中文摘要

補體系統屬先天性免疫(innate immunity)，由一群未被激活的血漿蛋白組成，主要參與由免疫複合體媒介的免疫反應。補體系統的活化有三條路徑：古典路徑(classical pathway)由抗原與抗體先行結合所引起；甘露糖結合凝集素路徑(mannose-binding lectin pathway)由補體成分與微生物表面特定糖分子結合引起；替代路徑(alternative pathway)為補體成分直接與微生物細胞壁成分結合所致；補體系統的生理作用如下：（一）清除免疫複合體（二）移除凋亡細胞（三）防禦病原菌感染（四）引起局部發炎反應（五）調節適應性免疫系統(adaptive immunity)。

當補體反應不足或補體成分缺乏時，會使得凋亡細胞碎片無法順利及時清除，造成自體抗原過度暴露進而導致自體免疫疾病的發生，如果缺乏反應路徑上較早期的補體成分則疾病發生機率會上升，病況嚴重程度也會加劇。依據先前紅斑性狼瘡致病機轉的研究，針對長期性發炎症狀，大量自體抗體形成、免疫複合體的沉積現象，顯著相關的染色體區域及老鼠遺傳模式，挑選出十一個候選基因，包括IL1B、IL1RN、LTA、TNF-α、LTB、C2、C4、PDCD1、FCGR2A、FCGR3A及FASLG。

第二補體成分歸屬於古典路徑，經由酵素激活後能與第四補體成分(C4)組成第三補體成分(C3)的轉化酶(convertase)主導後續的連鎖反應。另有研究顯示當第二補體成分蛋白缺乏時，病患會表現出全身性紅斑性狼瘡的症狀，以上結果使我們確信第二補體成分對於紅斑性狼瘡臨床病症具有其相關的重要性。

本研究探討的主題：第二補體成分基因多型性與紅斑性狼瘡臨床表徵的關聯性分析；第二補體成分基因的單一核苷酸多型性挑選主要依據美國國家生物技術與資訊中心(NCBI)的資料庫，在啟動子區域及可轉譯出蛋白質區域作搜尋，著重於轉錄因子結合或氨基酸序列改變的候選單一核苷酸多型性，選擇三個內含子區域的單一核
苷酸多型性(intronic SNPs：rs2844455，rs3130681，rs2734335)及三个外显子区域的
单一苷酸多型性(exonic SNPs：rs1042663，rs1042664，rs4151648)：利用连锁不平衡
检验并挑选小等位基因频率在病患组具有意义(minor allele frequency > 5%)，最
后决定了位于内含子区域(GC-rich region)的单一苷酸多型性(SNP rs2844455)依基
因型与病征、基因表现情形作后续分析。

首先完成病患资料建檔，包括临床症状、肾脏病征、全血檢查、补体成分浓度
及活性分析、自体抗體種類検測，接著為基因型之鑑定及基因表现之定量。结果显示
显示与正常人相互比較下，有较高频率的AA基因型会出现在病患组(22.1% vs. 9.5%，
P<0.05)；A对偶基因在病患组的频率则更高(40.5% vs. 28.4%，P<0.05)。

针对红斑性狼疮病人，A对偶基因与毛髮脱落、皮膚光敏感性、抗心磷脂質抗體
之存在均有显著相关；反之，G对偶基因与这些病症则为显著不相关。基因表现的
定量分析(qPCR)，可发现第二補體成分基因在病患組的相對表现情形(relative mRNA
level)明显高於正常人；再依個別基因型與相對表现量進一步分析可以發現，若與GG
基因型病人(中位数：35.76，四分位距：19.33-49.71)相比，AA基因型病人(中位数：
18.86，四分位距：11.36-22.43，P=0.002)或AG基因型病人(中位数：18.38，四分位距：
13.13-28.82，P=0.004)其第二補體基因轉錄情形皆为明显降低；在RNA转錄異
構体(transcript isoforms)的定性分析(RT-PCR)中，能看到所检测的異構體種類在病人
與正常人的表現不論在型式或比例上都有差異；另外分析了第二補體成分的血清浓
度，结果顯示病人的血清濃度明显低於正常人，依基因型分組則發現血清中補體濃
度與基因型沒有显著相關。

