國家科學及技術委員會補助專題研究計畫報告

辣椒素協助改善自體免疫疾病腸道暨陰道微菌叢以利孕婦健康

報 告 類 別 ： 精簡報告
計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 110-2629-B-016-001-
執 行 期 間 ： 110年08月01日至111年07月31日
執 行 單 位 ： 國防醫學院風濕免疫科

計 畫 主 持 人 ： 盧俊吉

計畫參與人員： 碩士級-專任助理：卓君蓉

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 11 月 15 日

中 文 摘 要 ： 全身性紅斑性狼瘡及類風濕性關節炎皆可侵犯生育年齡的女性，全身性紅斑性狼瘡及類風濕性關節炎患者皆已發現腸道微菌叢異常，且此微菌叢異常與疾病活性皆相關。全身性紅斑性狼瘡患者懷孕時疾病活性會增加，類風濕性關節炎女性患者在懷孕時疾病活性反而下降。目前沒有很明確的證據解釋為何全身性紅斑性狼瘡跟類風濕性關節炎女性在懷孕的過程當中會導致疾病活性截然不同的變化。目前文顯已知懷孕過程中媽媽陰道的微菌叢會影響胎兒的健康。研究顯示懷孕孕程腸道微菌叢異常會導致媽媽的腸道微菌叢改變及腸道上皮層免疫功能及生理功能異常而導致早產或流產。本研究假設女性陰道微菌叢可藉由會陰部的局部接觸影響腸道微菌叢及腸道陰道的微菌叢交互作用軸線存在。因此全身性紅斑性狼瘡及類風濕性關節炎女性患者可能藉由腸道微菌叢異常影響陰道微菌叢異常，因此影響患者懷孕過程異常腸道免疫反應及全身免疫反應，藉由討論備孕前，三個懷孕孕程及生產完後腸道及陰道微菌叢的交互作用及對自體免疫活性影響，有助於認知全身性紅斑性狼瘡跟類風濕性關節炎於女性懷孕期間的自體免疫活性變化。

營養素補充以調整腸道微菌叢已大幅使用在某些疾病的治療如大腸癌及糖尿病。本研究顯示紅斑性狼瘡患者包括腎炎患者，約30%病友日常飲食會包括辣椒素攝取。辣椒素可參與腸道微菌叢生理反應，增加腸道某些代謝氨基酸產物的細菌。辣椒素可以抑制全身抗發炎反應。然而辣椒素與腸道細菌的交互作用詳細的機轉並不清楚，本實驗顯示健康成年女性腸道與陰道微菌叢經過辣椒素補充之後，微菌叢組成成分與相關代謝物機轉會有所變化，包括蛋白質以及某些發炎反應的代謝物訊號。全身性紅斑性狼瘡女性停止攝取辣椒素之後，腸道微菌叢的組成及代謝反應亦有所改變。本研究結果顯示食物辣椒素攝取的確會影響實驗小鼠及健康成年人及紅斑性狼瘡女性的腸道及陰道的微菌叢，未來的研究需要更徹底的解析腸道微菌叢及陰道微菌叢如何藉由局部的免疫反應影響到胎盤的成長及全身免疫系統的異常活性。利用無菌小鼠動物實驗證實食物辣椒素對腸道微菌叢的影響與腸道本身微菌叢的細菌互相作用。我們希望藉由辣椒素能否調控腸道及陰道的微菌叢交互作用來協助治療紅斑性狼瘡及類風濕性關節炎並預防懷孕過程中的疾病活性變化以利婦幼健康。

中 文 關 鍵 詞 ： 全身性紅斑性狼瘡，類風濕性關節炎，陰道微菌叢，懷孕，辣椒素，腸道免疫

英 文 摘 要 ： Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) could affect women of childbearing age. Pregnancy can predispose to a lupus flare. Patients are recommended not to conceive until the disease has been quiescent for at least 6 months. However, disease activity of RA often attenuated during pregnancy, while disease flares occur during the postpartum period. RA patients in low disease activity in the first trimester are likely to have low disease activity or remission in the last trimester. Inflammation and immunosuppressants therapy influence the safety during implantation and early pregnancy in women

with RA and SLE. The evidence explaining why disease activities have diverse changes in SLE and RA pregnant women is lacking. However, quiescent disease activities of SLE and RA for six months on pregnancy-compatible medications are recommended to optimize maternal and fetal outcomes.

