行政院國家科學委員會專題研究計畫 成果報告

CD14, STAT6 及人類 E-cadherin 基因多形性在兒童氣喘之易感受性角色

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行政院國家科學委員會補助專題研究計畫成果報告

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中文摘要：

第二型輔助 T 淋巴球 (Th2 細胞) 和其他細胞激素在氣喘的致病過程中扮演著重要的角色。CD14 為細菌細胞壁成分的多功能接受器，並可能調節兒童時期早期 Th1-Th2 的比例。信號轉導和轉錄啟動因子(signal transducer and activator transcription [Stat]) 6 則為影響 Th2 細胞分化的重要轉錄因子。香菸暴露對於兒童氣喘發生的致病機轉，至今仍不清楚。並且部分兒童暴露於室內二手菸狀態下，並未具有氣喘之表現；因此易感受性因子在氣喘的成因上的角色，值得進一步的觀察。香菸已被顯示可減少上皮細胞的黏附以及增加分離，而細胞黏附分子上皮細胞黏附蛋白 (E-cadherin) 對於正常結構以及上皮組織的功能之形成與維持，扮演一個必要的角色，因此人類上皮細胞黏附蛋白基因 (CDH1) 可能相關於氣喘的發生。我們以一個醫院為基礎的病例對照研究檢視 CD14 -159、CD14 -260 以及 STAT6 G2964A、CDH1-160 基因多形性與兒童氣喘相關的假說，總計 357 名兒童參與本研究。研究對象的個人特徵資料，是經由面對面的問卷訪視所收集；兒童的室內二手菸暴露量是以每天平均暴露的香菸支數計算，亦即父母親於兒童在家時，抽菸支數的總和。四種台灣常見過敏原也被執行皮膚測試加以判定；基因型則是以聚合酶鏈鎖反應 (polymerase chain reaction [PCR]) 判定。我們的研究結果顯示，兒童氣喘發生顯著危險是相關於個體過敏原測試陽性以及 CD14 -159、STAT6 G2964A、CDH1 -160 基因型。過敏原反應呈陽性並且攜帶 CD14 -159 TT/CT 基因型 (RR_m = 6.3; 95% CI = 2.0-20.4)、STAT6 G2964A AA/GA 基因型 (RR_m = 8.9; 95% CI = 2.8-27.9)、以及 CDH1-160 AA/CA 基因型 (RR_m = 19.9; 95% CI = 4.6-87.0) 的兒童分別相較於過敏原反應呈陰性且攜帶 CD14 -159 CC 基因型、STAT6 GG 基因型、以及 CDH1-160 CC 基因型的兒童有較高之氣喘發病危險性。進一步地，評估室內二手菸暴露與易感受性基因型對於氣喘發生之危險。相較於過敏原測試陽性攜帶 CDH1-160 CC 基因型且每天暴露香菸支數 0-5 支的兒童，攜帶 CDH1-160 AA/CA 基因型且暴露香菸支數 0-5 支的兒童、與每天暴露香菸支數大於 5 支的兒童，分別呈現 1.9 倍 (95% C.I. = 0.9-4.2) 與 3.1 倍 (95% C.I. = 1.1-8.5) 的氣喘發生危險。我們的結果建議著表示，CD14、STAT6 和 CDH1 易感受基因型可能調控過敏與二手菸暴露之兒童的氣喘發病歷程。

關鍵詞：CD14 基因，STAT6 基因，CDH1 基因，兒童氣喘，過敏原

Abstract：

Type 2 T helper lymphocytes (Th2 cells) and their cytokine products are important in the pathogenesis of asthma. CD14 functions as a multifunctional receptor for bacterial cell wall components and is likely to play a role in the modulation of Th1-Th2 response during early childhood. Signal transducer and activator of transcription factor (Stat) 6 is a transcription factor essential for Th2 cell differentiation. The way passive smoking affects childhood asthma is still a mystery. A part of children at the indoor tobacco exposure were not have the asthma expression; therefore, the sensitive factor of a role at asthma will be observed. And cigarette smoke has been shown to decrease epithelial-cell adherence and to increase detachment, the cell adhesion molecule E-cadherin plays an essential role in the formation and maintenance of normal architecture and function of epithelial tissues; therefore, human CDH1 gene might be related to the cause of asthma. In the present study, the hypothesis that polymorphisms in the CD14 -159, CD14 -260, STAT6 G2964A and CDH1-160 genes are associated with childhood asthma were examined under a hospital-based case-control study. A total of 357 children were recruited into our study. Independent interviews with parents by our well-trained research assistants with a semi-structured clinical questionnaire during the study period. The smoking history of subject's family members will be included the number of cigarettes smoked daily, and the duration the child was exposed to environmental tobacco smoke. Allergen test was performed by an intracutaneous skin test with Taiwan common aeroallergens. The genotypes of CD14 -159, CD14 -260, STAT6 G2964A and CDH1-160 were identified by polymerase chain reaction (PCR). Our results revealed that allergen test-positive children with the CD14 -159 TT/CT genotype (RR_m = 6.3; 95% CI = 2.0-20.4), the STAT6 G2964A AA/GA genotype (RR_m = 8.9; 95% CI = 2.8-27.9), and the CDH1 -160 AA/CA genotype (RR_m = 19.9; 95% CI = 4.6-87.0) had a higher risk of asthma development than did allergen test-negative children with the CD14 -159 CC genotype, the STAT6 GG genotype, and CDH1-160 CC genotype, respectively. Further, assess the indoor tobacco exposure and sensitive genotype about the risk of asthma development. Children with the CDH1-160 AA/CA genotype and exposure 0-5 cigarettes smoked daily (RR_m = 1.9; 95% CI = 0.9-4.2), and exposure surpassed in 5 cigarettes smoked daily (RR_m = 3.1; 95% CI = 1.1-8.5) had a higher risk of asthma development than allergen test-positive children with the CDH1-160 CC genotype. These results suggested that susceptible CD14, STAT6 and CDH1 genotypes may modulate the asthma development in smoking-exposed children.

