) to steatosis with associated inflammation and fibrosis [ie. nonalcoholic steatohepatitis (NASH)]. The three major causes of NAFLD-related mortality include cardiovascular disease, all-cause malignancy, and liver-related death [10]. NASH can progress to cirrhosis and potentially to hepatocellular carcinoma [11], however, most patients with NAFLD outlive their liver disease and are more likely to develop fatal complications from cardiovascular disease or malignancy [11]. Currently, diet and exercise are the mainstay treatment for the majority of patients with NAFLD and no specific medication target NAFLD is available [9].

Several clinical studies show a profound gender dimorphism in liver diseases as women more commonly present with acute liver failure, autoimmune hepatitis, benign liver lesions, primary biliary cirrhosis, and toxin-mediated hepatotoxicity, but less commonly have malignant liver tumors, primary sclerosing cholangitis, viral hepatitis, liver transplant and hepatitis C virus (HCV)-associated decompensated cirrhosis than men do. In general, men are 2-fold more likely to die from chronic liver disease and cirrhosis than are women [12-14]. Interestingly, NAFLD is twice as common in postmenopausal women as in premenopausal women whose estrogen levels are higher than postmenopausal women, which suggests the protective role of estrogens in NAFLD [15]. Nonalcoholic steatohepatitis (NASH) patients who are candidates for liver transplantation seem to be generally older, female, and Asian; are most often affected by diabetes, hypertension, obesity, and cardiac disease [16]. Substantially, interactions between sex hormones, adipose distribution and sex hormone-binding globulin may explain the differences in the sex-specific liver diseases. However, the precise therapy to probe the gender dimorphism in NAFLD remained unidentified.

Secretory proteins derived from adipose tissue are collectively called adipokines [17]. Ectopic fat accumulation, including visceral obesity and fatty liver, can be considered a consequence of adipose tissue dysfunction and results in altered adipokine levels [18]. Increasing evidence indicates that adipkines regulate steatosis and inflammation in NAFLD [19-20]. Therefore, characterising the adipokine alterations in NAFLD may help to evaluate the prognosis of NAFLD. Moreover, the pattern of adipokine alterations are distinct between the men and women with NAFLD [20]. The sexual dimorphism of baseline adipokine has been attributed to the direct effects of sex hormones on adipocytokine secretion or to differences in the body fat composition of normal individuals [21]. Adiponectin, a 30-kDa adipokine, is highly expressed in adipocytes and is also expressed in hepatocytes [22]. However, increased visceral adipose tissue stores reduce the

abundance of circulating adiponectin [20]. Several insulin resistance (IR)-associated hormones such as insulin and catecholamines might dysregulate adiponectin expression [23]. Post-translational adiponectin modifications result in the secretion of oligomers of 90-kDa trimers, which are found in the circulation as low molecular weight (LMW) and high molecular weight (HMW) adiponectins. HMW adiponectin is more closely correlated with insulin sensitivity than LMW adiponectin [24]. Adiponectin mediates its effects on target cells via at least two adiponectin receptors, adiponectin receptor I (AdipoR1) and receptor II (AdipoR2). AdipoR1 is abundantly expressed in skeletal muscle and the liver, whereas AdipoR2 is primarily expressed in the liver [25]. Adiponectin and its receptors might protect hepatocytes from triglyceride accumulation by increasing β-oxidation, decreasing the de novo synthesis of fatty acids, and promoting the uptake and inhibiting the production of glucose in the liver [26-27]. Thus, hepatitis steatosis is usually associated with low levels of adiponectin [27]. In addition, statin-inducted hypolipidemia is associated with hyperadiponectinemia [28]. Moreover, adiponectin has anti-inflammatory, anti-atherosclerotic and anti-apoptotic properties [29]. Paradoxically, circulating adiponectin has been positively correlated with heart failure, coronary artery disease and all-cause mortality [30-31]. Some animal studies had showed the beneficial effect of adiponectin in protecting obesity-related NAFLD [32]. In human studies, adiponectin had been documented as biomarkers for NAFLD [33], NASH [34-36], fibrosis [37], insulin resistance [38-39] and glucose metabolism [40]. Species and gender differences in plasma levels, tissue or cell distribution and hormonal regulation have been reported for adiponectin [41]. Higher adiponectin levels are usually noted in females than age-matched males [20]. For women, the lowest levels of adiponectin are observed during the postovulatory period [42]. Plasma adiponectin levels correlated negatively with body fat percentage in older males but not in older females [43].