The gut microbiota during pregnancy is a critical determinant of offspring health. Vertical transmission of bacteria from mother to newborn contributes to developing the microbiota of the infant gut. Changes in metabolic, microbiota and epithelial permeability of intestine contribute to intestinal bacterial homeostasis. Microbes interact with each other in women through a periurethral contamination. The vaginal microbiota increases levels of vaginal inflammatory cytokines, which, in turn, increases the risk of spontaneous preterm birth. The relationship between the immune system, gut microbiota, vaginal microbiota, and metabolism of pregnant women is unclear. We hypothesize the importance of the gut-vaginal microbiota interaction of women in the outcomes of pregnancy and emphasize that changes in bacteria composition at gut and vagina are associated with pregnancy outcomes of SLE or RA patients. Population-specific studies should be able to shed light on the regulation of the vaginal microbiota as therapeutic strategies, which include immune modulators and microbiome-based therapeutic approaches, the pharmacomicrobiomics.

Diet nutrients supplement help regulate the gut microbiome and could represent an innovative therapeutic strategy. Spicy food, especially capsaicin (CAP), has been recently drew attention from their benefits on the gut microbiota. Dietary CAP could help improve obesity, glucose homeostasis, and insulin sensitivity. However, the mechanisms how CAP reshapes the intestinal and vaginal microbiota are not completely elucidated. Considering the increasing interests in intestinal and vaginal microbiota regulation, and the emerging data linking CAP to the gut and vaginal microbiota and relevant metabolites pathways, we aim to underline the possible mechanisms by which diet CAP exerts its influence in the gut and vaginal dysbiosis in patients of SLE and RA. Our data indicated that diet CAP could influences the intestinal and vaginal microbiota in healthy women. SLE women who have regular diet CAP intake would have altered gut microbiota after one week withdrawing CAP. CAP mediated intestinal microbiota changes is in a intestinal microbiota dependent manner in the germ free mice experiments. In a conclusion, diet CAP is engaged in the gut-vaginal microbiota interaction and metabolites

signaling. We will elucidate the detailed mechanism in the future.

英文關鍵詞：Systemic lupus erythematosus, rheumatoid arthritis, microbiota, pregnancy, capsaicin, vagina

前言

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease that affects women of childbearing age (1). Pregnancy can predispose to a lupus flare, especially if the disease is not adequately controlled (2). Factors associated with the adverse pregnancy outcomes include active lupus nephritis (LN), hypertension, high-dose steroids and adverse effects of immunosuppressants (3-5). Women with quiescent LN have no increased risk of maternal and fetal complications as compared to non-renal patients (6-8). A multidisciplinary panel of experts have developed evidence- based recommendations on the management of family planning and women's health issues in SLE (9). Disease activity of rheumatoid arthritis **(**RA) often attenuated during pregnancy, while disease flares occur during the postpartum period (10). RA patients in low disease activity in the first trimester are likely to have low disease activity or remission in the last trimester (11-13). Inflammation and immunosuppressants therapy influence the safety during implantation and early pregnancy in women with RA. The evidence explaining why disease activities have diverse changes in SLE and RA pregnant women is lacking. Quiescent disease activities of SLE and RA for six months on pregnancy-compatible medications are recommended to optimize maternal and fetal outcomes.

The gut microbiota during pregnancy is a critical determinant of offspring health (14-17). Vertical transmission of bacteria from mother to newborn contributes to developing the microbiota of the infant gut (18). Increased intestinal permeability in early pregnancy is associated with increased maternal levels of lipopolysaccharides, excessive inflammatory cytokines at the endometrial level and increased risk of pregnancy loss. Butyrate-producing bacteria taxa decline, whereas *bifidobacteria*, *proteobacteria*, and lactic acid–producing bacteria increase during the third trimester of pregnancy. Changes in metabolic, hormonal, and gastrointestinal permeability, the increased diffusion of glucose from the gut epithelium toward the lumen contribute to intestinal bacterial translocation (19-21). Gut microbiota also causes host weight gain during pregnancy due to changed metabolism of fatty acids and immune system modification (14,20-23).

Microbes interact with each other in women through a periurethral contamination. Female genital tract may be a potential target organ in SLE since cervical inflammation is associated to disease activity independently of sexual activity (24). The vaginal microbiota increases the levels of vaginal inflammatory cytokines, which, in turn, increases the risk of spontaneous preterm birth (25-27). To date, the role of the endometrial microbiota in female reproduction is not fully understood. Hormonal changes during and after pregnancy are linked with modifications in the maternal microbiota (28). The relationship between the immune system, gut microbiota, and metabolism of pregnant women is unclear. We hypothesize that the vaginal microbiota altered during the pregnancy in patients with SLE or RA or even healthy women. Thus, we further **propose the importance of the maternal microbiota in the outcomes of pregnancy and emphasize that changes in bacteria composition at gut and vagina are associated with pregnancy complications and disease activities of SLE or RA.** Thus, we collected vaginal samples since vaginal and endometrial environments influences with each other. Furthermore, population-specific studies should be able to shed light on the regulation of the vaginal microbiota as therapeutic strategies, which include immune modulators and microbiome-based therapeutic approaches, the pharmacomicrobiomics (52).