KEY WORDS: CD14 gene; STAT6 gene; CDH1 gene; childhood asthma; Allergen.

前言：

氣喘是種與呼吸道阻塞相關的支氣管失調，明顯地伴隨著反覆性突發的呼吸困難 (dyspnea)，以及因為支氣管痙攣收縮所導致的哮喘 (wheeze) [1]。重要的是，台灣兒童在 2002 年的氣喘盛行率已經增加到 12.2% [2]；而兒童是較成人容易產生氣喘，這根源於基因及環境因素的共同作用。傾向發展成氣喘的個體，是因為基因與環境因子決定其易感受性。有許多種誘因會導致氣喘發作的開始或惡化，包括暴露於過敏原 [3, 4]、病毒性呼吸道感染 [5, 6]、以及呼吸道刺激物，例如香菸 [7, 8]、與特定的環境污染物 [9, 10]。

兩種主要的 $CD4^+$ T 輔助細胞亞型為 Th1 和 Th2，各自分泌不同的細胞激素，並且相互調節不同種類的免疫反應 [11, 12]。Th1 細胞分泌細胞間白素 (interleukin [IL])-2、干擾素 (interferon [IFN])-γ、以及腫瘤壞死因子 (tumor necrosis factor [TNF])-β；而 Th2 細胞製造 IL-4、IL-5、IL-6、IL-10 及 IL-13。如果免疫反應趨向以 Th2 的表現來對抗環境中無害的抗原，則可能發生如氣喘的過敏性疾病。Th2 細胞激素控制具有發炎性氣喘反應的主要成分，包括免疫球蛋白 E (immunoglobulin [Ig] E) 同質型轉換 (isotype switching)、黏液的製造，以及嗜伊紅性白血球 (eosinophils) 的聚集與活化。

研究已經建議，幼兒的細菌感染可能保護其對抗過敏之發展 [13]；並且也被假設細菌訊息可藉由抑制可能引起異位性表現的 Th2 型態免疫反應 [14]，以扮演一個對於 Th1 型態免疫反應之成熟的功能性角色。微生物產物如脂多醣體 (lipopolysaccharide [LPS]) 可提供 Th1-成熟的活化訊息，而對於 LPS 與其他細菌細胞壁成分具有的重要高度親和力的接受器為 CD14 [15]，其為分子量 55k-D 之醣蛋白，大都表現在單核球及巨噬細胞的表面 [16]；LPS 與 CD14 的鍵結也需要脂多醣體結合蛋白 (lipopolysaccharide-binding protein) 的協助。

細胞激素 IL-12 以及 IL-4，分別促使原本的輔助 T 細胞分化成 Th1 與 Th2 細胞 [17, 18]。此外，原先的研究顯示就像是大部分其他的細胞激素，IL-4 和 IL-12 活化 Janus Kinase-訊息傳遞及活化轉錄因子 (signal transducer and activator transcription factor) (Jak-Stat) 的訊息路徑 [19-21]；在此訊息路徑中，細胞激素與其接受器的結合可導致接受器相關激酶之 JAK 家族成員的活化。這些激酶隨後透過酪氨酸 (tyrosine) 的磷酸化 (phosphorylation) 而活化原先存在於細胞質因子 Stats，酪氨酸的磷酸化允許了 Stat 蛋白質成二聚體化並且轉行至細胞核內，在此它們藉由與特定 DNA 成分結合來調節基因表現的改變。

IL-4 刺激 Jak1 和 Jak3 來活化 Stat6 [21]，相對地，IL-12 導致 Jak2 與 Tyk2 的活化，以及隨後的 Stat4 磷酸化；部分的研究團隊也已經探討在氣喘者中其 Stat6 的表達與活化。在氣喘和過敏性的病患之周邊血液淋巴球，相對於健康對照，其 Stat6 活性程度並沒有表現出顯著的不同 [22]，但是這些病患其呼吸道確實有較高的 Stat6 表達細胞之密度 [23]；有趣的是，在異位性的氣喘者中，Stat6 表達細胞的密度是顯著地高於非異位性的氣喘者。一項針對為嚴重氣喘的對象所進行的最近研究也證實，這些病患呈現顯著提升的呼吸道 Stat6 濃度 [24]。