根据以上的结果，我們知道位於第二補體成分基因的单一苷酸多型性(SNP
rs2844455)是一个在基因轉录表现上具調控功能的位点，不僅影响基因整體的表现量
與異構體種類，也明顯與臨床病徵相關如毛髮脫落、皮膚光敏感症狀及抗心磷脂質抗體的產生。

本研究以台灣華人族群遺傳背景為基礎探討第二補體成分基因多型性與全身性紅斑性狼瘡病徵之間的關聯性，我們將 A 對偶基因歸納為引發紅斑性狼瘡的危險因子，而 G 對偶基因較常出現於正常人及沒有明顯症狀的病人，相對於 A 對偶基因的高風險性，G 對偶基因應該是一個能對抗疾病的保護因子。

關鍵字：抗心磷脂質抗體 (anticardiolipin antibody)

第二補體成分 (complement component 2)

光敏感性 (photosensitivity)

單一核苷酸多型性 (single-nucleotide polymorphism)

全身性紅斑性狼瘡 (systemic lupus erythematosus)
Abstract

The complement system comprises a large number of plasma proteins participating in pathogen defense, apoptotic cell removal, immune-complex clearance and inflammatory response of innate immunity; it also has regulatory potencies affecting B cell maturation and altering T cell function.

Impaired complement function or complement deficiency is believed to induce defective clearance of apoptotic cells to drive the autoimmunity in systemic lupus erythematosus (SLE). Complement component 2 (C2), an early member of the classical complement pathway, is correlated with systemic manifestations of SLE. We hypothesize that C2 polymorphism may confer genetic susceptibility to complement dysfunction in SLE so we aim to investigate the clinical and serological associations of C2 variants in Taiwanese patients with SLE.

The single-nucleotide polymorphisms (SNPs) were selected according to the NCBI dbSNP database and analyzed using the Genomatix software suite; we also performed computational calculations to estimate the linkage disequilibrium (LD) relationships for effective selection including three intronic SNPs (rs2844455, rs3130681 and rs2734335) and three exonic SNPs (rs1042663, rs1042664 and rs4151648). These SNP allele frequencies were tested in normal control subjects; upon comparison with the allele frequency in SLE patients, if the minor allele frequency (MAF) exceeded 5%, the SNPs were chosen for further study.

The rs2844455 located in the GC-rich region of C2 gene was genotyped by direct sequencing in 95 SLE patients and 95 matched normal control subjects. The gene expression profiles were generated by quantitative real-time PCR (qPCR) and reverse transcription PCR (RT-PCR); the serum C2 levels were determined using commercial ELISA tests.
Our results showed that SLE patients had significantly higher frequencies of the AA genotype (22.1% vs. 9.5%, \( P<0.05 \)) and the A allele (40.5% vs. 28.4%, \( P<0.05 \)) when compared with normal control subjects. The A allele was strongly associated with the occurrence of hair loss, photosensitivity and anticardiolipin antibodies; whereas the G allele was associated with lower frequencies of these clinical presentations.

The relative expression levels of C2 mRNA were significantly lower in patients with the AA genotype [median: 18.86, interquartile range (IQR): 11.36-22.43, \( P=0.002 \)] and the AG genotype (18.38, IQR: 13.13-28.82, \( P=0.004 \)) than in those with the GG genotype (35.76, IQR: 19.33-49.71). Differential expression patterns of C2 gene were demonstrated while the gel showed that the banding patterns and their intensity were obviously related to the genetic variants in patients; however, serum protein assays showed no significant connection existed between rs2844455 genotypes and serum C2 level in SLE patients \( (n=10 \) for each genotype; ANOVA \( F=0.071, P>0.05 \)).

As expected, we confirmed the A allele as a risk factor for SLE development in a Taiwanese population, in contrast, the G allele might be a protective factor against the pathogenic autoantibody formation and cutaneous manifestations in SLE patients.

Key words: anticardiolipin antibodies; complement component 2; clinical features; single-nucleotide polymorphism; systemic lupus erythematosus