Taken together, targeting the adiponectin-associated pathway has the potentiality to dissect the basis of gender-dimorphism in NAFLD and provide the tailored follow-up and treatment protocols for NAFLD. By using conditional transgenic mice that over-express the hepatitis C virus (HCV) core in the liver, we had developed 3 transgenic mouse lines with low, intermediate or high core expression under the control of the tetracycline transactivator (tTA) [44-50], those mice exhibited non-obese NAFLD. While well-established commercialized db/db mutant mice [51] may serve as a suitable animal model of obese NAFLD. Thus, based on the results our previous studies investigating the roles of adiponectin in non-obese mice with hepatic steatosis [50], in human with NAFLD [20] and in human with chronic HCV infection [52-53] (which is the main cause for NAFLD [54]); and surveying the adipokine [55-57] and metabolic profiles [58-62] in chronic liver diseases, the present proposal is designed to dissect the impact of gender on NAFLD in Taiwan by conducting a prospective case-control cohort of NAFLD, with 3-year follow-up of hepatic and extra-hepatic manifestations, stratified by the menstruation period and the presence of menopause in women, and focused on the adiponectin-associated pathway. In parallel, the associated basis of will be probed by using the HCV core transgenic mice and db/db mice with equivalent phenotypes for human NAFLD. The current proposal holds promise to provide therapeutic interventions targeting crucial gender factors to control NAFLD-associated complications in Taiwan.

The **specific aims** of the current proposal are as follows:

1. Enroll patients with normal liver and NAFLD to systematically determine the associated gender-associated pathophysiology and prognosis.
2. Maintain and characterize the mice with NAFLD.
3. Dissect the basis for the various phenotypes of NAFLD in mice.

4. Survey adiponectin-specific sex hormone, adipokine, metabolic and liver profiles for the prognosis of NAFLD in humans with sex, menstruation period and presence of menopause stratification.
5. Elucidate the potential adiponectin-specific target of the associated pathways in the mice with various phenotypes equivalent to human NAFLD.

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Based on this grant, we had accomplished several papers, as follows:

1. **Chang ML*,** Yang Z, Yang SS. Roles of Adipokines in Digestive Diseases: Markers of Inflammation, Metabolic Alteration and Disease Progression. Int J Mol Sci. 2020 Nov 5;21(21):8308.
2. **Chang ML*,** Hu JH, Pao LH, Lin MS, Kuo CJ, Chen SC, Fan CM, Chang MY, Chien RN. Critical role of triglycerides for adiponectin levels in hepatitis C: a joint study of human and HCV core transgenic mice. BMC Immunol. 2021 Aug 11;22(1):54.
3. **Chang ML***. Fatty Pancreas-Centered Metabolic Basis of Pancreatic Adenocarcinoma: From Obesity, Diabetes and Pancreatitis to Oncogenesis. Biomedicines. 2022 Mar 17;10(3):692.

1. Roles of Adipokines in Digestive Diseases: Markers of Inflammation, Metabolic Alteration and Disease Progression

Ming-Ling Chang *, Zinger Yang, Sien-Sing Yang

Adipose tissue is a highly dynamic endocrine tissue and constitutes a central node in the interorgan crosstalk network through adipokines, which cause pleiotropic effects, including the modulation of angiogenesis, metabolism, and inflammation. Specifically, digestive cancers grow anatomically near adipose tissue. During their interaction with cancer cells, adipocytes are reprogrammed into cancer-associated adipocytes and secrete adipokines to affect tumor cells. Moreover, the liver is the central metabolic hub. Adipose tissue and the liver cooperatively regulate whole-body energy homeostasis via adipokines. Obesity, the excessive accumulation of adipose tissue due to hyperplasia and hypertrophy, is currently considered a global epidemic and is related to low-grade systemic inflammation characterized by altered adipokine regulation. Obesity-related digestive diseases, including gastroesophageal reflux disease, Barrett's esophagus, esophageal cancer, colon polyps and cancer, non-alcoholic fatty liver disease, viral hepatitis-related diseases, cholelithiasis, gallbladder cancer, cholangiocarcinoma, pancreatic cancer, and diabetes, might cause specific alterations in adipokine profiles. These patterns and associated bases potentially contribute to the identification of prognostic biomarkers and therapeutic approaches for the associated digestive diseases. This review highlights important findings about altered adipokine profiles relevant to digestive diseases, including hepatic, pancreatic, gastrointestinal, and biliary tract diseases, with a perspective on clinical implications and mechanistic explorations.

2. Critical role of triglycerides for adiponectin levels in hepatitis C: a joint study of human and HCV core transgenic mice

Ming-Ling Chang*, Jing-Hong Hu, Li-Heng Pao, Ming-Shyan Lin, Chia-Jung Kuo, Shiang-Chi Chen, Chun-Ming Fan, Ming-Yu Chang, Rong-Nan Chien

Background: Both hepatitis C virus (HCV) infection and adiponectin are critically involved in metabolism. The reversal and associations of altering adiponectin levels after sustained virological responses (SVRs) following direct-acting antivirals (DAA) in HCV-infected patients remained elusive.

Methods: A joint study was conducted in a prospective cohort of 427 HCV-infected patients and a line of HCV core transgenic mice.