Microbiota-derived metabolites can affect the autoimmune responses (30). Thus, remodeling the gut

microbiome by dietary supplements could represent an innovative therapeutic strategy targeting autoimmune diseases during their pregnancy. Spicy food, especially capsaicin (CAP), the major component in chili and a very popular worldwide food, has been recently drew attention from their benefits on the gut microbiota (31-33). Diets CAP and its derivatives could facilitate butyrate-producing bacteria colonization, reducing plasma total ghrelin and circulant proinflammatory cytokines (34). CAP could decrease the abundance of LPS-producing gram-negative bacteria and strengthen the intestinal barrier. CAP is able to inhibit pathogenic bacteria growth by a bactericidal effect. However, **the mechanisms how CAP reshapes the intestinal microbiota are not completely elucidated**. CAP could restore the gut dysbiosis and suppress the intestinal inflammation in patients with IBDs. However, high doses of CAP could alter the intestinal barrier, while common doses decrease the permeability of the gut intestinal barrier (34). CAP mediated reactions involving intestinal microbiota-metabolites pathways are needed to be deciphered. Personalized nutrition guidance with dietary CAP in patients with autoimmune diseases such as SLE and RA is warranted since how diets CAP help regulate gut microbiota is still unclear neither. Thus, more experimental and clinical trials are warranted before providing the optimal diets CAP guidance, adjustable according to individual enterotype and diverse pathological condition. **Considering the increasing interests in intestinal and vaginal microbiota regulation in women of childbearing age with autoimmune diseases, the emerging data linking diets CAP to the benefits for intestinal dysbiosis, and the proposed gut-vaginal axis via periurethral microbial contamination, we aim to underline the possible mechanisms by which diets CAP exerts its influence in the gut and vaginal dysbiosis in the outcomes of pregnancy in SLE and RA in this proposal.** We could collect how dietary CAP impacts diseases activities, maternal and fetal outcomes in human patients and decipher CAP mediated immune responses throughout the pregnancy by mice models.

研究目的

We hypothesize that gut enterotypes may influence the beneficial effects of dietary CAP and guide the personalized nutrition in patients with SLE or RA, especially during their pregnancy and breastfeeding. The aim of the research is to prospectively assess the impact of vaginal and intestinal microbiota on the pregnancy outcome in patients with SLE and RA and identify how the diets capsaicin regulates gut-vaginal microbiota axis and prevent complications of autoimmunity. We

Aim 1: Whether gut dysbiosis influences vaginal microbiota in a periurethral contamination manner in patients with SLE or RA and influences pregnant outcomes.

Aim 2: Diets capsaicin help regulate intestinal and vaginal dysbiosis, host systemic autoimmunity and disease activities in a metabolite dependent manner in patients with SLE or RA.

Aim3: Dietary capsaicin alters gut and vaginal dysbiosis in SLE and RA mice and further influences the systemic dysregulated autoimmunity during the pregnancy and postpartum.