人類 CD14 是位於染色體 5q31.1 [25]，而於 CD14 基因啟動者之多形性是靠近 Sp 辨識序列因子，其對於 CD14 表現是相當重要 [26]。在 CD14 基因啟動者-159 上游位置上產生 C→T 的變異，可能與過敏有關 [27]。在高加索小孩中，攜帶同型 TT 基因型者相較於 CC 基因型者具有較高的 sCD14 的血清濃度 [27]；此外，在皮膚過敏原測試陽性的小孩中，相較於攜帶 CC 與 CT 基因型者，攜帶同型 TT 基因型者有較低的血清總 IgE 與較少數目的陽性皮膚穿測結果 [27]。此外，Hubacek 等人 [28] 指出無急性疾病的健康者其-260 上游位置上 C→T 的變異可影響 CD14 基因表現程度，因此我們假設 CD14 的增加密度，可能由基因所決定。因此，CD14 基因的變異可能解釋兒童氣喘的累積。進一步地，人類 STAT6 基因是位於染色體 12q13.3-q14.1 [29]。雖然，Duetsch

等人 [30] 於 STAT6 辨識到 13 個單一基因多形性 (single-nucleotide polymorphisms [SNPs])，並且檢驗其與 108 對高加索小孩之氣喘相關，並未發現單一基因多形性與在 exon 1 上之 GT 重複序列顯示與氣喘關連，研究者則發現到於 STAT6 基因 3'未轉譯區域之 G2964A 基因型則與氣喘及輕微的異位性 [31] 及堅果過敏 [32] 相關。有趣的是，STAT6 G2964A 的對偶基因頻率在日本人中 (G 對偶基因 76%，A 對偶基因 24%) 與在英國族群中 (G 對偶基因 33%，A 對偶基因 67%) 是相當的不同 [31]，這可能建議著在族群中的基因異質性 (genetic heterogeneity) 表現。

人們大多數的時間是在室內；因此，近年來對於室內空氣污染為氣喘之一項危險因子的考量也逐漸提升。室內污染物是眾多的；通常的來源，像是香菸、塵蹣、潮濕、以及寵物。少數的研究已經建議易感受性的個體於室內暴露到環境二手菸 (environmental tobacco smoke [ETS])，與其氣喘症狀相關 [33, 34]；尤其是，環境二手菸暴露被發現與孩童氣喘之發展相關。然而，二手菸影響孩童氣喘的途徑仍然是個謎題；因此，對於環境二手菸誘發孩童氣喘之機制的瞭解是必需的。每口氣態的香菸中包含 10^{20} 個氧化分子 [35]，並且能夠導致呼吸道上皮的發炎 [36]。因此，氧化/抗氧化的不平衡對於暴露到香菸的個體其上皮細胞的通透性之增加上可能具有一定的角色 [40]；並且香菸也被顯示可減少上皮細胞的黏附以及增加分離 [41]。

氣喘是種呼吸道發炎疾病，而上皮細胞間葉單位顯然在氣喘的機制上是重要的 [42]；氣喘病人的支氣管上皮細胞相較於對照組對象的細胞，其發育較差也較脆弱 [43]。此外，依鈣性的細胞黏附分子上皮細胞黏附蛋白 (E-cadherin) 對於正常結構以及上皮組織的功能之形成與維持，扮演一個必要的角色 [44, 45]；而上皮細胞黏附蛋白減弱的表現，也被視為是項參與在細胞與細胞黏附系統之功用喪失的分子現象，因而加速疾病的發展。人類上皮細胞黏附蛋白基因 (CDH1) 位於染色體 16q22.1 的位置 [46]。Becker 等人 [47] 首先提出 E-cadherin 突變促使胃癌的組織病理學上之表現，可觀察到同型細胞與細胞間的交互作用減少；可能的機制被認為是 CDH1 突變、或是對偶基因之遺失，可能會影響上皮細胞黏附蛋白的功能。然而，CDH1 之分子角色在氣喘的發展仍然不清楚。更進一步地，Li 等人 [48] 辨識到 CDH1 啟動子在轉譯開始之-160 位置上有 C→A 的單一核苷酸多形性現象；在 *in vitro* 的研究建議這種多形性與前列腺細胞癌 (prostatic carcinoma) 細胞株的基因轉譯之減少具有相關 [48]。除此之外，CDH1 之 DNA 序列變異與胃癌之間的一項相關研究也在台灣人中已被證實 [49]，並且結果顯示病患相較於對照組，在於 CDH1 啟動子-160 位置上之 C→A 多形性，具有較低頻率的 AA 基因型。雖然這種變異已被推測為癌症的可能基因標記，但是具有易感受性 CDH1 的個體與氣喘增加危險的相關性是不清楚的。

因此，我們執行一個以醫院為基礎之病例對照研究來探討是否 CD14、STAT6 與 CDH1 多形性變異相關於兒童氣喘之危險。此外，二手菸暴露與 CD14、STAT6 與 CDH1 多形性變異在氣喘發展上具有交互作用的存在也將被探討。

