行政院國家科學委員會專題研究計畫 成果報告

台灣漢族華人之牙周炎與 cytokines, MMPs/TIMPs,CD14/ TLRs 基因多型性的關係之探討
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 98-2314-B-040-020-
執 行 期 間 ：98 年 08 月 01 日至 99 年 07 月 31 日
執 行 單 位 ：中山醫學大學牙醫學系（所）

計 畫 主 持 人：蔡吉政
共 同 主 持 人：林英助
計畫參與人員：學士級-專任助理人員：蕭郁丰

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 99 年 10 月 20 日

☑成果報告

行政院國家科學委員會補助專題研究計畫

□期中進度報告

台灣漢族華人之牙周炎與 cytokines, MMPs/TIMPs,CD14/ TLRs 基因多型性的關係之探討(1/3)

計畫類別：☑個別型計畫　□ 整合型計畫
計畫編號：NSC　98－2314－B－040－020
執行期間：98 年　08 月　01 日 至　99 年　07 月　31 日

計畫主持人：蔡吉政 教授

共同主持人： 林英助 副教授

計畫參與人員：陳俊呈副教授、張育超教授、何全城副教授、楊麗秋助理教授

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
☑赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年☑二年後可公開查詢

執行單位：中山醫學大學牙醫學研究所

中　華　民　國　　99 年　　10 月　　20 日

98年度國科會研究成果報告

(一)中文摘要：

MMPs 與牙周組織的破壞有強烈的相關性，因為 MMPs 是牙周組織的破壞時，膠原蛋白纖維破壞的重要介質。許多有關 MMPs 及 TIMPs 的基因多型性的研究探討它們與牙周炎的感受性及/或嚴重性。

有些過去的研究探討牙周炎的感受性與多種 interleukins 基因多型性的關係。牙周細胞積極參與 innate immune response 以對抗牙菌斑細菌，牙周細胞會表現各種的 Toll-like receptors(TLRs)。TLRs 的訊息傳遞結果使 innate immune response 釋出 anti-bacterial peptide 及募集 neutrophils 的 IL-8 ，因此 TLRs 的表現可謂是對細菌的侵犯時，激發炎性反應的工具，顯示 TLRs 在牙周炎扮演某一角色。 TLRs 的基因多型性與牙周炎的感受性在一些族群亦有被探討。

基因在宿主的免疫反應上具有一定的角色，但結果在族群間顯示有極大的差異。本計畫將對感受性基因，包括 MMP-1、-2、-3、-8、-9 及-12，TIMP-1、-2，IL-2、-4、-8、-17，CD14、TLR-2、TLR-4 以 polymerase chain-restriction fragment length polymorphism analysis 來探討。同時探討基因的 nucleotide transition 對宿主細胞激素(炎性介質)的表現(protein or mRNA)的影響。在過去的基因研究也有注意到對免疫調整(immune regulation)或新陳代謝(metabolism)有關的細胞激素(介質)與牙周炎感受性的角色，但與功能上的表現(functional implication)的關係的研究報告則尚缺乏。本計畫也要在基因多型性與牙周炎的關係間提供功能上的證據(functional evidence) ，這可藉由分析牙周炎患者全血細胞培養液的細胞激素(介質)濃度偵測得知，利用 ELISA 偵測 protein expression, RT-PCR 偵測 mRNA expression。

關鍵語: 基因多型性、牙周炎、基底質金屬蛋白酶、基底質金屬蛋白酶組織抑制分子、細胞激素、D14 接受器、Toll-like 接受器

(二)英文摘要：

MMPs have strong correlation to periodontal diseases, since MMPs are the major players in collagen breakdown during periodontal tissue destruction. Some studies in the past years had indicated the association between selected candidate gene polymorphisms and the susceptibility to periodontitis.

The TLR signaling results in innate immune responses involving the release of antibacterial peptides and neutrophil recruiting chemokine (IL-8). TLR signaling serves to limit pathogenic infections and to prevent commensal organisms from breaching the epithelial barrier. In the epithelium and connective tissue via TLR signaling, the stimulated cells could produce an array of cytokines, chemokines, and other mediators that promote inflammation and immune cell infiltration. Additional cytokines produced by infiltrated memory T cells would amplify the inflammatory reaction, leading to destruction of connective tissue and bone. Investigation into TLR SNPs in periodontitis individuals should provide some insight into the role of the immune system in maintaining periodontal health and combating periodontal disease.

During the past few years, several genes have been individually sequenced for association with periodontitis. Different studies have been conducted in varied populations and ethnicities. Frequencies of the genetic marker of interest may show large heterogenecity between races Variation in genotype frequencies across diverse population may affect the number of individuals at increased risk for a disease, and population substructure imbalances may create serious differences in genotype frequencies of the compared groups in gene-disease association studies Susceptible genes polymorphisms including MMPs(MMP-1、-2、-3、-8、-9 and -12)，TIMPs (TIMP-1、-2)、interleukin(IL-2、-4、-8、-17)、antigen-recognition-related receptors(CD14、TLR-2、TLR-4) shall be analyzed by polymerase chain-restriction fragment length polymorphism analysis. We shall also investigate whether the nucleotide transition will affect the protein and mRNA expressions of the cytokines/mediators which shall be analyzed with ELISA in the samples of the supernatants and RT-PCR of the cell lysates of whole-blood cell cultures respectively. Our data shall provide functional evidence in the association between selected gene plomoyphisms and the susceptibility to periodontitis.

Keywords:Single Nucleotide Polymorphisms (SNPs),Periodontitis,Cytokine,Matrix Metalloproteinases (MMPs), Tissue Inhibitors of MMPs, CD14 Receptors, Toll-like Receptors (TLRs**)**

前言：

牙周炎是牙周組織的一種炎性病變伴有牙齦腫大、齒槽骨喪失，及牙齒動搖的現象。牙周炎的原因是複雜的，且是與基因因子(genetic factors)及環境因子(例如：牙菌斑及生活習態/life style)有關。牙周炎之發生及進行是與宿主的免疫反應(immune responses)，且受口腔微生物及它們的衍生物的影響。雖然病原菌是引起牙周炎的必要(essential)條件，但牙菌斑的量也並不一定與疾病的嚴重度呈對比關係。因此強調宿主的免疫反應，而不只是細菌的原因，為疾病表徵的主要決定者是今日牙周病學所強調的。近代的研究模式強烈的指出牙周炎的病理-生理學(pathophysiology)具有基因的背景(genetic background)。促炎性階梯(proinflammatory cascade)被認為與牙周炎發展相關的炎性過程有關。因此，探討會影響促炎性介質(proinflammatory mediators)產生的基因多型性是具有重要的意義。Genotype 的頻率橫跨不同的組群可能影響某種疾病危險性的數目，且組群的結構不平衡(population structure imbalance)可能造成相比較的組間的genotype 頻率高低的很大差異；因此與牙周炎相關的基因於診斷上可能只限於特定組群，而不能用於全球或橫跨族群。 在考慮到以上所說的議題,在未來研究上要選擇一個較均一(homogenous)組群(年齡及種族之配對)是非常重要的。 近來的研究顯示基因是為牙周炎的嚴重性(severity)及進行性(progression)的重要決定因子。一些於炎性及免疫反應方面被懷疑於牙周炎的發生擔當某種角色。基因因子甚至被認為與50%的牙周炎感受性(susceptibility)有關。應用基因資訊及技術於牙周炎的診斷與治療，似乎已成為重要的觀念。不只患者的種族的(racial 與ethnic)背景資料應加考慮，研究更需要有足夠且慎選牙周炎與基因多型性的相關性之病例及控制(對照)組，方能使研究更加清楚。如此，牙周炎之感受性可由某些相關地普遍的高危險性基因多型性(relatively common high-risk polymorphisms)來解釋是有可能。的。因此，本研究的結果於牙周炎的發生防制策略(therapeutic intervention)上及個別化的治療模式(individualized approach)具有相當的參考價值。

研究目的：

本計畫將對感受性基因，包括MMPs(MMP-1、-2、-3、-8、-9 及-12) ，TIMPs (TIMP-1、-2 、-3) 、interleukin (IL)-2 、-4 、-8 、-17), antigen-recognition-related receptors (CD14 、TLR-2 、TLR-4) 將以polymerase chain-restriction fragment length polymorphism analysis 來分析與探討其與牙周炎的相關性。

文獻探討：

一、基底質金屬蛋白酶(matrix metalloproteinases, MMPs)與基底金屬蛋白酶組織抑制分子(tissue inhibitors of MMPs, TIMPs)的基因多型性:

於牙周炎的活動期間(active stage) ，研究顯示牙齦組織的破壞(degradation)至少部份是因 MMPs 的作用。MMPs 的 functional activity 是受血清或組織抑制分子(tissue inhibitors of MMPs,TIMPs) 的調控(1). MMPs 更與牙周病有強烈的關係,因為 MMPs 是牙周組織的蛋白酶(1,2-5). MMP-2 與 MMP-9 可能參與牙周炎時的組織破壞(6)。最近的研究指出 MMP-8 對牙周病時組織破壞的重要性。一種 MMP-8 的 chair-side test 已被開發出來(8)，偵測 GCF 中的 MMP-8 水平可能是很確實的一種檢驗方法，用來診斷及監測(monitoring)牙周病(7-9)。我們必須記住，單獨有 MMPs 的呈現於 GCF 及/或牙周組織不能就表示牙周組織內的酶的活性增加。因此，功能的控制(基因的)機轉可能較可以顯示 MMPs 在牙周炎的感受性(susceptibility)或/及嚴重性(severity)上的角色。Single nucleotide polymorphisms(SNPs)被認為與細胞激素(cytokines)的產生程度有關。許多 MMPs 及 TIMPs 的 SNPs。CaO 等(10)結論說 MMP-1 之-1607bp promoter region 的 SNP 可能與中國人的慢性牙周炎的嚴重 CP 有關。de Souza 等(11)的結論是 MMP-1 於 promoter region 的 SNP 的 genotype 是與嚴重慢性牙周病有關。Astalfi 等(12)認為非吸煙古巴人的 MMP-1 SNPs 與牙周炎的 susceptibility 似乎無關，但 MMP-3 gene SNP 與巴西人牙周炎的牙周組織破壞可能有關。Istagaki 等(13)說 MMP-1 及/或 MMP-3 gene promoter SNPs 不會影響日本人的牙周炎感受性。Holla 等(14)於捷克人的結果顯示 MMP-1 promoter 的 SNPs 可能對慢性牙周炎的病因只有小的影響。Holla 等(15)認為 CP 的 development 與 severity 與 MMP-2 gene promoter 的 SNPs 對 European Caucasians 的牙周炎個別感受性及/或嚴重性未具有意義的影響。Suzuk 等(16)認為 MMP-1,-2,-3,-9 的 SNPs 與日本人的侵犯性牙周炎(AgP)與 CP 無關。MMP-9 於 MMP 家族中是一重要份子，具有功能性的基因多型性於人類已經證實於-1562 處由 C 轉為 T 的改變產生兩種不同 alleles，且 C/T 與 T/T genotype promoter 的高活性會增加炎性疾病的危險性。