文獻探討

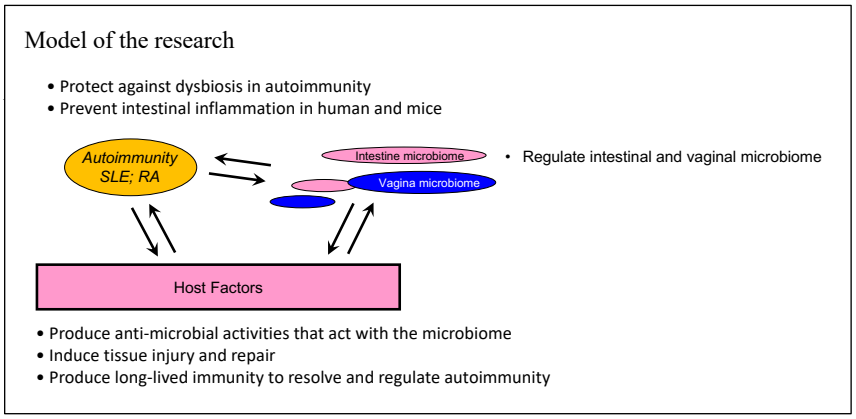
1. Pugh-Bernard AE, Cambier JC. B cell receptor signaling in human systemic lupus erythematosus. Curr Opin Rheumatol 2006;18:451–5.
2. Rahman A, Isenberg DA: Systemic lupus erythematosus. N Engl J Med 358: 929–939, 2008
3. Dall'Era M, Wofsy D. Clinical Features of Systemic Lupus Erythematosus. In: Firestein GS, Budd RC, Gabriel SE, McInnes LB, O'Dell JR (eds), Kelley and Firestein's Textbook of Rheumatology. Philadelphia, PA: Elsevier, 2017. p. 1345–1367.e3.
4. ClowseME.Lupusactivityinpregnancy. Rheum Dis Clin North Am 2007; 33: 237–252.
5. HuongDLT,WechslerB,Vauthier-BrouzesD, et al. Pregnancy in past or present lupus nephritis: A study of 32 pregnancies from a single centre. Ann Rheum Dis 2001; 60: 599–604.
6. Julkunen H, Jouhikainen T, Kaaja R, et al. Fetal outcome in lupus pregnancy: A retrospective case-control study of 242 pregnancies in 112 patients. Lupus 1993; 2: 125–131.
7. **Obstetric nephrology: lupus** and **lupus nephritis** in **pregnancy.** Clin J Am Soc Nephrol. 2012;7:2089-99
8. Koh JH, Ko HS, Lee J, et al. Pregnancy and patients with preexisting lupus nephritis: 15 years of experience at a single center in Korea. Lupus 2015; 24: 764–772.
9. Andreoli L, Bertsias GK, Agmon-Levin N, *et al.* EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. *Ann Rheum Dis* 2017;**76**:476–485.
10. De Man YA, Dolhain RJ, van de Geijn FE, et al. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241–8.

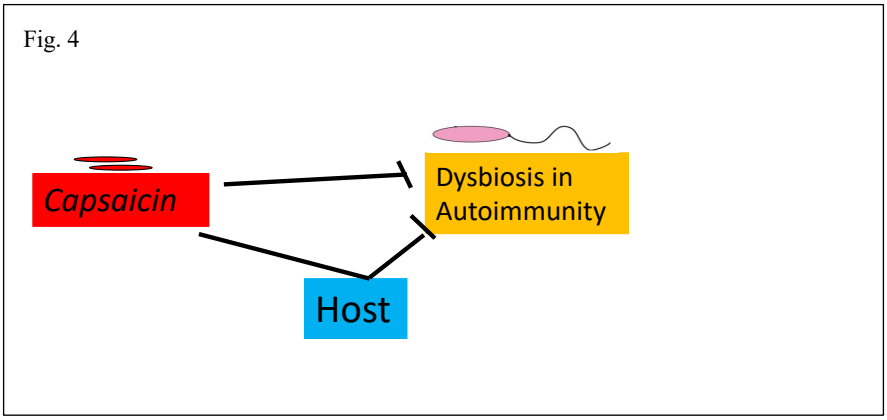
11. Ince-Askan H, Hazes JMW, Dolhain R. Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy. Arthritis Care Res (Hoboken) 2017;69:1297–303.
12. McHugh NJ, Reilly PA, McHugh LA. Pregnancy outcome and autoantibodies in connective tissue disease. J Rheumatol 1989; 16:42–6.
13. Kaplan D. Fetal wastage in patients with rheumatoid arthritis. J Rheumatol 1986;13:875–7.
14. Nyangahu DD, Jaspan HB. Influence of maternal microbiota during pregnancy on infant immunity. Clin Exp Immunol (2019) 198:47–56.
15. Gomez-Arango LF, Barrett HL, McIntyre HD, et al. Connections Between the Gut Microbiome and Metabolic Hormones in Early Pregnancy in Overweight and Obese Women. Diabetes (2016) 65:2214–23.
16. Donnet-Hughes A, Perez PF, Doré J, Leclerc M, et al. Potential role of the intestinal microbiota of the mother in neonatal immune education. Proc Nutr Soc (2010) 69(3):407–15.
17. Dunlop AL, Mulle JG, Ferranti EP, et al. Maternal Microbiome and Pregnancy Outcomes That Impact Infant Health: A Review. Adv Neonatal Care (2015) 15(6):377–85.
18. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr (1999) 69:1035S–45S.
19. Mesa MD, Loureiro B, Iglesia I, et al. The Evolving Microbiome from Pregnancy to Early Infancy: A Comprehensive Review. Nutrients (2020) 12:E133.
20. Neuman H, Koren O. The Pregnancy Microbiome. Nestle Nutr Inst Workshop Ser (2017) 88:1–9
21. Gosalbes MJ, Compte J, Moriano-Gutierrez S, et al. Metabolic adaptation in the human gut microbiota during pregnancy and the first year of life. EBioMedicine (2019) 39:497–509.
22. Nuriel-Ohayon M, Neuman H, Koren O. Microbial Changes during Pregnancy, Birth, and Infancy. Front Microbiol (2016) 7:1031.
23. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell (2012) 150(3):470–80.
24. M V Febronio. Inflammatory cervicovaginal cytology is associated with disease activity in juvenile systemic lupus erythematosus. *Lupus* (2007) **16**, 430–435
25. Anahtar MN, Gootenberg DB, Mitchell CM, et al. Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. Cell Host Microbe (2018) 23:159–68.
26. Hyman RW, Fukushima M, Jiang H, et al. Diversity of the vaginal microbiome correlates with preterm birth. Reprod Sci (2014) 21(1):32–40
27. Fettweis JM, Serrano MG, Brooks JP. The vaginal microbiome and preterm birth. Nat Med (2019) 25:1012–21. doi: 10.1038/s41591-019-0450-2
28. Recent Insights on the Maternal Microbiota: Impact on Pregnancy Outcomes. Front. Immunol. 2020; 11:528202.
29. R Mattsson, A Mattsson, R Holmdahl, et al. Maintained pregnancy levels of oestrogen afford complete protection from post-partum exacerbation of collagen-induced arthritis. *Cin. exp. Immunol.* (1991) 85. 41-47
30. Yurkovetskiy LA, Pickard JM, Chervonsky AV. Microbiota and autoimmunity: exploring new avenues. Cell Host Microbe (2015) 17:548–52