材料與方法：

病例確認與對照選擇

本研究經由中山醫學大學倫理委員會的認可後執行。年齡介於四至十二歲的研究對象，是從位於中台灣的中山醫學大學附設醫院中選取，此醫院對於來自所有社會經濟階級的病患皆具有可近性。病例是從中山醫學大學附設醫院小兒氣喘門診選取，經由小兒科醫生確認，並且是符合美國胸腔協會 (American Thoracic Society) 所制定的可逆性呼吸道疾病標準 (如支氣管氣喘) [50]。相同於病例，對照是從相同醫院所選取；兒童先前不具氣喘的診斷，醫師證實沒有異位性疾病史、沒有如哮喘和咳嗽等氣喘症狀者，在研究中被定義為對照。本研究並以 1：2 之病例與對照比例進行配對；合計，119 名病例和 238 名對照組被納入本研究分析。健康對照是個別地與病例之年

齡 (±5 歲)、性別進行配對。所有對象必須能夠提供臨床病歷以及同意書。

問卷訪視

研究對象的個人特徵資料，在獲取所有參與者的家長同意書後，經由面對面的問卷訪視所收集。問卷所涵蓋的問題包括人口學特質、生活型態如與兒童共同生活之家戶成員抽菸狀態、室內其它污染狀態如燒香、潮濕度、是否飼養寵物、以及一等親氣喘家族史。研究對象的家戶成員抽菸狀態包括每天抽菸支數及抽菸年數；兒童的室內二手菸暴露量是以每天平均暴露的香菸支數計算，亦即父母親於兒童在家時，抽菸支數的總和。住家的潮濕程度在最近一年內符合以下條件之一者即定義為潮濕：可以看見家戶內部表面具有黴菌滋生、家戶內積水、或漏水。氣喘家族病史則是以受測者之一等親家族具有氣喘來加以定義。

皮膚過敏原測試

本研究針對四種台灣常見過敏原進行皮膚測試 [51]，分別是家中的灰塵 (*House Dust*)、家塵蹣 (Mite, *Housedust Dermatophagoides pteronyssinus* [*D.P.*] 與 *Housedust Dermatophagoides farinae* [D.F.])、以及美洲蟑螂 (*Cockroach, American*)。用過敏原及八爪皮膚測敏器 (Greer Laboratories，USA) 進行皮膚測試 [52]；以組織胺為陽性對照組，以甘油 (glycerin) 為陰性對照組。如紅斑直徑超過陽性對照組時，代表此人為皮膚過敏原測試陽性反應者；如紅斑直徑小於陰性對照組時，代表此人為皮膚過敏原測試陰性反應者。

基因多形性分析

所有研究對象的靜脈血液被收集在含肝素的採血管中，然後進一步地萃取出DNA。簡而言之，對於CD14 -159基因之分析所使用的方法是根據2001年由Koppelman等人 [53] 所描述之研究，來進行聚合酶鏈鎖反應 (polymerase chain reaction [PCR]) 增幅後，接續執行限制片段長度多形性 (restriction fragment length polymorphism [RFLP]) 分析，用以偵測在AvaII限制酵素辨識點之差異。用以增幅CD14 -159基因的引發子序列為5’-TGA GGA TCA TCC TTT TCC CAC AC-3’以及5’-CAG GCT TCA CAC TTG TGA ACT CTT C-3’。 0.5-μL的DNA模版被加入至包含有200 ng的引發子、1.5 mM $MgCl_2$、0.2 mM之去氧核苷三磷酸 (dNTPs)、50 mM KCl、10 mM Tris-HCl (pH = 8.3)、以及0.1%的胎牛血清白蛋白 (BSA)，最終體積以蒸餾水調成50 μl。PCR循環參數組成為變性：94ºC30秒，重鍊：57ºC30秒，以及延展：72ºC30秒。PCR產物再由AvaII進行消化；消化後的產物在2.0%的瓊膠中進行電泳後，以ethidium bromide染色進行判讀。同型CC基因型的個體表現出一段497-bp的產物片段；同型TT基因型的個體顯示出一段353-bp及一段144-bp的產物片段，而異型CT基因型的個體則有所有三段產物片段。CD14 -260基因多形性的分析是根據Zee等人 [51] 的研究來進行偵測。CD14 -260基因引發子序列為 5’-TGA GGA TCA TCC TTT TCC CAC AC-3’及5’-CAG GCT TCA CAC TTG TGA ACT CTT C-3’。相似於CD14 -159基因多形性分析所描述，除變性：94ºC30秒，重鍊：58ºC45秒，延展：72ºC1分鐘。反應產物再進行HaeⅢ限制酶消化。同型TT基因型者顯現出一段318-bp的產物片段；同型CC基因型者顯現出一段172-bp及一段146-bp的產物片段，而異型CT基因型的個體則有所有三段產物片段。

STAT6 G2964-BsaHI 基因多形性的分析，是依照 Amoli 等人 [55] 所發展的方法所修飾。用以增幅 STAT6 基因的引發子序列為 5’- GAA GTT CAG GCT CTG AGA GAC -3’以及 5’- CCA TCA CCC TCA GAG AGC -3’。PCR 循環參數組成為 95^{o}C 三分鐘的先前培養，接續 35 回合的循環，包括變性：95ºC 一分鐘，重鍊：57ºC 一分鐘，以及延展：72ºC 一分鐘；反應於最後的 72^{o}C 五分