Keles 等(17)發現 MMP-9(-1562 C/T)的 CT genotype，CT 與 TT genotype 於 CP 組的頻率較 CC genotype 的頻率低,顯示 MMP-9 promoter gene SNP 似乎與土耳其人的嚴重廣泛性 CP 有關。Gürkan 等(18)結論說 MMP-2(-735 C/T)、MMP-9(-1562)及 MMP-12(357 Asn/Ser) SNPs 與土耳其人的 CP susceptibility 及嚴重性無關，而 MMP-9(-1562)的 T allele gene 可能與減低 CP susceptibility 有關。Gürkan 等(19)認為 MMP-2 -735 C/T 與 MMP-12 357 Asn/Ser SNPs 是與 GAgP 無關。Chen 等(20)指出 TIMP-2 -418 的 allele G/C 與中國漢人的 GAgP 是有相關性的,GAgP 組的 C allele frequency 的增加對病人可能是不好的。 最近 Kubota 等(21)研究日本人的 MMPs 及其 inhibitors 之 gene expression 看起來，MMP 表現的上升與 MMP/ TIMP ratio 的增加顯示 extracellular matrix 的 degradation 與 synthesis 於牙周炎侵犯的牙齦組織處不平衡(imbalance)的可能性，此種過程可能就是牙周炎時增加的組織破解的原因。

二、細胞激素基因多型性(Cytokine gene polymorphisms)

細胞激素基因多型性(cytokine gene polymorphisms)，各個人之間對於感染(infections)的反應有極大的差異性，基因因子(genetic factors)可能解釋這些個別的差異(variations)(22)。再進一步而言，炎性標的(genetic polymorphisms of inflammatory markers)與嗜中性白血球接受器(receptors)之基因多型性最近已證實與細菌的感染與瘧疾(malaria)的盛行率(prevalence)有關(24,25)。瞭解這些不同的

反應，可促進我們對這些感染性疾病的 pathogenesis 且可幫助感染的治療與控制(23)。侵犯性牙周炎(AgP)是一種牙周組織(periodontium)的感染性疾病。影響年青成年人引起牙齒支持組織的傷害，導致骨吸收及牙齒喪失(26,27)，在 AgP，宿主反應(由基因決定)與微生物-生物的因素(microbiological factors)似乎是主要的決定分子(components)，由此可激發或引起疾病(28,29)。這種觀念導致認為 *A. a.*、*P. g.*與 *T. f.*為主的牙周病菌感染較易發生在一些特異感受性的人。另一在 CP 病人的研究(30)亦指出如何特殊 genotype 可能使宿主防禦機轉較易對特殊性牙周致病菌產生抵抗。因此，有假說認為在 AgP 或 CP，宿主 genotype 會影響牙齦下微生物組成。因此我們想探討國人的 AgP 或 CP 是否與重要的炎症-相關分子的 genes 的 polymorphisms 有關。以下就對炎症標的(inflammatory markers)的 gene polymorphisms 與牙周炎的相關性文獻作一回顧。

(1). Interleukin-2(IL-2):

IL-2 是一種促炎性的(pro-inflammatory)細胞激素(cytokines)。Scarel-Caminaga 等(31)於巴西人)的研究結果顯示 IL-2 gene -330 (T→C) SNP 是與牙周病的嚴重程度有關，IL-2 SNPs 於牙周病的 pathogenesis 上應有積極的角色。

(2). Interleukin-4(IL-4):

IL-4 具有多重性的角色。Michel(32)探討日耳曼人之 IL-4 SNPs 與 early onset periodontitis 的結果指出 IL-4 -590 C→T 多型性與 CP susceptibility 沒有關係。 Kara 等(33)的研究顯示 IL-4 SNPs 與土耳其人之嚴重廣範性 CP 無關。Pontes 等(34)也發現 IL-4 SNPs 與巴西人的牙周炎的 susceptibility 無關。Kang 等(35)於韓國人亦發現無論在 allele, genotype 及 haplotype 的分佈上，CP 組與 HC 組間並無差異。Gonzales 等(36)也結論說日本人與 Caucasians 的 IL-4 SNPs 與 risk of AgP 無關。Gonzales 等(37)在日耳曼人的 IL-4 與 IL-13 promoter region gene 的 SNPs 研究說：IL-4-509 T/T 與 IL-4-34 T/T genotype 與 AgP 有關。Hooshmand 等(38)的結果指出 IL-4(C-590T)與 IFN-γ(G5644A)與伊朗人的 poriodontitis suseeptibility 無關。雖然 IL-4 與 IFN-γ 位於 promoter region 的 gene 的 mutation 有報告說會影響此兩激素的蛋白質表現，但從以上的文獻回顧來看，IL-4/IFN-γ 的 SNPs 於各種族群的 AgP/CP 的 suseeptibility 與 severity 的關係仍然有許多爭議之處。

(3). Interleukin-16(IL-16):

IL-16 與多種促炎細胞激素像 monocytes 的 TNF-α、IL-1β、IL-6 及 IL-15 的表現有關。IL-16 的表現有意義地於多種慢性炎性疾病時有升高的報告(39-41)。反之，IL-16 的濃度於牙周炎患區與控制(健康)組織相比較時，顯得有意義的減低(42)，因此，低程度的 IL-16 表現被認為與牙周組織破壞有關，但是 Folwaczny 等(43)的結果並未發現 IL-16 SNP(-295 T→C)與 CP 的關係(日耳曼族群)。

(4). Interleukin-17(IL-17):

IL-17 只由活化的 T-cells 形成，這種細胞激素能引起炎性反應，支持 Th_1 免疫反應及與 RNAK 及 RANKL 共同刺激 osteoclastic bone resorption。IL-17 的生物功能被認為慢性炎性牙周病的 pathogenesis 與 progression 及控制(control)有關，但 T-cells 對組織破壞的確實貢獻則尚未充分瞭解。IL-17 共同與其他細胞激素於牙周病的病因病理方面具有可能的角色。Beklen(44)發現於牙周炎時，IL-1β、TNF-α 與 IL-17 有上昇的現象；這些 cytokines 引起牙齦造纖維細胞的 pro-MMP-1 及 MMP-3 的產生，但不影響 MMP-8 與 MMP-9 的表現。 IL-17 對 MMP 的直接激發較 IL-1β 與 TNF-α 為弱，但會刺激 macrophages 的 TNF-α 之產生，及牙齦造纖維細胞之 IL-6 及 IL-8 的產生，綜合而言，MMP-1 與 MMP-3 於牙周炎時有增加，牙齦造纖維細胞透過 MMP-1 與 MMP-3 的產生可能

對牙周炎時組織的破壞具重要的角色，在此過程中，IL-17 擔任著主宰的角色，固此 IL-17 的 SNPs 與牙周炎的感受性的關係值得探討(45)。

(5). Antigen recognition-related polymorphisms CD14 receptor：

CD14 分子是認知 LPS 的一種 receptor，啟動對細菌的侵入產生 innate immune response。SNP-159 C→T 會提升 CD14 的表現(47)、活性(48)及密度(density)(49)。另有四個 SNPs 會影響可溶性 CD14 的水平(50)。Folwaczny 等(51)報告說-159 C/T genotype 與日耳曼女性牙周炎有關(與男性的牙周炎無關)，此結果與 Donati 等(46)的結果相一致。Holla 等(52)報告說捷克人的 CP 嚴重度與-1359 C/T genotype 有關，但與-159 C/T genotype 無關。Yamazaki 等(53)的報告說日本人的牙周炎發生與 CD14 -159 C/T polymorphism 沒有關係，但是在牙周炎患者中，有此 C→T SNP 的表現與 early disease activity 有關(53)。Laine 等(54)認為 CD14 -260 T/T genotype 對 Dutch Caucasians 罹患 severe periodontitis 的 susceptibility 有影響。Trevonen 等(55)亦建議 CD14 -260 的 polymorphisms 與牙周病的關係。Donati 等(46)發現 CD14 -159 gene polymorphisms 與 North European origin 的 Caucasians 之 CP 有相關性。

(6). Toll-like receptor (TLR)-4(TLR4)、-2(TLR2):

細菌性牙菌斑刺激宿主的炎性反應導致組織損傷。對細菌的侵入，宿主是透過pattern-recognition receptors稱為toll- like receptors(TLRs)來擊發炎反應(56)，因此，TLRs可能在牙周炎有扮演一些角色。TLRs的功能對innate immune system擔任主角的角色(57)，TLRs能認知(recognize)且鑑別(distinguish)微生物的構造(structures)，此構造稱為pathogen-associated molecular patterns (PAMP)，包括LPS、peptidoglycan、lipoproteins、bacterial DNA與double-stranded DNA。在innate immune system，TLRs感覺到微生物(包括bacteria、viruses、fungi及protozoa)的入侵而擊發免疫反應來清除病原菌(pathogens)，在與微生物的PAMP作用後，TLRs透過intracellular signaling pathways傳遞此訊息而活化innate immune cells。以TLRs來媒介的innate immune response對adaptive immune system的發展與方向亦是很具關鍵性的角色。由於牙齦是經常持續地曝露在細菌的PAMP之下，牙周組織的TLRs之sensing與signaling極可能在innate immune responses與維持牙周健康上扮演重要的角色。牙周病是一種慢性的細菌感染。於牙周組織內的PAMP之慢性TLRs刺激可導致過度的促炎性介質的產生，結果是組織的破壞。人類的牙齦上皮細胞內生性地(constitutively)表現TLRs：TLR2、TLR6與TLR9(58)。TLR4的表現可因IFN-γ而增加(59)。TLR2的表現於spinous epithrelial layer比在basal epithelial layer更密集(denser)(58)，於接近囊袋上皮處的結締組織內由於是出現在上皮組織的最外層，會持續性地與口腔細菌衍生物接觸(58，60)。TLR2 signaling 與*P. g.*對細胞釋出IL-8的反應相關(61)。牙齦細胞亦會表現TLR3與TLR9(58)，這些TLRs提升上皮細胞對virus與bacteria反應的能力。牙齦造纖維細胞在受細菌及其產物的刺激，也會產生各種炎性細胞激素，包括 IL-1、IL-6及IL-8(62-64)。人類牙齦造纖維細胞(HGFs)會內生性地表現TLR2、TLR4、TLR9及其他TLR相關的分子(64-67,69)。DNA microarray分析顯示人類牙齦造纖維細胞的TLR2、TLR4及CD14之表現於牙周炎患者比健康者高(70)。HGFs在受*P.g.* LPS刺激後會增加表現TLR2、TLR4、CD14 (66)。 因此*P.g.* LPS可能與TLR2、TLR4與CD14於牙周炎時的上升表現有關。來自炎性牙齦組織的IFN-γ能提HGFs的CD14表現(71)，同樣的細胞激素表現的提昇也可見於IFN-γ處理的HGFs受*A.a.* LPS的刺激(71)時發生 。*P.g.*, *A.a.*的LPS與INF-γ會提昇TLR2、TLR4與CD14的表現之結果建議說透過增加對來自口腔牙菌斑細菌的TLR2與TLR4 ligands 的反應，TLR2與TLR4可能對擴大牙周結締組織內