31. Wang, F.; Huang, X.; Chen, Y.; et al. Study on the Effect of Capsaicin on the Intestinal Flora through High-Throughput Sequencing. *ACS Omega* **2020**, *5*, 1246–1253
32. Marini, E.; Magi, G.; Mingoia, M.; et al. Antimicrobial and Anti-Virulence Activity of Capsaicin Against Erythromycin-Resistant, Cell-Invasive Group A Streptococci. *Front. Microbiol.* **2015**
33. Qiu, J.; Niu, X.; Wang, J.; et al. Capsaicin protects mice from community-associated methicillin-resistant Staphylococcus aureus pneumonia. *PLoS ONE* **2012**, *7*, e33032.
34. Rosca AE, Iesanu MI, Zahiu CDM, et al. Capsaicin and Gut Microbiota in Health and Disease *Molecules* **2020**, *25*, 5681
35. Nyangahu DD, Jaspan HB. Influence of maternal microbiota during pregnancy on infant immunity. Clin Exp Immunol (2019) 198:47–56.
36. Yurkovetskiy LA, Pickard JM, Chervonsky AV. Microbiota and autoimmunity: exploring new avenues. Cell Host Microbe (2015) 17:548–52.
37. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. Cell Host Microbe (2018) 23:159–68.
38. Hyman RW, Fukushima M, Jiang H, Fung E, Rand L, Johnson B, et al. Diversity of the vaginal microbiome correlates with preterm birth. Reprod Sci 2014;21:32–40.
39. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, et al. The vaginal microbiome and preterm birth. Nat Med 2019;25:1012–21.
40. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Ann Rheum Dis. 2019;78:1151-1159.
41. CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
42. RJ. Measuring disease activity and functionality during pregnancy . Arthritis Rheum 2007;57:716-22.
43. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004; 15: 241–250.
44. Srinivasan S, Hoffman NG, Morgan MT, et al. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PloS one. 2012;7(6):e37818.
45. Mahe F, Rognes T, Quince C, et al. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ. 2014;2:e593.
46. Salipante SJ, Kawashima T, Rosenthal C, et al. Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. Applied and environmental microbiology. 2014;80(24):7583-91.
47. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335-6.
48. Theodoridis, G., Gika, H. G., & Wilson, I. D. (2008). LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics. TrAC Trends in Analytical Chemistry, 27(3), 251-260.
49. Zhou, B., Xiao, J. F., Tuli, L., & Ressom, H. W. (2012). LC-MS-based metabolomics. Molecular BioSystems, 8(2), 470-481.
50. Bergeron,F.;Bouin,M.;D'Aoust,L.;et al. Food avoid ance in patients with inflammatory bowel disease: What, when and who? *Clin. Nutr.* **2018**, *37*, 884–889.