Results: Of 427, 358 had completed a course of DAA therapy and 353 had SVRs. At baseline, male sex (95% CI β: - 1.44 to - 0.417), estimated glomerular filtration rate (eGFR) (- 0.025 to - 0.008), triglycerides (- 0.015 to - 0.005), and fibrosis-4 levels (0.08-0.297) were associated with adiponectin levels; BMI (0.029-0.327) and triglycerides levels (0.01-0.03) were associated with homeostatic model assessment for insulin resistance (HOMA-IR) in HCV-infected patients. At 24-week post-therapy, in SVR patients, male sex (- 1.89 to - 0.5) and eGFR (- 0.02 to - 0.001) levels were associated with adiponectin levels, levels of BMI (0.094-0.335) and alanine transaminase (0.018-0.078) were associated with HOMA-IR; compared with baseline levels, adiponectin levels decreased (6.53 ± 2.77 vs. 5.45 ± 2.56 μg/mL, $p < 0.001$). In 12-month-old HCV core transgenic mice with hepatic steatosis, triglyceride levels (0.021-0.111) were associated with adiponectin levels, and hepatic adipopnectin expression was comparable with that of control mice.

Conclusions: Triglycerides and hepatic fibrosis are associated with HCV-specific alteration of adiponectin levels, and adiponectin may affect insulin sensitivity through triglycerides during HCV infection. In DAA-treated patients, after SVR, adiponectin levels decreased and the linking function of triglycerides between adiponectin and insulin sensitivity vanished. Moreover, HCV core with hepatic steatosis might affect extrahepatic adiponectin expression through triglycerides.

3. **Fatty Pancreas-Centered Metabolic Basis of Pancreatic Adenocarcinoma: From Obesity, Diabetes and Pancreatitis to Oncogenesis**

Ming-Ling Chang*

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancer, and it is currently the third most common cause of cancer death in the U.S.A. Progress in the fight against PDAC has been hampered by an inability to detect it early in the overwhelming majority of patients, and also by the reduced oxygen levels and nutrient perfusion caused by new matrix formation through the activation of stromal cells in the context of desmoplasia. One harbinger of PDAC is excess intrapancreatic fat deposition, namely, fatty pancreas, which specifically affects the tumor macro- and microenvironment in the organ. Over half of PDAC patients have diabetes mellitus (DM) at the time of diagnosis, and fatty pancreas is associated with subsequent DM development. Moreover, there is a strong association between fatty pancreas and fatty liver through obesity, and a higher intrapancreatic fat percentage has been noted in acute pancreatitis patients with DM than in those without DM. All these findings suggest that the link between fatty pancreas and PDAC might occur through metabolic alterations, either DM-related or non-DM-related. Based on clinical, in vivo and in vitro evidence, the current review highlights the etiologies of fatty pancreas (including fatty infiltration and replacement) and the fatty pancreas-associated metabolic alterations involved in oncogenesis to provide crucial targets to prevent, detect, and/or effectively treat PDAC.

110年度專題研究計畫成果彙整表

計畫主持人：張明鈴			**計畫編號：**110-2629-B-182-001-		
計畫名稱：非酒精性脂肪肝的性別差異: 聚焦於脂聯素的研究（第二年及第三年）					
成果項目			量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	3	篇	1. Chang ML*, Yang Z, Yang SS. Roles of Adipokines in Digestive Diseases: Markers of Inflammation, Metabolic Alteration and Disease Progression. Int J Mol Sci. 2020 Nov 5;21(21):8308. 2. Chang ML*, Hu JH, Pao LH, Lin MS, Kuo CJ, Chen SC, Fan CM, Chang MY, Chien RN. Critical role of triglycerides for adiponectin levels in hepatitis C: a joint study of human and HCV core transgenic mice. BMC Immunol. 2021 Aug 11;22(1):54. 3. Chang ML*. Fatty Pancreas-Centered Metabolic Basis of Pancreatic Adenocarcinoma: From Obesity, Diabetes and Pancreatitis to Oncogenesis. Biomedicines. 2022 Mar 17;10(3):692.
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	1		吳麗謹
	非本國籍	大專生	0		

		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）			無		

國家科學及技術委員會補助研究計畫
涉及臨床試驗之性別分析報告

日期：2022 年 10 月 28 日

計畫編號	MOST 110-2629-B-182-001 -		
研究人員姓名	張明鈴		
任職機關系所	長庚大學醫學系	職稱	教授
計畫名稱	非酒精性脂肪肝的性別差異: 聚焦於脂聯素的研究 (第二年及第三年)		

說明：

本年度專題研究計畫涉及臨床試驗且進行性別分析，請於計畫進度報告/成果報告時一併繳交「性別分析報告」。

項次	項目	說明	備註
1	本計畫之研究結果已進行性別分析。	Yes	
2	本計畫之收案件數及其性別比例。	1:1	
3	本計畫研究結果之性別差異說明。 如無性別差異，亦請說明。	Male sex is associated with low adiponectin levels.	