的炎性反應具關鍵性的角色。基於以上的發現，對調控TLRs的表現的基因(promoter region) polymorphism有一些學者進行進一步的研究。Folwaczny 等(68)結論說TLR2與TLR4 gene的various mutations與日耳曼人的CP沒有相關。Schröden等(72)指出TLR4(Asp299 Gly,Thr399 Ile) SNPs只與德國柏林人的CP有關(與AgP無關)。Fukusaki等(73)發現TLR4的genetic variation與日本人的moderate與severe periodontitis有關。Besdelin等(74)雖然報告說TLR2與TLR4 2的SNPs與CP susceptibility沒有關係，但是TLR SNPs可能影響宿主對微生物病原菌的反應能力。 Izakovicovaet等(75)未能證明捷克人的CP與TLR4(Asp299 Gly，896A>G，Thr399Ile及1196 C>T)SNPs的關係。 James等(76)於UK，Belfast Western European Caucasians的研究指出Asp299Gly TLR4 SNP是與減少AgP的risk相關，與CP的risk則沒有關係，CD14 gene不影響得炎性牙周炎的susceptibility，對此Rangsini等(77)指出CD14與TLR4認知細菌成分的結果會導致NF-kappa B有關的炎性反應，但是Emingil等(78)則未能證明TLR2(Arg753Gln，Arg677Trp)與TLR4(Asp299Gly，Thr399 Ile)SNPs與AgP的關係。Zhu等(79)於中國人的牙周炎的研究結論說：TLR2 Arg753Gln 的SNPs可能與中國人的AgP或CP無關。從以上的文獻回顧，我們知道牙周細胞會積極參與innate immune response以抵抗牙菌斑細菌。這些牙周細胞表現不同的TLRs。牙齦上皮細胞表現TLR2，與TLR6與TLR9的mRNA(58)讓它們能認知各種的PAMP。於感染早期，於牙齦上皮表現的TLRs與附著在牙齒表面的口腔細菌交互作用，TLR signaling導致於antibacterial peptides與neutrophils recuitment的chemokine(IL-8)的釋出;因此TLR signaling作為限制病原性感染與預防細菌破壞上皮性屏障。持續性的大量neutrophils移至口腔內(80,81)，是因牙齦上皮細胞對口腔細菌反應產生IL-8的結果。健康的牙銀上皮細胞會釋出antibacterial peptide：β-defensin-2(82)，顯示β-defensin-2有可能是牙齦上皮細胞對口腔細菌的反應時透過TLR signaling而產生的。有些細菌會侵入dentogingival epithelial barrier，使細菌成分有機會活化Langerhans cells、interstitial dendritic cells、macrophages、fibroblasts、endothelial cells、osteoblasts與osteoclasts等細胞;在此過程中，這些位於上皮深層與結締組織內的細胞是透過TLR signaling而活化的這些受刺激的細胞會釋出一系列的cytokines， chemokines及其他促進發炎與免疫細胞浸潤(infiltration)的介質(mediators)。另外從memory T cells產生的cytokines會加強炎性反應，導致結締組織與骨組織的破壞。牙周炎是高度複雜與多因素的炎症，探討TLR SNPs與牙周炎的關係應可提供免疫系統於維持牙周健康與抵抗牙周病的機轉方面提供有意義的資料。

研究方法：

(I)研究對象之選擇：

慢性牙周炎(CP)、侵犯性牙周炎病人(AgP)及與病人無家族性相關性(unrelated)的健康控制組個體(HC)將為收案對象。 所有個案均為居住於台灣地區的的漢民族(Han Chinese ethnicity)且具有至少 18 顆牙齒。牙周狀態評估包括：探測囊袋深度(probing pocket depth)，附連喪失水平(clinical attachment loss)及齒槽骨破壞的 X-光特徵。 病人診斷為 CP 或 AgP 的依據是 1999 年世界牙周病研討會及美國牙周病醫學會(The World Workshop for Periodontics and The American Academy of Periodontology 1999)所頒佈的條件(criteria)。 簡而言之，個案為大於 35 歲，一顆以上的牙齒具有臨床附連喪失等於或大於 5mm，有超過 3 個 sites 是大於或等於 6mm 的探測囊袋深度，及至少有兩顆牙齒的病灶是分佈於每一象限(quadrant)者是為 CP；個案有超過 8 顆牙之臨床附連喪失為超過 5mm 及探測囊袋深度為大於 6mm，且至少三顆患牙不是第一大臼齒或切齒者是為 AgP(其年齡

盡量控制在 35 歲以下)；個案無超過一個 site 附連有喪失，且探測深度小於 3mm 及無牙周病的病史者是為健康控制組(HC)，控制組盡量是為 35 歲以上者，以避免錯誤分類。 吸菸狀態則紀錄為〝未吸菸者〞及〝吸菸者〞，於收案時仍吸菸或以前有吸菸過均規為〝吸菸者〞群。

本研究計畫將經高雄醫學大學,中山醫學大學及台北醫學大學的〝倫理委員會〞(Ethics Committee)審查通過，且於進行收案前，須經個案對象簽同意書(informed consent)。

(II)血液樣本收集：

一、每一位個案人，經由靜脈穿刺抽取 20c.c.的週邊血，轉置入含有 EDTA 的試管內，然後於 4℃，1,600xg 離心 10 分鐘，分別收集中層白血球部分及上層血漿，白血球部分進行 DNA 萃取。另外，部分血(1ml)置於含 heparin 的試管內，將進行全血細胞培養(whole blood cell culture)。

二、萃取 DNA (DNA extraction)

血經離心約 10 分鐘,取其中段含白血球部份，以 standard phenol/chloroform extraction techniques [83] 萃取 DNA,再以 ethanol 沉澱 DNA。利用 UV spectrophotometry 測定 DNA 濃度。

三、Genotyping：

利用 polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP)來測定所選定之基因型。如有必要再度確定結果，代表性的 gel-purified PCR oroducts 將 directly sequenced by an ABI PRISM 377 DNA analyzer (Perkin-Elmer, Foster City, CA, USA)。 Sequence 之結果也須符合 NCBI Genome 發表的 sequence。

1.MMP-2 -1306 C/T；-790T/G；-735C/T:

(1)MMP-2 -1306 (C→T)

Forward primer：5’-CTTCCTAGGCTGGTCCTTACTGA-3’
Reverse primer：5’-CTGAGACCTGAAGAGCTAAAGAGCT -3’

共 188bp 限制酶為 ***XspI***

PCR 條件：95℃, 10 min., 35 cycles：95℃, 45s.; 55℃ ,45 s; 72℃, 45 s.

限制酶判定：188、162、26 bp→CT；188 bp→CC；162、26 bp→TT

(2)MMP-2 -790(T→G)

Forward primer：5’-GGGTCTTTTGTGACCTCGATC-3’
Reverse primer：5’-GGTAAAATGAGGCTGAGACCTG-3’

共 118bp 限制酶為 ***PvuI***

PCR 條件：95℃, 10 min., 40 cycles：95℃, 45s.; 55℃ ,45 s; 72℃, 45 s.

限制酶判定：118、99、19 bp→CT；118 bp→TT；99、19 bp→TT

(3) MMP-2 -735(C→T)

Forward primer：5’-ATAGGGTAAACCTCCCCACATT-3’
Reverse primer：5’-GGTAAAATGAGGCTGAGACCTG-3’

共 300bp　限制酶為 ***HinfI***

PCR 條件：95℃, 10 min., 40 cycles：95℃, 45s.; 55℃ ,45 s; 72℃, 45 s.

限制酶判定：300、254、46 bp→CT；300bp→CC；254、46 bp→TT

2. MMP-9 -1562C/T:

Forword primer：5-GCCTGGCACATAGTAGGCCC-3
Reverse primer：5-CTTCCTAGCCAGCCGGCATC-3

共 436bp　限制酶為 ***Sph I***

PCR條件：94°C for 10 minutes followed by 35 cycles of denaturation of 94°C for 30 seconds, annealing at 59°C

限制酶判定：194、242、436bp→CT；436 bp→CC；194、242 bp→TT

3.TIMP-2 -418 G/C: primers 已設計完成。

Forward primer：5’-CGTCTCTTGTTGGCTGGTCA-3’
Reverse primer：5’-CCTTCAGCTCGACTCTGGAG-3’

共 304bp　限制酶為 ***BsoBI***

PCR 條件：94℃, 4 min., 35 cycles：94℃, 1 min.; 56℃ ,45 s; 72℃, 45 s.

限制酶判定：G allele 有 two *BsoBI* restrictionsites, 經enzyme digestion 後產生230, 51 及 23 bp

bands;但 Callele 缺少 one *BsoBI* restriction site, 產生253 及51 bp 的two fragements。

4.MMP-8 -799C/T:

Forward primer：5’-CTGTTGAAGGCCTAGAGCTGCTGCTCC -3’
Reverse primer：5’- CATCTTCTCTTCAAACTCTACCC-3’

共 255bp 限制酶為 ***BglII***

PCR 條件：95℃, 10 min., 40 cycles：95℃, 30s.; 56℃ , 30s; 72℃, 60s.

限制酶判定：Homozygotes for the –799T allele yielded two restriction fragments of 224 and 31 bp after *Bgl*II digestion, homozygotes for –799C remained uncut (255-bp band), and heterozygotes yielded all three of these bands.

5. CD14 (C→T)：
Forward primer：5’- GTGCCAACAGATGAGGTTCAC -3’
Reverse primer：5’- GCCTCTGACAGTTTATGTAATC -3’

共497bp 限制酶為***AvaII***

PCR 條件：95℃, 15 min., 35cycles：94℃, 30s.; 57℃ , 30s; 72℃, 30s.

限制酶判定：497、345、143 bp→CT；497bp→CC；354、143 bp→TT

6.IL-4 -590(C→T)：

Forword primer：5’- ACTAGGCCTCACCTGATACG -3’
Reverse primer：5’- GTTGTAATGCAGTCCTCCTG -3’

共 252bp 限制酶為 ***BsmFI***

PCR 條件：95℃, 10 min., 40 cycles：94℃, 60s.; 57℃ , 60s; 72℃, 60s.

限制酶判定：252、192、60 bp→CT；252bp→CC；192、60 bp→TT

7. IL-17F 7488 (C→T)

Forward primer：5’- AGCTGGGAATGCAAACAAAC -3’
Reverse primer：5’- GTTCCCATCCAGCAAGAGAC -3’

共412bp(Arg161Arg)　限制酶為***NlaIII***

PCR 條件：95℃, 3 min., 40 cycles：94℃, 30s.; 60℃ , 30s; 72℃, 30s.

限制酶判定：412 288 124 bp→GA；252bp→GG；288/124 bp→AA

8. IL-13 (-1112 C/T)
Material and methods
Study subjects

The total number of participants in this study was 359, comprising 60 with aggressive periodontitis (31 men and 29 women), 204 with chronic periodontitis (111 men and 93 women) and 95 who were periodontally healthy (52 men and 43 women). The smoking status of participants was recorded as non-smoker or current smoker. Subjects who had never smoked or had quit smoking for at least 6 mo before the start of the study were recorded as nonsmokers. The betel nut chewing status of participants was recorded as for smokers (i.e. subjects who had never chewed betel nut or had quit chewing betel nut for at least 6 mo before the start of the study were recorded as non-betel nut chewers).