51. Mu et al. Pregnancy and lactation interfere with the response of autoimmunity to modulation of gut microbiota. Microbiome (2019) 7:105
52. Scher JU, et al. Pharmacomicrobiomics in inflammatory arthritis: gut microbiome as modulator of therapeutic response. Nat Rev Rheumatol. 2020;16:282-292

研究方法





1.1 Patient selection This is an observational analytical prospective cohort study. Healthy controls, SLE and RA women will be recruited from the Rheumatology and Obstetrics clinics of Tri-Service general Hospital and Taipei Veterans General Hospital. Data on disease activity and medication use will be collected before conception, at each trimester during pregnancy, and 6 months postpartum (10)[10]. All SLE or RA patients receive regular visits to the Rheumatology clinic for at least 6 months before their pregnancy. Upon diagnosis of pregnancy, the patients will be referred to Obstetrics clinic where they will be followed up prospectively during pregnancy and for 6 months after it ended. Patients were classified according to the 2019 American College of Rheumatology (ACR) criteria or for SLE (53)[40]. Improvement and deterioration of the disease activity in SLE and RA patients during and postpartum pregnancy were calculated as differences in the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria and DAS28, respectively (41,42).

1.2 Data collection before and after pregnancy

1.2.1 Patients are subjected to thorough assessment including full history taking and monthly assessment. Laboratory tests, including blood cell counts, serum biochemistry data, inflammatory biomarkers such as erythrocyte sedimentation rate and c-reactive protein, serum immunological profiles including autoantibodies and cytokines, serum complement levels and urine analysis are

performed every 1 month guided by the patients' condition. We would collect serum for metabolomic analysis as well. The World Health Organization class of LN will be documented when available (43). Follow up by an obstetrician is performed at all trimesters. Follow up of the enrolled patients will be continued for the 6 months after pregnancy ended, including maternal complications throughout pregnancy and fetal complications 6 months after delivery. Clinical features, laboratory parameters and medications received by the patients will be recorded. Diets CAP habit will be recorded in the enrolled patients additionally.

1.2.2 Vaginal and fecal microbiome data collection and calculation

1.2.2.1 Longitudinal sample collections of vaginas and feces for microbiome, metabolomic and host genes analysis. Vaginal and fecal samples will be collected. We collected the samples at different time points, including the time before pregnancy, at each three trimester throughout the pregnancy, and postpartum. Vaginal samples are collected from the posterior fornix, and feces are collected based on our previous protocol to avoid contaminations. All samples will be divided them for enzyme-linked immunosorbent assays, 16S rRNA gene sequencing of microbiota, metabolite analysis and gene expression analysis of host cells. The collected samples will be stored at −80°C within 30 minutes.

1.2.2.2 Taxonomic assignment of 16S rDNA sequences.

1.2.2.3 Metabolomic analysis. Serum, fecal and vaginal samples will be used for metabolites analysis by using Ultra high performance Liquid Tandem Chromatography Quadrupole Time of Flight Mass Spectrometry (UHPLC-QTOFMS) and Multivariate Analysis as previously (48-50).

1.2.2.4 Gene expression analysis. RNAseq will be performed.

1.3 Statistical analysis. For all subjects, descriptive statistics were calculated as numbers, percentages, means, and standard deviation (SD). Comparisons between the study groups were done using the Mann-Whitney test for the quantitative variables and the chi-square tests for the qualitative variables. The odds ratio was calculated with a 95% confidence interval. Wilcoxon signed-rank tests was used for the comparison between paired data. A 2-sample Student t test was used to detect differences in mean disease activity scores. P values < 0.05 were considered statistically significant. Statistical analyses were performed using STATA software for Windows or Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) for Microsoft Windows.

2.1. Longitudinal intestinal and vaginal microbiota monitoring in SLE or RA mice. MRL/MpJ-Fas^{lpr}/J mice will be maintained in a CV facility at the National defense Medical Center as a pregnant SLE animal model, while female DBA/1 mice will be mated with normal DAB/1 males as a pregnant RA mice model. Mice above will be purchased from The Jackson Laboratory (USA) and Taiwan National Laboratory Animal Center.

2.1.1 SLE mice model. 8-week-old female MRL/MpJ- Fas^{lpr}/J mice will be mated with age-matched male mice.

2.1.2. RA mice model. Subcutaneous type II collagen injection will be injected in 12-week-old DAB/1 mice. The boost type II collagen injection will be performed in 13-week-old mice.