鐘的延展後終止。PCR 產物再由 BsaHI 進行消化。同型 GG 基因型的個體表現出一段 93-bp 的產物片段；同型 AA 基因型的個體顯示出一段 74-bp 及一段 19-bp 的產物片段，而異型 GA 基因型的個體則有所有三段產物片段。對於 CDH1 基因之分析所使用的方法是根據 2002 年由 Verhage 等人 [53] 所描述之研究。用以增幅上皮細胞黏附蛋白基因的引發子序列為 5'-TCC CAG GTC TTA GTG AGC CA-3'及 5'-ACG ACT AAC CGA CAC CGG-3'。在以下的條件執行基因增幅：變性：94°C 1 分鐘，重鍊：57°C 40 秒，延展：72°C 40 秒。PCR 的反應產物再以 BstEⅡ限制酶消化；攜帶同型 AA 基因型的個體呈現出一段 190-bp 之產物片段，攜帶同型 CC 基因型的個體有 111-bp 以及 79-bp 兩段產物片段；而攜帶異型 CA 基因型的個體則有全部三段產物片段。

統計分析

對於 CD14 基因型、STAT6 基因型、CDH1 基因型、氣喘家族史、室內二手菸暴露狀態、室內燒香狀態、在家是否做紡織類的工作、過去是否在臥房內觀察到蟑螂、寵物飼養、家戶潮濕狀態、以及過敏原測試等對於兒童氣喘發生的配對之相對危險性 (matched relative risk [RR_m]) 以及相對應的 95%信賴區間 (95% confidence interval [CI]) 是使用條件式對數迴歸模式 (conditional logistic regression model) 來進行評估。此外，二手菸暴露狀態與易感受性 CD14 基因型、STAT6 基因型、CDH1 基因型也將一起以多變項條件式對數迴歸模式來決定其與氣喘發展的相關。所有的 *P* 值將是以用雙尾檢定來判定。

結果：

總計，357 名兒童 (234 名男生與 123 名女生) 參與本研究，其年齡範圍從四至十二歲 (平均 9.8 歲)。研究對象之基本特徵及 CD14、STAT6、CDH1 基因型分布頻率被呈現於表一。84.9%之研究對象的父母其教育程度達高中以上；並且父母的教育程度為大學以上 (RR_m = 4.2; 95% C.I. = 2.0-9.2, $P < 0.01$) 或高中 (RR_m = 1.6; 95% C.I. = 0.8-3.5) 之兒童相較於父母的教育程度為高中以下之兒童，分別具有較高的氣喘發生危險性。相較於對照，顯著較高比例的氣喘家族史在我們的病例中被發現 (RR_m = 3.5; 95% C.I. = 1.8-6.9, $P < 0.01$)。攜帶 CD14 -159 TT 基因型或 CT 基因型的兒童相較於攜帶 CD14 -159 CC 基因型的兒童，分別呈現 2.0 倍 (95% C.I. = 1.0-3.9, $P = 0.05$) 與 1.5 倍 (95% C.I. = 0.8-3.0) 之氣喘發生危險性。而攜帶 STAT6 G2864A AA 基因型或 GA 基因型的兒童也相較於攜帶 STAT6 GA 基因型的兒童，分別呈現 2.0 倍 (95% C.I. = 1.0-3.8, $P = 0.05$) 與 1.5 倍 (95% C.I. = 0.8-2.6) 之氣喘發生危險性。同樣地，攜帶 CDH1 AA 或 CA 基因型的兒童相較於攜帶 CDH1 CC 基因型的兒童，也分別呈現 2.4 倍 (95% C.I. = 1.1-5.5, $P = 0.03$) 及 2.6 倍 (95% C.I. = 1.5-4.6, $P < 0.01$) 之氣喘發生危險性。然而，並未有顯著相關性在 CD14 -260 基因型與我們的兒童氣喘發生危險性間被觀察到。

各種環境因子對於氣喘發生之配對後相對危險性被呈現於表二。父母親具有抽菸習慣的兒童相較父母親沒有抽菸習慣的兒童，具有較低的氣喘發生危險 (RR_m = 0.8; 95% C.I. = 0.4-1.3, P = 0.29)，但並未達到統計顯著性。同樣地，反向的相關也在家中燒香與兒童氣喘發生間被發現 (RR_m = 0.3; 95% C.I. = 0.2-0.5, $P < 0.01$)。家中有從事紡織類工作之兒童則相較家中沒有從事紡織類工作的兒童，呈現顯著較高的氣喘發生危險性 (RR_m = 2.7; 95% C.I. = 1.2-5.8, $P = 0.01$)。而兒童氣喘增加危險也是相關於過敏原測試陽性，相較於過敏原測試為陰性者 (RR_m = 6.2; 95% C.I. = 3.6-10.5, $P < 0.01$)。然而，並無顯著相關於二手菸暴露、飼養寵物、寢室是否有蟑螂及家中潮溼度與孩童氣喘發展間被發現。