Analysis of the IL-13 genotype

The IL-13 genotype at position -1112 from the transcription start site was determined using the polymerase chain reaction–restriction fragment length polymorphism method . For analysis of the IL-13 -1112 C/T polymorphism, the following primers were used: 5’-GGAATCCAGCATGCCTTGTGAGG-3’ and 5’-GTCGCCTTTTCCTGCTCTTCCCGC-3’. The reaction conditions and cycling parameters were as follows: 100 ng of genomic DNA (l μL) was used for PCR amplification in a reaction mixture containing 2.5 μL of 10 reaction buffer, 2.0 μL of 2.5 mM dNTP, 0.3 μL of Taq polymerase (Taq DNA polymerase, 5 U/ μL; Supertherm, UK),18.7 μL of distilled water and 0.25 μL of each primer. The PCR conditions were as follows: samples were dena--tured at 95℃ for 10 min. followed by 40 cycles of 95℃ for 45 s, 55℃ for 30 s, 72℃ for 50 s and then a final extension for 10 min at 72℃. Restriction digestion was accomplished using BstUI (NEB, Schwalbach am Taunus, Germany) at 60℃ for 1 h. For the IL-13 -1112 C/T genotype, a 224 bp PCR fragment was generated for allele C and a 247 bp fragment was generated for allele T. Sequencing was performed on approximately 25% of randomly selected samples, to confirm the genotype results.

Statistical analyses

All statistical analyses were performed using the JMP 6.0 package (SAS, Cary,NC, USA). The two-sample t-tests or chi-square tests were used to compare means and proportions between the control and the periodontitis groups. To determine whether an association existed between periodontal disease and the IL-13 genotype and allele frequency, the significance of the difference in the distribution of

genotypes and alleles between periodontitis patients and control subjects was calculated using chi-square statistics and validated by the p-value. All p-values were two-sided. A p-value of < 0.05 was considered to be statistically significant. Association of IL-13 genotypes and periodontitis was analyzed by logistic regression of periodontitis patients vs. controls. To control the potential confounding effects, gender, age, smoking status and betel nut chewing habit were used as independent variables for adjustment.

9. FcRIIIB

Materials and methods

Study subjects

93 aggressive periodontitis (AgP) patients (40 females, 53 males), 372 chronic periodontitis (CP) patients (164 females, 208 males) and 158 ethnic-matched periodontally healthy (HC) subjects (82 females, 76 males).

Sample collection and DNA extraction

Twenty milliliters of heparin anticoagulated peripheral blood was collected from each study subject. Genomic DNA was extracted from the peripheral blood leukocytes by standard phenol/chloroform extraction techniques and precipitation with ethanol (Blin and Stafford, 1976). The DNA concentration was determined by ultraviolet (UV) spectrophotometry.

FcγRIIIb allotyping (NA1 and NA2)

FcγRIIIb allotyping was carried out using allele-specific primers(Bux et al., 1995). Two μl of genomic DNA was added to 25 μl reaction mixture containing 1 x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 U *Taq* polymerase (JMP Holdings, London, UK) and 1.0 μM of each primer. In the NA1-ASPA (allele-specific primer annealing), the sense primer is 5'-CAG TGG TTT CAC AAT GTG AA-3', the antisense primer is 5'-CAT GGA CTT CTA GCT GCA CCG-3'. In the NA2-ASPA, the sense primer is 5'-CTC AAT GGT ACA GCG TGC TT -3', the antisense primer is 5'-CTG TAC TCT CCA CTG TCG TT-3'. The PCR procedure consisted of initial denaturation at 95℃ for 9 minutes, followed by 35 cycles of denaturation at 95℃ for 30 sec, annealing at 56℃ for 30 sec and extension at 72℃ for 30 sec, followed by a final extension at 72℃ for 10 minutes. The products for NA1 and NA2 were 141 bp and 169 bp, respectively. The genomic DNA samples from two subjects representing NA1NA1, and NA1NA2 genotypes which were identified by DNA sequencing were used as the internal controls in each PCR procedure. All PCR products were analyzed by electrophoresis on 2% polyacrylamide gels containing ethidium bromide and were visualized under UV light.

(III). 統計分析:

各組之描述性統計量是以 mean±SD 及 proportion 表現。基因型及對偶基因之頻率(genotype and allelic frequencies)於各疾病組(chronic periodontitis, generalized aggressive periodontitis)與控制組(controls)相比較時，將採用 Chi-square (χ^2) tests,以 ANOVA 檢定是否有組間差異。若 p 值小於 0.05, 則視為有統計上的顯著差異(statistic significant difference)。各對偶基因或基因型與疾病危險的相關性(risk association)以 simple logistic regression 分析,以 odds ratio (OR)及 95% confidence intervals (CI)

來表示。較罕見之同合子將與異合子合併後再分析與疾病危險之相關性,以提高統計效力。為了避免年齡、性別及抽菸狀態之干擾作用(confounding effect),將這些變數加入multiple logistic regression, 進行調整(adjustment)。以上分析是採用 JMP software (SAS Institute, Cary, NC, USA)。

結果：

I.FcRIIIB

The demographic characteristics of the study subjects. The gender distribution was similar for these three groups. The mean age of the AgP patients was significantly lower than that of the CP patients and the HC. A significant difference was observed in the smoking status either between CP patients and HC (p=0.0008), or between CP and AgP patients (p=0.036), but not between AgP patients and HC (p=0.091). The CP patients were more likely to be current smokers.

The genotype distribution of FcγRIIIb in HC satisfied the Hardy-Weinberg equilibrium (p>0.05). In this study population, heterozygote (NA1NA2 genotype) was the most abundant (64.8%), followed by NA1 homozygote (NA1NA1 genotype, 22.3%) and NA2 homozygote (NA2NA2 genotype, 12.8%). The genotype distribution in AgP was significantly different from that in HC and that in CP (p=0.0002 for AgP versus HC, p=0.0034 for AgP versus CP), while the genotype distribution did not show significant difference between CP and HC (p=0.0612). No significant difference was observed in allelic frequency among groups.

Since the genotype distribution in AgP was significantly different from HC and CP, we analyzed further the association of AgP and FcγRIIIb genotype (table 3). The proportion of FcγRIIIb-NA2 carrier was higher in AgP patients as compared to the CP patients and HC. The crude odds ratio (OR) for NA2 carrier (NA1NA2 and NA2NA2 genotypes combined compared with the NA1NA1 genotype) to be associated with AgP was 3.27 (95% CI=1.57-7.51, p=0.0027) and 2.94 (95% CI=1.49-6.48, p=0.0037) as compared to HC and CP, respectively. After adjustment for age, gender and smoking status, the association was still significant (adjusted OR=2.74, 95% CI=1.07-7.67, p=0.043 for AgP versus HC; adjusted OR=2.65, 95% CI=1.06-7.29, p=0.046 for AgP versus CP). It means that the NA2 carriers (NA1NA2 or NA2NA2 genotype) had higher risk having aggressive periodontitis than the non-NA2 carriers (NA1NA1 genotype). No significant difference was observed in the distribution of NA2 carrier between CP and HC.

II.IL-13 (- 1112)

Demographical data and clinical parameters

A total of 60 patients with generalized aggressive periodontitis, 204 patients with chronic periodontitis and 95 healthy controls took part in the study. Clinical characteristics, smoking, betel nut chewing, age and gender distribution are summarized. The values of the clinical parameters (probing pocket depth and clinical attachment loss) were higher in aggressive periodontitis and chronic periodontitis groups. The mean age was significantly younger in the aggressive periodontitis group than in the other groups.

Distribution of genotypes

The distribution of genotypes in periodontitis patients and the healthy control group, as well as the results of Fisher_s exact test, are shown in Table 2. Homozygosity for the IL-13 C allele at position)1112 was found in 78.3% of the patients with aggressive periodontitis, in 57.4% of the patients with chronic periodontitis and in 54.7% of the healthy controls. A total of 21.7% individuals with aggressive periodontitis, 41.2% of individuals with chronic periodontitis and 43.2% of healthy controls were heterozygous (C/T). The prevalence of the T allele homozygote was very low in our study population: none was found in patients of the aggressive periodontitis group, and the prevalence was 1.5% in patients of the chronic periodontitis group and 2.1% in the healthy control group. The distribution of the genotypes (CC/CT+TT) between patients with aggressive periodontitis and healthy controls was significantly different ($p < 0.05$). The frequency of the CC genotype and C allele were highest among the patients with aggressive periodontitis, followed by those with chronic periodontitis and then healthy controls. The distribution of IL-13 genotypes at position)1112 in patients and controls did not differ from Hardy – Weinberg equilibrium.

Association of genotypes and periodontitis

. There was no significant difference between patients with chronic periodontitis and healthy controls. The CC genotype of the patients with aggressive periodontitis was significantly higher than in healthy controls (78.3:54.7%). Using T or CT+TT as the reference, the crude odds ratio (OR) was 2.55 for C/T and 2.98 for CC/CT+TT in comparison with patients with aggressive periodontitis and healthy controls. The association of the IL-13)1112 gene polymorphism and aggressive periodontitis still existed, even after adjusting for age, gender, smoking and betel nut chewing status by logistic regression analysis [C/T: adjusted OR = 3.58, 95% confidence interval (CI) = 1.35 – 10.89, $p = 0.015$; CC/CT+TT: adjusted OR = 6.45, 95% CI = 1.99 – 23.72, $p = 0.003$]. The results indicated that patients with aggressive periodontitis had a higher distribution of the CC genotype and C allele frequency than healthy controls.

Impact of the IL-13 polymorphism on the risk for periodontitis

The study subjects were further stratified by their smoking status to examine whether the smoking factor would augment the impact of the IL-13

polymorphism on the risk for periodontitis. As the frequency of the TT genotype was very small after grouping, we combined CT and TT as the reference in this analysis. A significant difference of the IL-13 genotype between aggressive periodontitis and healthy controls was only found in the nonsmoking group. This significant association still remained after adjustment for age, gender and betel nut chewing (aggressive periodontitis vs. healthy controls: crude OR = 3.08, $p = 0.007$; adjusted OR = 4.48, 95% CI = 1.31 – 16.93, $p = 0.020$).

III.IL-4

We evaluated gene polymorphisms of IL-4 -590C>T, in patients with chronic periodontitis (CP, n=150) and generalized aggressive periodontitis (AgP, n=63) in comparison with controls (H, n=83). Sixty-three patients with generalized aggressive periodontitis (AgP), 150 patients with chronic periodontitis (CP) and 83 matched healthy controls (H) participated in the study. Blood samples were collected and DNA isolated. The PCR-RFLP technique was used to investigate the polymorphism in the promoter region. Genotype and allele frequencies among study groups were compared using nominal logistic regression with $p < 0.05$ considered significant. Pearson's X2 test was used for analysis of Hardy-Weinberg equilibrium.