2.2 16S rDNA sequencing from fecal and vaginal contents. Fecal and vaginal contents will be collected from all mice upon sacrifice in specific subaim 3.1, and stored at -80 C. We will initially process 10 replicate samples from all tested mice. The decision to sequence additional replicates and samples will be based on the analysis of this data. The protocol is the same as mentioned in the subaim 1.2.

2.3. Taxonomic assignment of 16S rDNA sequences. Sequences will be classified by sequence identity as described previously. The protocol is the same as written in the subaim 1.2.

2.4 Metabolites and host genes collection, isolation and calculation of feces and vaginal samplings. Serum, fecal and vaginal contents will be collected from all mice upon sacrifice in specific subaim 3.1, and stored at -80 C. Metabolomics study and host cell gene expressions will analyzed by the same protocols as mentioned in the aim 1.2.

2.5 Statistical analysis. For all subjects, descriptive statistics were calculated as numbers, percentages, means, and standard deviation (SD). Comparisons between the study groups were done using the same methods described in the aim 1.3.

結果與討論

1. We would collect the fecal and vaginal microbiota in a sequential manner, including the timing when preparing for pregnancy, every trimester of the pregnancy and post-partum. **We expect to identify the gut-vagina axis, which influences autoimmunity in SLE and RA women during their pregnancy.** Our data revealed that *Firmicutes* and *Lactobacillaes* are predominant in the healthy vaginas (Fig. 1). Vaginal microbiota of healthy women would alter after short term diet CAP supplement.

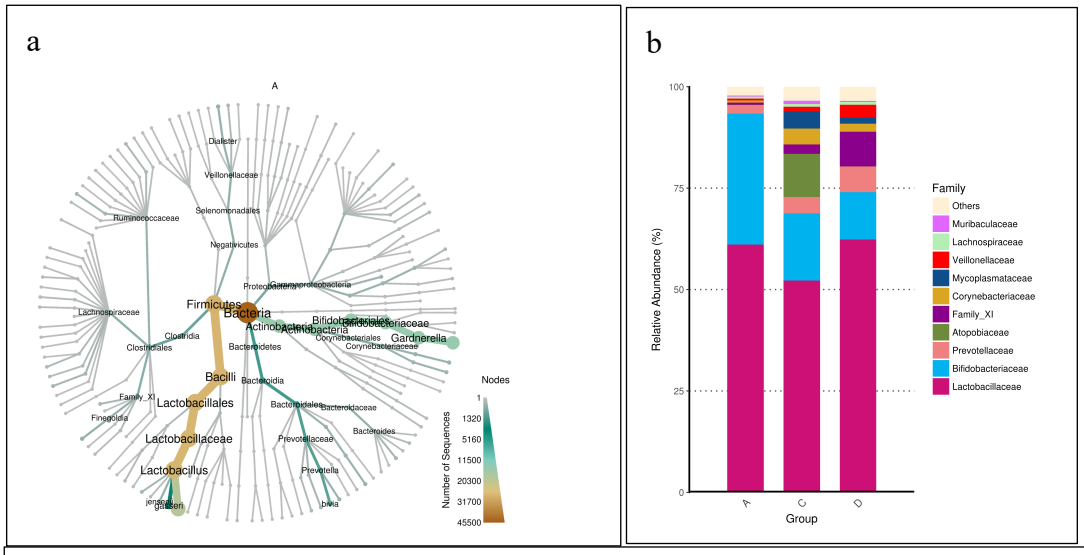


Fig. 1 *Firmicutes and Lactobacillaes* are predominant in vaginal microbiota of healthy people (a). One-week diets capsaicin (CAP) supplement changed the composition of vaginal microbiota in the healthy people who originally did not take diet CAP (b). CAP: capsaicin; A: vaginal microbiota in diet CAP naïve healthy people. C: vaginal microbiota in healthy people, who used to take daily diet CAP supplement but have stopped diet CAP for one week. D: vaginal

2. **Diets CAP could be able to impact on intestinal and vaginal microbiome via gut-vagina axis**. We also have identified that one-week dietary CAP eliminated a specific *Acinetobacter_johnsonii* bacteria strain in the vaginal sample of healthy people and that one-week diets CAP contributes to different composition of vaginal microbiota and *Clostridiales family* colonization in healthy women by 16S rRNA sequencing and metagenomic sequencing (Fig. 2). Because of difficulty of human experiments in COVID era, we took the advantage of mice experiments. Our data indicated diets CAP could have an impact on the composition of gut microbiota without induing intestinal inflammation in healthy B6 mice (Fig 3).