兒童氣喘之相對危險性被計算以探討過敏原測試結果與易感受性基因型之合併效應，結果呈

現於表三。在調整氣喘家族史、父母親教育程度、家中燒香、家中從事紡織類工作等變項之效應後，當我們將 CD14 -159 TT 與 CT 基因型合併考慮，以過敏原測試陰性且攜帶 CD14 -159 CC 基因型為參考組 (RR_m = 1.0)，則過敏原陰性且攜帶 CD14 -159 TT/CT 基因型的兒童有 1.2 倍的氣喘發生危險 (95% C.I. = 0.4-4.3)，過敏原陽性且攜帶 CD14 -159 CC 基因型的兒童有 5.2 倍的氣喘發生危險 (95% C.I. = 1.4-20.0)，過敏原陽性且攜帶 CD14 -159 TT/CT 基因型的兒童則有 6.3 倍的氣喘發生危險 (95% C.I. = 2.0-20.4)。同樣地，當我們將 CD14 -260 TT 與 CT 基因型合併考慮，以過敏原測試陰性且攜帶 CD14 -260 CC 基因型為參考組 (RR_m = 1.0)，則過敏原陰性且攜帶 CD14 -260 TT/CT 基因型的兒童有 1.1 倍的氣喘發生危險 (95% C.I. = 0.3-3.8)，過敏原陽性且攜帶 CD14 -260 CC 基因型的兒童有 3.0 倍的氣喘發生危險 (95% C.I. = 0.8-11.4)，過敏原陽性且攜帶 CD14 -260 TT/CT 基因型的兒童則有 6.4 倍的氣喘發生危險 (95% C.I. = 2.0-20.3)。同樣以過敏原測試陰性且攜帶 STAT6 G2964A GG 基因型為參考組 (RR_m = 1.0)，則過敏原陰性且攜帶 STAT6 G2964A AA/GA 基因型的兒童有 1.6 倍的氣喘發生危險 (95% C.I. = 0.5-5.3)，過敏原陽性且攜帶 STAT6 G2964A GG 基因型的兒童有 4.1 倍的氣喘發生危險 (95% C.I. = 1.2-14.6)，過敏原陽性且攜帶 STAT6 G2964A AA/GA 基因型的兒童則有 8.9 倍的氣喘發生危險 (95% C.I. = 2.8-27.9)。當我們將 CDH1 AA 與 CA 基因型合併考慮，同樣以過敏原測試陰性且攜帶 CDH1 CC 基因型為參考組 (RR_m = 1.0)，則過敏原陰性且攜帶 CDH1 AA/CA 基因型的兒童有 3.9 倍的氣喘發生危險 (95% C.I. = 0.9-17.9)，過敏原陽性且攜帶 CDH1 CC 基因型的兒童有 8.3 倍的氣喘發生危險 (95% C.I. = 1.7-39.0, P < 0.01)，過敏原陽性且攜帶 CDH1 AA/CA 基因型的兒童則有 19.9 倍的氣喘發生危險 (95% C.I. = 4.6-87.0, P < 0.01)。

隨後，在具有不同過敏原反應的兒童中，評估室內二手菸暴露與 CD14、STAT6、CDH1 基因型對於氣喘發生之合併危險，結果如表四。在過敏原測試陽性的兒童中，以每天暴露香菸支數 0-5 支並且攜帶 CD14 -159 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數 0-5 支並且為 CD14 -159 TT/CT 基因型者，有 1.5 倍兒童氣喘發生之危險 (95% C.I. = 0.6-3.9)；而每天暴露香菸支數大於 5 支的兒童，其 CD14 -159 基因攜帶 TT/CT 基因型者被觀察到有較高兒童氣喘發生之危險 (RR_m =1.7; 95% C.I. = 0.6-5.0)。在過敏原測試陰性的兒童中，同樣以每天暴露香菸支數 0-5 支並且攜帶 CD14 -159 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數大於 5 支並且攜帶 CD14 -159 CC 基因型、以及每天暴露香菸支數 0-5 支並且攜帶 CD14 -159 TT/CT 基因型的兒童分別有 2.4 倍 (95% C.I. = 0.1-41.9) 以及 1.3 倍 (95% C.I. = 0.5-6.1) 的氣喘發生危險；而每天暴露香菸支數 5 支以上並且攜帶 CD14 -159 TT/CT 基因型的兒童，則有 2.1 倍 (95% C.I. = 0.4-12.3) 的氣喘發生危險。在過敏原測試陽性的兒童中，以每天暴露香菸支數 0-5 支並且攜帶 CD14 -260 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數 0-5 支並且為 CD14 -260 TT/CT 基因型者，有 2.2 倍兒童氣喘發生之危險 (95% C.I. = 0.9-7.9)；而每天暴露香菸支數大於 5 支的兒童，其 CD14 -260 基因攜帶 TT/CT 基因型者被觀察到有較高兒童氣喘發生之危險 (RR_m = 2.6; 95% C.I. = 0.9-7.9)。在過敏原測試陰性的兒童中，同樣以每天暴露香菸支數 0-5 支並且攜帶 CD14 -260 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數大於 5 支並且攜帶 CD14 -260 CC 基因型、以及每天暴露香菸支數 0-5 支並且攜帶 CD14 -260 TT/CT 基因型的兒童分別有 2.1 倍 (95% C.I. = 0.1-33.4) 以及 1.2 倍 (95% C.I. = 0.3-6.1) 的氣喘發生危險；而每天暴露香菸支數 5 支以上並且 CD14 -260 TT/CT 攜帶基因型的兒童，則有 2.0 倍 (95% C.I. = 0.4-11.2) 的氣喘發生危險。在過敏原測試陽性的兒童中，以每天暴露香菸支數 0-5 支並且攜帶 STAT6 G2964A GG 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數 0-5 支並且為 STAT6 G2964A AA/GA 基因型者，有 1.8 倍兒童氣喘發生之危險 (95% C.I. = 0.8-3.9)；而每天暴露香菸支數大於 5 支的兒童，其 STAT6 G2964A 基因攜帶 AA/GA