Table 1. The demographic characteristics of participants

		CP N=150		AgP N=63		H N=83			
		N	%	N	%	N	%	X^2	p-value
Age		54.65 ± 9.71		39.03 ± 7.05		48.84 ± 10.87			<0.0001
Gender									
	Male	75	50.0	44	69.8	43	51.8	4.776	0.0218
	Female	75	50.0	19	30.2	40	48.2		
Smoking status									
	Smokers	23	15.3	11	17.5	5	6.0	6.037	0.0489
	Non-smokers	127	84.7	52	82.5	78	94.0		

Table 2. Genotype distribution in CP , AgP , and H groups

Genotype	CP N=150		AgP N=63		H N=83			
	N	%	N	%	N	%	X^2	p-value
IL-4 -590							1.14	0.8879
CC	88	58.7	40	63.5	46	55.4		
CT	53	35.3	19	30.2	32	38.6		
TT	9	6.0	4	6.3	5	6.0		

Table 3. Frequencies of IL-4 - 590 SNP in CP, AgP and H groups

Genotype IL-4 -590	H	CP	AgP	CP Development	AgP Development	CP Development in Non-smokers	AgP Development in Non-smokers
	N = 83 (%)	N =150 (%)	N = 63 (%)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
CC	46 (55.4)	88 (58.7)	40 (63.5)	1.00	1.00	1.00	1.00
CT	32 (38.6)	53 (35.3)	19 (30.2)	0.90 (-0.64 ~ 0.38)	0.58 (-0.78 ~ 0.58)	0.85 (-0.67 ~ 0.42)	0.49 (-0.77~0.81)
TT	5 (6.0)	9 (6.0)	4 (6.3)	0.84 (-0.62 ~ 0.98)	0.50 (-1.36~0.68)	1.04 (-0.78~0.97)	0.25 (-2.05~0.44)
CT+TT	37 (44.6)	62 (41.3)	23 (36.5)	0.94 (0.54~ 1.66)	0.56 (-0.11~0.69)	0.87 (-0.23~0.37)	0.44 (-1.69~0.02)
CC+CT	78 (94.0)	141 (94.0)	59 (93.7)	1.00	1.00	1.00	1.00
TT	5 (6.0)	9 (6.0)	4 (6.3)	1.23 (-0.94~1.46)	0.57 (-0.47 ~ 1.05)	1.10 (-0.72~0.59)	0.32 (-3.16~0.56)
C allele	124 (74.7)	229 (76.3)	99 (78.6)	1.00	1.00	1.00	1.00
T allele	42 (25.3)	71 (23.7)	27 (21.4)	0.99 (-0.23~0.23)	0.62 (-0.57~0.08)	0.93 (-0.28~0.21)	**0.48 (-0.72~-0.02)**

The genotype distribution (Table 2) of IL-4 -590 C/T showed no significant difference among the three groups (p=0.8879). No significant differences in the IL-4 -590 C->T allele (adjusted OR=0.99, 95%CI=-0.23~0.23, p>0.05) and genotype frequencies were found between H and CP (Table 3). There was a similar frequency of the -590 genotype in patients with AgP compared with H, however, AgP nonsmokers with T allele as compared with C allele could be lesser susceptible to AgP (OR= 0.48, 95%CI=-0.72~-0.02, p=0.0412) than smokers.

IV. MMP-8 -799 C/T

The frequency of -799 C/C, C/T and T/T genotypes in the AgP were 35.4%, 52.0%, 12.5%; in the CP were 33.7%, 53.0% and 13.3% ; in H were 50.0%, 37.7% and 12.3% respectively. On the basis of logistic regression analysis with adjustment of age, sex, smoking and betel nut chewing habits, significant associations with risk of periodontitis were observed with MMP-8 -799 C/T. An increased risk of CP was found to be associated with -799C/T genotype with OR being 2.04, 95%CI=1.26 – 3.32, p=0.004; an increased risk of AgP with OR=2.21, 95%CI=1.18 -4.16, p= 0.013 compared with the -799 C/C genotype.. An increased risk of CP was found also to be associated with -799 alleles C/T + T/T. An increased risk of CP was found to associated with -799 C/T +T/T alleles with OR=1.90, 95%CI=1.12 -3.00, p= 0.006; an increased risk of AgP with OR=2.0, 95%ci=1.11 -3.61, p=0.022 compared with -799 CC allele.

Table 1. Genotype and allele frequencies of MMP-8 polymorphisms in patients of aggressive periodontitis (AgP), chronic periodontitis (CP), and healthy (H) controls

	AgP(96)	CP(353)	H(106)	AgP vs. H	CP vs. H
Genotype	N(%)	N(%)	N(%)	p value	p value
CC	34(35.4)	119(33.7)	53(50.0)	0.07	0.02 *
CT	50(52.0)	187(53.0)	40(37.7)		
TT	12(12.5)	47(13.3)	13(12.3)		
CC	34(35.4)	119(33.7)	53(50.0)	0.03*	0.006*
CT+TT	62(62.5)	234(66.3)	53(50.0)		

Chi-square test *

p<0.05

Table 2. Analysis of genotype and allele frequencies of MMP-8 polymorphisms in chronic periodontitis (CP) patients and healthy (H) controls

	CP	H	Crude OR	p value	Adjust OR	p value
Genotype	N (%)	N (%)	95% CI		95% CI	
CC	119(33.7)	53(50.0)	1		1	
CT	187(53.0)	40(37.7)	2.07 (1.28-3.36)	0.003 *	2.04 (1.26-3.32)	0.004*
TT	47(13.3)	13(12.3)	1.47 (0.73-2.99)	0.283	1.44 (0.71- 2.94)	0.314
CC	119(33.7)	53(50.0)	1		1	
CT+TT	234(66.3)	53(50.0)	1.92 (1.22-3.02)	0.005 *	1.90 (1.21-3.00)	0.006*

OR=odds ratio of CP vs. H; adjusted by age, gender, smoking and betel nut chewing by logistic regression analysis

Table 3. Analysis of genotype and allele frequencies of MMP-8 polymorphisms in chronic periodontitis (AgP) patients and healthy (H) controls

Genotype	AgP N (%)	H N (%)	Crude OR 95% CI	p value	Adjust OR 95% CI	p value
CC	34(35.4)	53(50.0)	1		1	
CT	50(52.1)	40(37.7)	2.07 (1.12-3.82)	0.020 *	2.21 (1.18-4.16)	0.013 *
TT	12(12.5)	13(12.3)	1.37 (0.54-3.42)	0.507	1.50 (0.59- 3.83)	0.399
CC	34(35.4)	53(50.0)	1		1	
CT+TT	62(62.6)	53(50.0)	1.89 (1.06-3.38)	0.031 *	2.00 (1.11-3.61)	0.022 *

OR=odds ratio of CP vs. H; adjusted by age, gender, smoking and betel nut chewing by logistic regression analysis

Table 4. The association of MMP-8 genotype and risk of periodontitis, comparisons performed by logistic regression analysis

	CT+TT	CC	Crude OR 95% CI	p value	Adjust OR 95% CI	p value
CP	234(66.3)	119(33.7)	1.92 (1.22-3.02)	0.005 *	1.90 (1.21-3.00)	0.006*
HC	53(50.0)	53(50.0)	1		1	
AgP	62(62.6)	34(35.4)	1.89 (1.06-3.38)	0.031 *	2.00 (1.11-3.61)	0.022 *
HC	53(50.0)	53(50.0)	1		1	
CP	234(66.3)	119(33.7)				
AgP	62(62.6)	34(35.4)				

OR=odds ratio of CP vs. H; adjusted by disease group, gender, and smoking by logistic regression analysis

V. MMP-2 -1306 C/T, -735 C/T, -790 G/T and TIMP-2 -418 C/G

	人數	比例
總人數	192	
CP	87	45%
AP	40	21%
H	63	33%

MMP2-1306			MMP2-735		
CC	168	92%	CC	117	64%
CT	13	7%	CT	53	29%
TT	1	1%	TT	13	7%
MMP2-790			TIMP2-418		
GG	125	69%	CC	127	74%
GT	45	25%	GC	39	23%
TT	11	6%	GG	5	3%

	MMP2-1306					
	CP	比例	AgP	比例	H	比例
CC	81	97%	34	97%	53	90%
CT	2	2%	1	3%	6	10%
TT	1	1%	0		0	

	MMP2-735					
	CP	比例	AgP	比例	H	比例
CC	52	62%	23	66%	40	66%
CT	25	30%	9	26%	18	30%
TT	7	8%	3	8%	3	4%

	MMP2-790					
	CP	比例	AgP	比例	H	比例
GG	1	1%	1	3%	10	18%
GT	21	26%	10	29%	10	18%
TT	59	73%	23	68%	36	64%

	TIMP2-418					
	CP	比例	AgP	比例	H	比例
CC	65	80%	21	64%	40	78%
CG	14	17%	10	30%	10	20%
GG	2	3%	2	6%	1	2%

VI. Others (IL-17F, CD14, MMP-9)

Primers of these gene polymorhisms have been prepared for PCR. Restriction digestions has shown the selective bps of allele i.e. the PCR-RFLP techniques are ready for the investigations of these polymorphisms in the promoter region.

討論：

I. FcRIIIB

The results of this study indicate that the FcγRIIIb polymorphism is associated with the susceptibility to aggressive periodontitis in Taiwanese. The FcγRIIIb-NA2 carriers were found to be over-presented in aggressive periodontitis patients as compared to the healthy controls and chronic periodontitis patients. The results are consistent with the findings in Japanese by Yoshihara et al.(Yoshihara et al., 2001) and Kobayashi et al.(Kobayashi et al., 2000a) and in Caucasians by de Souza et al.(De Souza and Colombo, 2006) that carrying at least one FcγRIIIb-NA2 allele exhibited increased susceptibility to aggressive periodontitis, but are disparate to the findings of Nibali et al.(Nibali et al., 2006) reported that the NA1NA1 genotype was associated with aggressive periodontitis.

Although the association of FcγRIIIb polymorphism and susceptibility to periodontitis in Taiwanese had been evaluated (Chung et al., 2003), the sample size was small (28 AgP, 50 CP, and 74 HC) in the study. Because sample size is critical in association study, we carried out this case-control study with relative larger sample size (93 AgP, 372 CP, and 158 HC) in order to get the more reliable result. Our results showed the FcγRIIIb-NA2 carrier was associated with increased risk of aggressive periodontitis, and 12.8% of our study subjects were NA2 homozygote. On the contrary, no association could be observed between FcγRIIIb polymorphism and periodontitis, and no NA2 homozygote could be detected in the aforementioned study. Another study surveyed the FcγRIIIb genotype distribution in 583 Taiwanese showed that 15.1% study subjects were NA2NA2 genotype. The genotype distribution and allelic frequencies of our study population (623 subjects, NA1NA1 22.3%, NA1NA2 64.8%, NA2NA2 12.8%; NA1 allele 54.7%, NA2 allele 45.3%)

were similar to that raised on a southern Chinese population (413 subjects, NA1NA1 28.1%, NA1NA2 56.9%, NA2NA2 14.5%; NA1 allele 56.5%, NA2 allele 43.0%) (Tong et al., 2003). We consider that no NA2NA2 genotype could be observed in Chung's study might be related to small sample size.

Host immune reactions triggered by periodontal pathogens result in periodontal tissue destruction. High levels of IgG1 and IgG3 subclass was found in GCF taken from periodontitis sites(Wilton et al., 1993, Takahashi et al., 1997). The efficient catch and phagocytosis of IgG1- and IgG3-opsonized bacteria via FcγRIIIb, then clearance of immune complexes are crucial and critical for host defense in periodontal tissue. Neutrophils bearing FcγRIIIb-NA2 allele have lower receptor affinity to IgG3, which is the predominant anti-*Porphyromonas gingivalis* serum IgG for rapidly progressive periodontitis (it has been changed to aggressive periodontitis) patients (Whitney et al., 1992), and lower phagocytic capacity. It is considered that neutrophils expressing the NA2 allele are less efficient in the capture of IgG-opsonized periodontal pathogens (Kobayashi), and triggering less potent effector functions of neutrophils, being indicated to that humoral immune response of FcγRIIIb-NA2 carrier might be ineffective to clear the *P. gingivalis* or other periodontal pathogens. The relative impairment of IgG-immune complexes clearance by NA2 allele is supposed to favor deposition of immune complexes, persistent bacterial infections and consequently prolonged proinflammatory reactions in periodontal tissue. The functional depression of the first line of host-defensive cell population in periodontium of the FcγRIIIb-NA2 carrier would early initiate the development and enhance the progression of periodontitis.