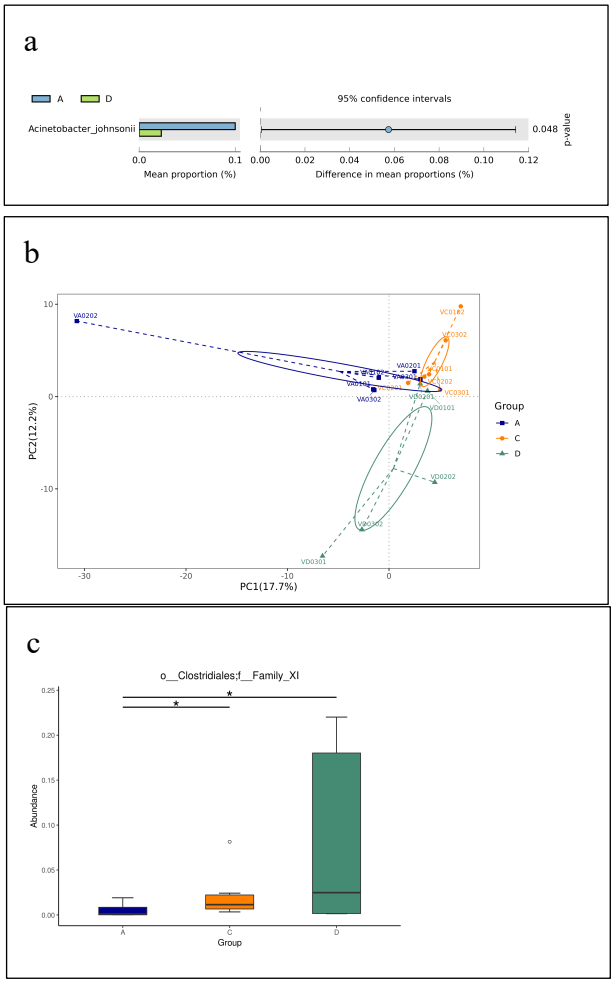


Fig. 2 We have identified that one-week diet CAP supplement eliminated a specific *Acinetobacter_johnsonii* bacteria strain in the vaginal sample of healthy people (a) and that one-week diet CAP supplement contributes to different composition of vaginal microbiota and *Clostridiales family* colonization in healthy people by 16S rRNA sequencing (b) and metagenomic sequencing (c). A: vaginal microbiota in diet CAP naïve healthy people. C: vaginal microbiota in healthy people, who used to take daily diet CAP supplement but have stopped diet CAP for one week. D: vaginal microbiota in healthy people who did not take diet CAP supplement but has received one-week diet CAP supplement.

結論與建議

Our data indicated that diet CAP could influences the intestinal and vaginal microbiota in healthy women. SLE women who have regular diet CAP intake would have altered gut microbiota after one week withdrawing CAP. CAP mediated intestinal microbiota changes is in an intestinal microbiota dependent manner in the germ free mice experiments. In a conclusion, diet CAP is engaged in the gut-vaginal microbiota interaction and metabolites signaling. We will elucidate the detailed mechanism in the future.

執行計畫過程遇到之困難或阻礙

1. It′ s hard to enroll SLE, RA and healthy pregnant women in the COVID era, which make us take the advantage of mice experiments. However, it′ s also difficult to import the SLE and RA mice from US due to COVID issue. Our mice and human experiments are delayed then.
2. We have great thanks to NSTC to support our proposal. However, the multi-omics research is expensive research. We need to apply multiple years grant to support such a longitudinal research.

110年度專題研究計畫成果彙整表

計畫主持人：盧俊吉	**計畫編號：**110-2629-B-016-001-
計畫名稱：辣椒素協助改善自體免疫疾病腸道暨陰道微菌叢以利孕婦健康	

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	1		2021年中華民國免疫醫學會年會口頭報告
		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	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	
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	8		協同計畫主持人共8位博士， 包括6位臨床醫師
		專任人員	1		碩士級研究助理一位
	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。）	2021年中華民國免疫醫學會年會口頭報告, 台灣風濕病學界首次闡述陰道與腸道微菌叢的交互作用, 利用飲食辣椒素影響腸道及陰道微菌叢的初步結果亦為國際前驅實驗, 有助於建立台灣本土系統性疾病之腸道免疫資料庫及如何善用台灣本土習慣飲食於常見風濕病之運用, 目前亞洲日本韓國及歐美皆有自身腸道菌叢資料庫及食療對治療疾病資料庫, 台灣資料庫仍待建立, 藉本研究苦協助建立先驅實驗模式, 有助於未來大規模研究設計