基因型者被觀察到有較高兒童氣喘發生之危險 (RR_m = 2.6; 95% C.I. = 0.9-7.1)。在過敏原測試陰性的兒童中，同樣以每天暴露香菸支數 0-5 支並且攜帶 STAT6 G2964A GG 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數大於 5 支並且攜帶 STAT6 G2964A GG 基因型、以及每天暴露香菸支數 0-5 支並且攜帶 STAT6 G2964A AA/GA 基因型的兒童分別有 4.4 倍 (95% C.I. = 0.4-46.4) 以及 2.9 倍 (95% C.I. = 0.6-15.6) 的氣喘發生危險；而每天暴露香菸支數 5 支以上並且攜帶 STAT6 G2964A AA/GA 基因型的兒童，則有 4.2 倍 (95% C.I. = 0.6-30.2) 的氣喘發生危險。

在過敏原測試陽性的兒童中，以每天暴露香菸支數 0-5 支並且攜帶 CDH1 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數 0-5 支並且為 CDH1 AA/CA 基因型者，有 1.9 倍兒童氣喘發生之危險 (95% C.I. = 0.9-4.2)；而每天暴露香菸支數大於 5 支的兒童，其 CDH1 基因攜帶 AA/CA 基因型者被觀察到有較高兒童氣喘發生之危險 (RR_m = 3.1; 95% C.I. = 1.1-8.5)。在過敏原測試陰性的兒童中，同樣以每天暴露香菸支數 0-5 支並且攜帶 CDH1 CC 基因型者為參考組 (RR_m = 1.0)，每天暴露香菸支數大於 5 支並且攜帶 CDH1 CC 基因型、以及每天暴露香菸支數 0-5 支並且攜帶 CDH1 AA/CA 基因型的兒童分別有 3.3 倍 (95% C.I. = 0.2-60.8) 以及 5.0 倍 (95% C.I. = 0.6-41.0) 的氣喘發生危險；而每天暴露香菸支數 5 支以上並且攜帶 CDH1 AA/CA 基因型的兒童，則有最高的 8.5 倍 (95% C.I. = 0.9-85.4) 的氣喘發生危險。

討論：

在我們的研究中，兒童氣喘發生顯著危險是相關於個體過敏原測試之結果，也顯著相關於 CD14 -159、STAT6 G2964A、CDH1 -160 基因型。並且，分別相較於過敏原陰性且攜帶 CD14 -159 CC、STAT6 G2964A CC、CDH1 -160 CC 基因型的兒童，過敏原陽性且攜帶 CD14 -159 TT/CT、STAT6 G2964A AA/GA 基因型或 CDH1 -160 AA/CA 基因型的兒童也有顯著較高的氣喘發生危險。進一步地，每天於室內暴露香菸支數大於 5 支並且攜帶 CDH1 -160 AA/CA 基因型者，相較於每天於室內暴露香菸支數小於 5 支並且攜帶 CDH1 -160 CC 基因型者，具有較高的兒童氣喘發生之危險。

氣喘是多因子的症狀，許多種誘因會導致氣喘發作的開始或惡化，包括暴露於過敏原 [3, 4] 以及香菸 [7, 8]。過敏原試驗已常被用來檢驗是否個體具有異位性疾病 [3, 51]，在我們的研究中，台灣常見過敏原 [51]，包括家中的灰塵、家塵蟎與美洲蟑螂則被用來加以評估。過敏原致敏是氣喘發生的重要危險因子之一 [3, 4]，在我們現今的研究中，81.5%的氣喘兒童病例呈現具有至少一種過敏原致敏反應。過敏原的暴露可能會增加呼吸道上皮細胞的增生及發炎 [57]，而過敏性呼吸道的發炎反應可能是藉由 Type 2 T 輔助細胞產生的細胞激素所造成，進一步形成氣喘 [58]。一個關鍵的控制機制在於輔助 T 淋巴球細胞及其相關之細胞激素產物的角色 [11, 12]。IL12 刺激 Th1 淋巴球細胞的發展，這也相關於 IFN-γ及 IL-2 細胞激素的產生；而這些 Th1 細胞激素在細胞調節免疫的發展上扮演一個關鍵角色。IL-4 則促進 Th2 淋巴球細胞的發展，並且相關於 IL-5 及 IL-13 細胞激素的產生，以及促進 IgE 分泌與嗜伊紅性白血球的聚集。而這些 Th2 相關機制對於環境中抗原之過敏發展則是相當地關鍵。

CD14是LPS與其他細菌細胞壁成分具有高度親和力的接受器 [15]，CD14與細菌成分的結合則相關於藉由抗原呈現細胞強化的IL-12反應 [59]；並且IgE的反應已經明確地知道是藉由Th1型細胞所衍生的抑制訊號和Th2型細胞所提供的刺激訊號所調控 [11]。在CD14基因啟動者區域，一個C→T的變異在從主要轉錄處-159上游位置上已經被辨識，並且也被報告與過敏相關 [53]。在高加索小孩中，攜帶同型TT基因型者相較於同型CC基因型者具有較高的sCD14的血清濃度 [53]；此外，在皮膚過敏原測試陽性的小孩中，相較於攜帶CC與CT基因型者，攜帶同型TT基因型者有較