The genetic studies for association between periodontitis and FcγRIIIb polymorphism exhibit inconsistent findings (Table 4). Some investigations have associated the FcγRIIIb-NA2 allele with recurrence(Kobayashi et al., 1997), severity(Kobayashi et al., 2001), severity and extent of bone loss(Meisel et al., 2001) of chronic periodontitis and progression of periodontitis(Yoshihara et al., 2005). The FcγRIIIb-NA2 allele(Yoshihara et al., 2001) and FcγRIIIb NA2NA2 genotype were overrepresented in generalized aggressive periodontitis patients(Kobayashi et al., 2000a, De Souza and Colombo, 2006), and were associated with susceptibility to localized aggressive periodontitis in African-Americans(Fu et al., 2002) . On the contrary, a study reported FcγRIIIb NA1NA1 genotype was significantly associated with generalized aggressive periodontitis in Caucasian(Nibali et al., 2006). Several reports pointed the FcγRIIIb polymorphism did not contribute to periodontitis (Kobayashi et al., 1997, Chung et al., 2003), likely due to very small number of NA2NA2 homozygotes in some ethnic populations. FcγRIIIb genotype was reported not to associate with severity of periodontitis (Meisel et al., 2001, Loos

et al., 2003, Wolf et al., 2006) and treatment response of periodontitis (Colombo et al., 1998, Wolf et al., 2006). In addition, FcγRIIIb genotype also had been reported to be related to interindividual difference in resistance to periodontitis. NA1 allele was significantly overrepresented in periodontitis-resistant group compared with the periodontitis-susceptible group(Sugita et al., 2001).

Several issues might account for the conflicting results between the different studies : variations at the ethnic backgrounds of the study populations, the different definition and classification of periodontal disease, and the relative small sample size. The NA1 and NA2 allelic frequencies observed in different ethnic groups were quite different(Hessner et al., 1996). In Caucasian, NA2 is the most frequent allele (NA1:NA2=0.37:0.63). On the other hand, NA1 is more popular in Asian populations, including Taiwanese and Japanese (NA1:NA2=0.68:0.32). Based on the literature, the prevalence of FcγRIIIb genotype differs widely among distinct ethnic background, making the comparisons of the findings among studies quite difficult. Therefore, the evaluation of any FcγR genotype as the risk factor for periodontal diseases should be examined in different ethnic populations. The sample size is critical in association studies. The number of study subjects of several previous studies was relatively smaller. The interpretation of conclusions from these studies should be cautious.

Smoking is a known risk factor for periodontitis. In this study, we just used the smoking status as an independent variable for adjustment, not stratified the study subjects by their smoking status, because the current smokers in aggressive periodontitis patients (13 in 93) and healthy controls (12 in 158) were quite less.

Our study has several limitations. There are three subclasses of FcγRs (FcγRIIa, FcγRIIIa and IIIb) bearing functionally allelic polymorphisms can interact with IgG1 and IgG3 subclasses. The IgG-mediated leukocyte effector function is regulated by the interaction of FcγRs. Human FcγRs genes are clustered in very close proximity on chromosome 1q21-24. The findings of previous studies supported the FcγR polymorphisms might be non-randomly distributed (Torkildsen et al., 2005, Van der Pol et al., 2003). Linkage disequilibrium may exist between FcγRIIIb gene and the other FcγRs genes (Hatta et al., 1999) and/or other adjacent genes. Cross-linking of FcγRIIa and FcγRIIIb on neutrophils showed a synergistic intracellular Ca^{2+} response(Vossebeld et al., 1995), and increased phagocytosis and respiratory burst(Kocher et al., 1997). FcγRIIIb can enhance FcγRIIa function in an allele-sensitive manner, that is NA1 homozygotes of FcγRIIIb showed greater activation of FcγRIIa than the NA2 homozygotes (Salmon et al., 1995). Therefore, in the investigation of FcγR polymorphisms and susceptibility to periodontal disease, FcγR combined genotypes may represent more relevant risk factors than single FcγR

genotype. Some composite genotypes of FcγRs have been reported to be associated with periodontal diseases. For instance, the FcγRIIIa-158V and FcγRIIIb-NA2 composite genotype was associated with the severity of chronic periodontitis in Japanese(Kobayashi et al., 2001), the FcγRIIIb NA2NA2 and FcγRIIa H/H131 composite genotype may be associated with generalized aggressive periodontitis in Caucasian(De Souza and Colombo, 2006). We did not investigate the combination effect among FcγRs. We can not rule out the possibility that the association of FcγRIIIb polymorphism with susceptibility to aggressive periodontitis may result from the linkage disequilibrium with the real susceptibility genes located in the neighboring FcγR genes.

In summary, we have identified that the FcγRIIIb allelic polymorphism is associated with the susceptibility to aggressive periodontitis. However, the clinical implications of the FcγRIIIb polymorphism are not yet clear(De Haas et al., 1995a). Further investigations about the clinical implications of the FcγRIIIb polymorphism and the effects of FcγR composite genotypes on neutrophils are needed to clarify the significance of the findings of this study.

II. IL-13

Several different factors may contribute to the pathogenesis of periodontitis. Recent data suggest that the genetic background may also play a potential role in disease manifestation and evolution. However, so far, little is known about the IL-13 gene and its exact role in periodontitis. The results of our study show that the genetic variant of periodontitis is associated with the -1112T polymorphism (genotype CC) of the IL-13 gene. To our knowledge, this is the first study investigating the IL-13 -1112 C/T polymorphism in Chinese patients with periodontitis. Several reports have indicated the presence of IL-13 in periodontal lesions. However another study only detected IL-13 in patients with gingivitis and in subjects with healthy gingiva . It has been proposed that the shift from gingivitis to periodontitis may be caused by an imbalance between Th1 and Th2 cells. Many other studies also point out that IL-13 shares biological features with IL-4 in controlling differentiation to Th2 cells, which respond with the progression from gingivitis to periodontitis. As the gingival concentrations of IL-13 were nearly 10 times greater than those of IL-4 within the tissues examined, IL-13 may be more important than IL-4 for maintaining the Th2 response. The results of our present study are different from the data of Gonzales et al. in which the genotype and the allele frequencies of the IL-13 polymorphisms were not different between the aggressive periodontitis and control groups of Caucasians. The IL-13 -1112C polymorphism (genotype CC) is known to lead to a lower transcription rate for IgE production . In our present study, the CC

polymorphism was detected at a higher frequency in patients with aggressive periodontitis than in patients with chronic periodontitis or in healthy controls. The difference might be caused by different ethnicity. Our previous studies showed that ethnic factors were considered to be a major variable for evaluating the predisposition to aggressive periodontitis. The distribution of the IL-13 -1112C/T genotype of our result is similar to the results of studies of the Chinese Han Nationality in China. The authors concluded that theIL-13 -1112C/T was associated with an increased serum IgE level and might be an important candidate gene for asthma. The results of this study showed that patients with aggressive periodontitis had a higher distribution of the CC genotype or a higher C-allele frequency than healthy controls. When considered together, we suggest that the IL-13 polymorphism -1112 CC genotype, or the C allele, may be a predisposing factor in the development of aggressive periodontitis in Taiwanese people of Han-ethnicity. The association of the IL-13 polymorphism -1112 CC genotype or C allele and aggressive periodontitis is demonstrated both in non-adjusted and adjusted models. From a study of prognostic factors in the treatment of generalized aggressive periodontitis, data showed that current smoking was strongly associated with non-responding patients (OR =3.8). According to the results, smokers had more lymphocytes and higher levels of IFN-r and IL-13. The authors suggest that the increased Th cell activity, and specifically an elevated Th2 profile in smokers, may constitute a risk for patients who smoke that may induce conversion of periodontal stability into progressive disease. When our study subjects were further subgrouped by their smoking status to examine whether the smoking factor synergistically impacted the IL-13 -1112 polymorphism, increasing the risk for periodontitis, no association was found in the smoking group. However, as mentioned earlier, there was an association in the nonsmoking group. That means that the influence of the IL-13 -1112 polymorphism on aggressive periodontitis was noticeable in the non-smoking group, but not in the smoking group. This seems to suggest that the influence of smoking on the development of aggressive periodontitis was stronger than the impact of the IL-13 gene polymorphism. The data also agree with our previous finding that smoking and genetic factors may influence the progression of aggressive periodontitis in different ways. However, the impact of genetics seems to be less than the impact of the smoking status.

III. IL-4

Conclusion: This study demonstrated no association between the IL-4 -590 C->T polymorphisms and the susceptibility to CP. However, the T allele in nonsmokers might be associated with a lower susceptibility to AgP. (Grant supported by NSC,

Taiwan)

IV. MMP-2, TIMP-2

At this report, we have to finished more samples to have statistical analysis for the association of the development of periodontitis and the SNPs of MMP-2 -1306 C/T, -735 C/T, -790 G/T and TIMP-2 C/G.. The results have been submitted to the 2011 IADR Annual Meeting in the U.S.A.

IV. MMP-8

Conclusion: From our results, we concluded that -799 C/T polymorphisms of MMP-8 were associated with the risk of development of periodontitis in Taiwanese of Han ethnicity. (supported by the grant NSC 98-2314-B-040-020- ,Taiwan, ROC).

參考文獻:

(1) Birkedal-Hansen H. J Periodontol 1993; 64: 474-84.
(2) Ma¨kela¨ M, Salo T, Uitto V-J, Larjava H. J Dent Res 1994;/73:/1397-406
(3) Lee W, Aitken S, Sodek J, McCulloch CAG. Periodontal Res 1995;/30:/23-33.
(4) Van Der Zee E, Everts V, Beersten W. J Periodontal Res 1996;/31:/483-8.
(5) Ejeil A, Igondjo-Tchen S, Ghomrasseni S, Pellat B, GodeauG, Gogly B. J Periodontol2003;/74:/188-95.
(6) Blavier L, Delaisse JM. J Cell Sci 1995; 108: 3649-59.
(7) Kinane DF, Darby IS, Luoto H, Sorsa T, Tikanoja S,Ma¨ntyla¨ P. J Periodontal Res 2003; 38: 400-4
(8) Ma¨ntyla¨ P, Stenman M, Kinane DF, Tikajona S, Luoto H,Salo T, et al. J Periodontal Res 2003; 38: 436-9
(9) Sorsa T, Tjaderhane L, Salo T. Oral Dis 2004;10:311-18.
(10) Cao Z, Li C, Zhu G. Tissue Antigens. 2005 Jul; 68(1):38-43.
(11) de Souza AP et al. J Clin Periodontol. 2003 Feb;30(2):154-8.
(12) Astolfi CM, Shinohara AL, da Silva RA, Santos MC, Line SR, de Souza AP. J Clin Periodontol. 2006 Oct; 33(10):699-703.
(13) Istagaki M, Kubota T, Tai H, Shimada Y, Morozumi T, Yamazaki K. J Clin Periodontol. 2004, Sep; 31(9):764-9.
(14) Holla LI, Jurajda M, Fassmann A, Dvorakova N, Znojil V, Vacha J. J Clin Periodontal. 2004 , Aug;31(8)685-90.
(15) Holla LI, Fassmann A, Vasku A, Goldbergova M, Beranek M, Znojil V, Vanek J, Vacha J. J Periodontol. 2005 Jul;76(7)1056-60.
(16) Suzuki A, Ji G, Numabe Y, Muramatsu M, Gomi K, kanazashi M, Ogata Y,

Shimizu E, Shibukawa Y, Ito T, Sugaya A, Arai T, Yamada S, Deguchi S, Kamoi K. Biochem Biophys Res Commun 2004; 317:887-892.
(17) Keles CC, Gunes S, Sumer AP, Sumer M, Kara N, Bagci H, Koprulu H. J Periodontol. 2006, Sep; 77(9):1510-4.
(18) Gürkan A , Emingil G, Saygan BH, Atila G, Cinarcik S, Jose T, Berdeli A. Arch Oral Biol. 2008, Apr;53(4):337-45.
(19) Gürkan A , Emingil G, Saygan BH, Atila G, Cinarcik S, Jose T, Berdeli A. J Periodontol 2007 Dec; 78(12):2338-47.
(20) Chen D, Wang Q, Ma ZW, Chen FM, Chen Y, Xie GY, Wang QT, Wu ZF. J Clin Periodontol. 2007; 34(5):384-9.
(21) Kubota T. et al. J Periodontol. 2008 Jan; 79(1):166-73.
(22) Wang JE (2005). Crit Care Med 33:695-696.
(23) Cooke GS, Hill AV (2001). Nat Rev Genet 2:967-977.
(24) Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, Looareesuwan S, et al. (2002). Parasitai Int 51:361-366.
(25) Sutherland AM, Walley KR, Russell JA (2005). CWI Care Med 33:638-644.
(26) Papapanou PN (1999). J Int Acad Periodontol 1(4): 110-116.
(27) Tonetti MS, Mombelli A (1999). Ann Periodontol 4:39-53.
(28) Loos BG, John RP Laine ML (2005). J Clin Periodontol 32(Suppl 6):159-79.
(29) Tonetti MS, Claffey N, L Clin Periodontol 32(Suppl 6):210-213.
(30) Socransky SS, Haffajee AD, Smith C, Duff GW (2000). J Clin Periodontol 27:810-818.
(31) Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Line SR. J Clin Periodontol 2002: 29: 587–591
(32) Michel J, Gonzales JR, Wunderlich D, Diete A, Herrmann JM, Meyle J. J Clin Periodontol 2001: 28: 483–488.
(33) Kara N, Keles GC, Sumer P, Gunes SO, Bagei H, KopruluH, Bek Y. Acta Odontol Scand. 2007, Oct; 65(5):292-7.
(34) Pontes CC, Gonzales JR, Novaes AB Jr, Junior MT, Grisi MF, Michel J, Meyle J, de Souza SL. J Dent 2004: 32: 241–246.
(35) Kang BY, Choi YK, Choi WH, Kim KT, Choi SS, Kim K, Ha NJ. Arch Pharm Res 2003: 26: 482–486.
(36) Gonzales JR, Kobayashi T, Michel J, Mann M, Yoshie H, Meyle J. J Clin Periodontol 2004: 31: 384–389.
(37) Gonzales JR, Mann M, Stelzig J, Bodeker RH. J Clin Periodontol. 2007 June; 34(6):473-9.

(38) Hooshmand B, Hajilooi M, Rafiei A, ManiKashani KH, Ghasemi R. J Periodontal Res 2008;43:111-115.
(39) Laberge S, Ernst P, Ghaffar O, et al. Am J Respir Cell Mol Biol. 1997;17:193–202. (A98-11)
(40) Blaschke S, Schulz H, Schwarz G, et al. J Rheumatol. 2001;28:12–21.
(41) Schreiber S. Gut. 2001;49:747-8
(42) Tsai IS, Tsai CC, Ho YP, et al. 2005;31:34–40. (A98-14)
(43) Folwaczny M, Glas J, Torok HP, Tonenchi L, Paschos E, Malachova O, Bauer B,Folwaczny C. Clin Exp. Immunolo. 2005 Oct; 142(1):188-92.
(44) Beklen A, Ainola M, Hukkanen M, Gurgan C, Sorsa T, Kontinen YT. J Dent Res. 2007 Apr; 86(4):347-51.
(45) Gaffen S.L, Hajishengallis G. J Dent Res 87(9):817-828, 2008
(46) Donati M, Berglundh T, Hytönen A-M, Hahn-Zoric M, Hanson L-Å, Padyukov L. J Clin Periodontol 2005: 32: 474–479.
(47) Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG. Lancet 2003: 361: 567–571.
(48) LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, Vercelli D. J Immunol 2001: 167: 5838–5844.
(49) Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. Am J Respir Cell Mol Biol 1999: 20: 976–983.
(50) Vercelli D, Baldini M, Martinez FD. T. Int Arch Allergy Immunol 2001: 124: 20–24.
(51) Folwaczny M, Glas J, Török H-P, Fricke K, Folwaczny C. J Clin Periodontol 2004: 31: 991–995.
(52) Hollá LI, Buckova D, Fassmann A, Halabala T, Vasku A, Vacha J. J Med Genet 2002: 39: 844–848.
(53) Yamazaki K, Ueki-Maruyama K, Oda T, Tabeta K, Shimada Y, Tai H, Nakajima T, Yoshie H, Herawati D, Seymour GJ. J Dent Res 2003: 82: 612–616.
(54) Laine ML , Morre SA, Murillo LS, van Winkelhoff AJ, Pena AS. J Dent Res. 2005 Nov; 84(11):1042-6.
(55) Trevonen T, Raunio T, Knuuttila M, Karttunen R. J Clin periodontal. 2007 May; 34(5): 377-83.
(56) Takeda K, Akira S. T. Int Immunol 2005: 17: 1–14.
(57) Janeway CA Jr, Medzhitov R. Annu Rev Immunol 2002: 20: 197–216.
(58) Kusumoto Y, Hirano H, Saitoh K, Yamada S, Takedachi M, Nozaki T, Ozawa Y, Nakahira Y,Saho T, Ogo H, Shimabukuru Y, Okada H, Murakami S. J Periodontol 2004: 75: 370–379.

(59) Uehara A, Sugawara S, Takada H. J Med Microbiol 2002: 51: 626–634.
(60) Mori Y, Yoshimura A, Ukai T, Lien E, Espevik T, Hara Y. Oral Microbiol Immunol 2003: 18: 54–58.
(61) Asai Y, Ohyama Y, Gen K, Ogawa T. Infect Immun 2001: 69: 7387–7395.
(62) Takada H, Mihara J, Morisaki I, Hamada S. Infect Immun 1991: 59: 295–301.
(63) Tamura M, Tokuda M, Nagaoka S, Takada H. Infect Immun 1992: 60: 4932–4937.
(64) Yamazaki K, Ikarashi F, Aoyagi T, Takahashi K, Nakajima T, Hara K, Seymour GJ. Oral Microbiol Immunol 1992: 7: 218–224.
(65) Nonnenmacher C, Dalpke A, Zimmermann S, Flores-De-Jacoby L, Mutters R, Heeg K. Infect Immun 2003: 71: 850–856.
(66) Tabeta K, Yamazaki K, Akashi S, Miyake K, Kumada H, Umemoto T, Yoshie H. Infect Immu*n* 2000: 68: 3731–3735.
(67) Hatakeyama J, Tamai R, Sugiyama A, Akashi S, Sugawara S, Takada H. Oral Microbiol Immuno*l* 2003: 18:14–23.
(68) Folwaczny M, Glas J, Török HP, Limbersky O, Folwaczny C. Clin Exp Immunol 2004: 135: 330–335.
(69) Hiraoka T, Izumi Y, Sueda T. Oral Microbiol Immunol 1998: 13: 246–252.
(70) Wang PL, Ohura K, Fujii T, Oido-Mori M, Kowashi Y, Kikuchi M, Suetsugu Y, Tanaka J.Biochem Biophys Res Commun 2003: 305: 970–973.
(71) Mochizuki S, Kobayashi M, Suzuki T, Oikawa A, Koseki T, Nishihara T, Hasegawa K. J Periodontal Res 2004: 39: 333–343.
(72) Schröder NW, Meister D, Wolff V, Christan C, Kaner D, Haban V, Purucker P,Hermann C, Moter A, Gobel UB, Schumann RR. Genes Immun 2005: 6: 448–451.
(73) Fukusaki T, Ohara N, Hara Y, Yoshimura A, Yoshiura K.J Periodontal Res. 13 :Aug 2007, online article.
(74) Berdeli A, Emingil G, Han Saygan B, Gurkan A, Atilla G, Kose T, Baylas H. J Clin Periodontol. 2007 Jul; 34(7):551-7.
(75) Izakovicova Holla L, Buckva D, Fassmann A, Rouballkova L, Vanek J. J Periodontal Res. 2007 Aug; 42(4):340-4.
(76) James JA, Poulton KV, Haworth SE, Payne D, McKayIJ, Clarke FM, Hughes FJ, Linden GJ. J Clin Periodontol. 2007 Feb; 34(2):111-7.
(77) Rangsini M and Sathit P. Periodontology 2000 Feb. 2007-vol. 43 Issue 1 41-55.
(78) Emingil G, Berdeli A, Baylas H, Saygan BH, Gurkan A, Kose T, Berdeli A. J Periodontol. 2007 Oct;78(10):1968-77.

(79) Zhu G, Li C, Cao Z, Corbet EF, Jin L. Quintessence Int. 2008 Mar; 39(3):217-26.
(80) Attstrom R. J Periodontal Res Suppl 1971: 8: 1–15.
(81) Raeste AM, Tapanila T, Tupakka R. J Periodontal Res 1977: 12: 444–449.
(82) Weinberg A, Krisanaprakornkit S, Dale BA. Crit Rev Oral Biol Med 1998: 9:399–414.
(83) Ioannidis JP, Cappelleri JC, Lau J. JAMA 1998: 279: 1089–1093.
(84) Thomas DC, Witte JS. Cancer Epidemiol Biomarkers Prev 2002: 11:505–512.
(85) Stephens JC, Schneider JA, Tanguay DA, et al. Science 2001: 293: 489–493.
(86) Page RC. J Periodontal Res 1999; 34:331-9.
(87) Michalowicz BS, Aeppil D, Virag JG, et al. Genes Immun 2000; 1:293-4. Michalowicz BS, Diehl SR, Gunsolley JC, et al. J Periodontol 2000: 71: 1699–1707.

出席國際學術會議心得報告

計畫編號	NSC 98-2314-B-040-020
計畫名稱	台灣漢族華人之牙周炎與cytokines, MMPs/TIMPs, CD14/ TLRs 基因多型性的關係之探討 Evaluation of Cytokines (IL-2,-8,-16,-17,-TGF- β 1), MMPs/TIMPs, CD14/TLRs Gene Polymorphisms and Periodontitis in a Taiwan Han-Chinese Population.
出國人員姓名 服務機關及職稱	蔡吉政 高雄醫學大學口腔醫學院　教授
會議時間地點	July 14-17, 2010 Centre Convensions International Bacelona (CCIB) Bacelona, Spain
會議名稱	(中文)2009 年國際牙醫學研究學會年會 (英文)2009 IADR General Session
發表論文題目	1833 Response of Osteoblast-like Cells to LPS and/or Mechanical Tensile Stress 2745 Halitosis of Patients at a Neurosurgery Intensive Care Unit 3402 IL-4 gene polymorphism in individuals with periodontitis in Taiwanese 3403 IL-13 Gene Polymorphism in Individuals with Periodontitis in Taiwanese

一、參加會議經過：

本次國際牙醫學會(IADR)2010 年會在西班牙 Bacelona 舉行，正式會期為 7 月 14 日至 7 月 21 日。大會主題共 25 項，論文(口頭報告與壁報貼示)計 4900 多篇。此次我們一齊出席的人士包括台大、中山、中國與高醫牙醫學系(所)的教師、主治醫師、住院醫師及部分家屬，共計 35 人。

二、與會心得：

在行程上，除參加會議外我們也順便參觀了 Bacelona 與 Madrid 的歷史文物建築，如大教堂、畢加索美術館、音樂廳、米羅美術館、善拉多美術館等。

在會議期間，我還是參閱以個人主題相關的論文發表，例如 molecular mediators of alveolar bone modeling and orthodontic tooth movement, oral malodor: etiology and treatment; current baric and clinical research and technology transfer, periodontal research-therapy-outcome of treatment of aggressive periodontitis, microbiology/immunology-Porphylomonas gingivalis/Aggregatibacter actinomycetemcomitans, oncology (association of COL6A2 polymorphisms with oral squamous cell carcinoma), periodontal research-diagnosis/epidemiology-bacterial, blood, saliva and GCF-derived biomarkers, periodontal research (therapy-periodontal wound healing and regeneration, in vitro studies), periodontal research (therapy-the impart of periodontal therapy on systemic diseases), periodontal research (pathogenesis-genetics and gene expression), periodontal research (pathogenesis-host/bacterial interactions)等等。令我個人感到驕傲的是多年前研究發現的 A.a.菌 JP_2 成為國際流用的研究菌種，而牙周病的基因多重型表現研究也有不少論文發表(包括對岸中國大陸)。

感謝國科會歷年來的支持，本人有機會參加 IADR 的年度學術研討會，獲益良多，早年

主在學習作實驗的技術，中年則想發現新的研究主題，晚年則感慨以前認識的學者專家們逐漸凋老，當然自己也更加成熟，可以與專家們互相討論研究心得。因此建議國科會能多鼓勵年輕人出國參加國際會議，以增廣見識。

三、所發表論文：

1833 Response of Osteoblast-like Cells to LPS and/or Mechanical Tensile Stress

Thursday, July 15, 2010: 4:45 p.m. - 6 p.m.

Location: Exhibit Hall (CCIB)

K.-Y. HO, Kaohsiung Medical University Hospital, Department of Dentistry; Kaohsiung Medical University,College of Dental medicine, Kaohsiung, Taiwan, Y.-P. HO, Kaohsiung Medical University Hospital, Department of Dentistry; Kaohsiung Medical University, College of Dental Medicine, Kaohsiung, Taiwan, C.-C. TSAI, Chung Shan Medical University & College of Oral Medicine, Taichung, Taiwan, and Y.-T. HSU, Kaohsiung Medical University, Kaohsiung, Taiwan

Objectives: Receptor activator of nuclear factor-kappa B (NF-kappa B) ligand (RANKL) and osteoprotegerin (OPG) expression on osteoblasts are essential to the formation and resorption of bone tissue. Although LPS (lipopolysaccharide) is an important local factor in bone metabolism, the effects of its combination with mechanical stress on osteoblasts have not yet been well established. We examined the effects of LPS and tensile stress on RANKL and OPG expression in an osteoblast-like cell line, MC3T3-E1 cells. Methods: MC3T3-E1 cells were treated with E. coli LPS for 24 hours and with two types of cyclic tensile strain (2% elongation with 60 cycles/min and 18% elongation with 6 cycles/min generated by a Flexercell Strain Unitt®). RANKL and OPG expressions were examined by real-time polymerase chain reaction (RT-PCR). Results: The addition of LPS induced the highest change of RANKL expression and was significantly higher than the LPS combined with tensile stress treated cells ($p < 0.05$). Cycle tensile strain increased the ratio of OPG/RANKL expression, but was suppressed by LPS. Furthermore, the result of linear regression showed that COX-2 expression was significantly related to RANKL synthesis induced by LPS. Conclusion: These results demonstrated that LPS induced RANKL expression, and cyclic tensile strain may inhibit osteoclastogenesis through the modulation of RANKL and OPG expression in osteoblasts.

2745 Halitosis of Patients at a Neurosurgery Intensive Care Unit

Friday, July 16, 2010: 3 p.m. - 4:15 p.m.

Location: Exhibit Hall (CCIB)

Y.-H. CHOU1, K.-F. HU1, F.Y. WU2, and C.-C. TSAI3, 1Kaohsiung Medical University Hospital. Department of Periodontics, Kaohsiung City, Taiwan, 2Kaohsiung Medical University, Kaohsiung City, Taiwan, 3Chung Shan Medical University & College of Oral Medicine, Taichung, Taiwan

Objectives: Volatile sulfur compounds may be the main source of oral malodor. The aim of this study was to clarify the relationship between the levels of volatile sulphur compounds (VSCs) and halitosis and to evaluate the improvement of several halitosis-related outcomes by triple oral hygiene practice (mechanical tongue cleaning, tooth brushing and chlorhexidine mouthwash). Materials and Methods: Thirty-four patients with oral endotracheal tube at a neurosurgery intensive care unit with heavy tongue coating were assessed for oral malodor. Oral malodor was evaluated by

measuring the levels of volatile sulfur compounds using Oral ChromaTM and the organoleptic test score. Twenty-six participants were randomly selected for subsequent experiments: triple oral hygiene practice consisting of mechanical tongue cleaning, tooth brushing and chlorhexidine (Scody®) mouthwashing. Results: Significant correlations were observed between the organoleptic test score and hydrogen sulfide (H2S), methyl mercapton (CH3SH), tongue coating score and volatile sulfur compounds (VSCs). VSCs were also significantly correlated with tongue coating score. Daily practice of the triple oral hygiene could reduce over one half of halitosis than baseline. Conclusions: Volatile sulfur compounds, with H2S and CH3SH as the main components in mouth air are the prominent elements of malodor. Daily oral hygiene practice (mechanical tongue cleaning, tooth brushing and chlorhexidine mouthwash) could reduce oral malodor and VSCs for the patients with oral endotracheal tube in the neurosurgery intensive care unit.

3402 IL-4 gene polymorphism in individuals with periodontitis in Taiwanese

Friday, July 16, 2010: 4:45 p.m. - 6 p.m.

Location: Exhibit Hall (CCIB)

K.-F. HU1, C.-C. TSAI2, Y.-H. CHOU1, K.-Y. HO3, Y.-P. HO4, and Y.-C. LIN5, 1Kaohsiung Medical University Hospital. Department of Periodontics, Kaohsiung City, Taiwan, 2Chung Shan Medical University & College of Oral Medicine, Taichung, Taiwan, 3Kaohsiung Medical University Hospital & University, Kaohsiung, Taiwan, 4Kaohsiung Medical University & University, Kaohsiung, Taiwan, 5Kaohsiung Medical University, Kaohsiung, Taiwan

Objective: A polymorphism at position -590 (C->T) of the IL-4 gene promoter has been identified, and was shown to influence the susceptibility to systemic disease. In this study the relationship between the -590 (C->T) polymorphism and the susceptibility to periodontitis was investigated. Material and Methods: Forty-one patients with generalized aggressive periodontitis (AgP), 100 patients with chronic periodontitis (CP) and 100 matched healthy controls (C) participated in the study. Blood samples were collected and DNA isolated. The PCR-RFLP technique was used to investigate the polymorphism in the promoter region. Genotype and allele frequencies among study groups were compared using nominal logistic regression with $p < 0.05$ considered significant. Pearson's x2 test was used for analysis of Hardy-Weinberg equilibrium. Results: No significant differences in the IL-4 -590 C->T allele (p=0.9462) and genotype (adjusted OR=1.24,95%CI=0.59-2.68)frequencies were found between C and CP, however, nonsmokers(vs. smokers) with C/C genotype as compared with C/T+T/T could be less susceptible to CP (OR= 2.87 vs. 3.31). There was a higher frequency of the -590 CT genotype in patients with AgP compared with CC (OR=2.36, 95%=0.054 –0.817, p=0.0296). Conclusion: This study demonstrated no association between the IL-4 -590 C->T polymorphisms and the susceptibility to CP. However, the -590 C->T polymorphism was associated with the susceptibility to AgP. (Grant supported by NSC, Taiwan)

3403 IL-13 Gene Polymorphism in Individuals with Periodontitis in Taiwanese

Friday, July 16, 2010: 4:45 p.m. - 6 p.m.

Location: Exhibit Hall (CCIB)

Y.-M. WU1, H.-L. CHUANG2, Y.-P. HO1, K.-Y. HO1, and C.-C. TSAI3, 1Kaohsiung Medical University Hospital & University, Kaohsiung, Taiwan, 2Division of Periodontics, Department of Dentistry, Antai Tian-Sheng Memorial Hospital, Pingtung County, Taiwan, 3Chung Shan Medical University & College of Oral Medicine, Taichung, Taiwan

Objective: The IL-13 –1112 C/T polymorphisms have been analyzed in patients with aggressive periodontitis in a North European population. The present study was to investigate the association of the polymorphisms in the IL-13 gene and susceptibility to periodontitis in Taiwanese. Material and Methods: The genotyping of IL-13 -1112 C/T polymorphism in 60 aggressive periodontitis (AgP), 204 chronic periodontitis (CP) patients, and 95 healthy controls (H) was carried out by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) technique. Genotypes and allele frequencies among study groups were compared with Fisher's exact test ($p<0.05$). Pearson's ÷2 test was used for analysis of the Hardy-Weinberg equilibrium. Results: The distributions of CC genotypes and C alleles between AgP and H were significantly different ($p = 0.034$ and 0.046). After adjustment for age, gender, betel nut chewing, and smoking status by logistic regression analysis, the odds ratio (OR) was 6.45(95% confidence interval (CI) = 1.99–23.72, $p = 0.003$) for AgP. However, the CC genotype was only significantly associated with the risk of aggressive periodontitis in the nonsmoking group (OR = 4.48, 95% CI = 1.31 – 16.93, $p = 0.020$). Conclusion: The CC genotype or C allele appears to increase the risk of developing AgP in Taiwanese.

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：蔡吉政	計畫編號：98-2314-B-040-020-
計畫名稱：台灣漢族華人之牙周炎與 cytokines, MMPs/TIMPs,CD14/ TLRs 基因多型性的關係之探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	7	8	90%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	15	18	90%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	1	1	100%		

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

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

近來的研究顯示基因是為牙周炎的嚴重性(severity)及進行性(progression)的重要決定因子。 一些於炎性及免疫反應方面被懷疑於牙周炎的發生擔當某種角色。基因因子甚至被認為與 50%的牙周炎感受性(susceptibility)有關。應用基因資訊及技術於牙周炎的診斷與治療，似乎已成為重要的觀念。不只患者的種族的(racial 與 ethnic)背景資料應加考慮，研究更需要有足夠且慎選牙周炎與基因多型性的相關性之病例及控制(對照)組，方能使研究更加清楚。如此，牙周炎之感受性可由某些相關地普遍的高危險性基因多型性(relatively common high-risk polymorphisms)來解釋是有可能的。因此，本研究的結果於牙周炎的發生防制策略(therapeutic intervention)上及個別化的治療模式(individualized approach)具有相當的參考價值。