低的血清總IgE與較少數目的陽性皮膚穿測結果 [53]。然而，近期的研究也指出，在非過敏性氣喘與食物過敏之患者中，其CD14 -159 T對偶基因型相較於健康者有較高的比例 [60]。此外，一個在CD14 -260上游位置上的C→T變異也被辨識，如此的變異可影響CD14基因表現程度。在我們的研究中，攜帶CD14 -159 TT/CT基因型或CD14 -260 TT/CT基因型的過敏原測試為陽性之兒童相較於攜帶CD14 -159 CC或CD14 -260 CC基因型的過敏原測試陰性之兒童，分別有較高的氣喘發生危險。我們的結果建議著CD14基因變異可能改變CD14表現，並且如此可能調節經環境過敏原刺激後改變之Th2和Th1細胞比例，因而增加隨後氣喘的發生。

除了CD14，在我們的研究中，過敏原測試為陽性且攜帶STAT6 G2964A AA/GA基因型的兒童也呈現較高的氣喘發生表現。STAT6被建議參與在IL-4和IL-13訊息傳遞路徑中 [37, 38]；IL-4刺激T淋巴球細胞的增生，並且此對Th2細胞的分化很重要 [11]。已知在支氣管黏膜以及支氣管肺泡灌洗液 (bronchoalveolar lavage fluid) 中，活化的Th2細胞、巨大細胞以及嗜伊紅性白血球的增加數量是氣喘的固定表徵 [39]，並且也與過敏型態之增加血清IgE濃度相關。雖然，Duetsch等人 [30] 於STAT6上辨識到13個單一核苷酸多形性，並且在108對高加索人之手足間進行與氣喘的相關測試，則並未發現單一核苷酸多形性與在表現序列 (exon) 1中之GT重複片段與氣喘呈現連鎖/相關；兩個研究團隊發現在STAT6基因的3’端未轉譯區域之G2964A基因型與氣喘、輕度異位性過敏 (mild-type atopy) [52] 及堅果過敏症 (nut allergy) [32] 具有相關性。一項動物研究也指出起始的氣膠抗原誘發在無需Stat6之過程，允許有限數目的特定Th2細胞進入肺臟中 [39]；一旦進入肺臟中，Th2細胞就會分泌細胞激素，例如IL-4和IL-13，以活化肺臟細胞，並且在需要Stat6調控的行為下分泌化學激素來活化Th2細胞以及嗜伊紅性白血球；這些化學激素然後藉由吸引更大量的Th2細胞及嗜伊紅性白血球來放大Th2反應，而被收集過來的Th2細胞隨後在Stat6調控的行為下引發黏液產生以及呼吸道過度反應。然而，我們的資料指出STAT6常見的基因變異與較多氣喘的相關，可能就是顯示在抗原刺激與氣喘及過敏間的複雜關係。許多研究已經報告過敏原暴露和氣喘間之正向相關，但是沒有將適當的分子基因變異列入考慮。有趣地是，我們進一步的分析顯示過敏原測試陽性且攜帶易感受性CD14 -159 TT/TC或STAT6 G2964A AA/GA基因型的兒童是較可能發生氣喘。綜合以上的結果，建議著在CD14表達下具有嚴重過敏承受之個體，透過Th-2免疫訊息之STAT6基因變異，即可能加強氣喘的發展與維持。未來，探討過敏原暴露和氣喘與過敏間的相關之流行病學研究應需要考慮在Th-2免疫訊息上之基因變異。

在我們的研究中，我們也觀察到 CDH1 -160 基因型與兒童氣喘之發生是具有相關性。依鈣性的細胞黏附分子上皮細胞黏附蛋白對於正常結構以及上皮組織的功能之形成與維持，扮演一個必要的角色 [44, 45]。此外，氣喘病人的支氣管上皮細胞相較於對照組對象的細胞，其發育較差也較脆弱 [43]；而先前的研究也指出 CDH1 -160A 對偶基因具有較少的基因轉譯 [48]，較缺乏轉譯因子結合強度，因此可能相較於 C 對偶基因是較無法表現上皮細胞黏附蛋白的功能。先前研究已經證實室內二手菸的暴露與氣喘間的關連性 [7, 8]，但是室內二手菸暴露對於兒童氣喘發生的致病機轉，至今仍不清楚。氣態的香菸中包含氧化分子，並且能夠導致呼吸道上皮的發炎 [35, 36]；進一步地促使上皮細胞產生損害並且需要進行結構重塑 (remodeling)。而香菸也被顯示可減少上皮細胞的黏附以及增加分離 [41]。在我們的研究中，相較於攜帶 CDH1 -160 CC 基因型的兒童，攜帶 CDH1 -160 AA/CA 基因型的兒童具有顯著較高的氣喘發生危險；特別是暴露於室內二手菸支數較多的兒童。重要的是，我們的過敏原陽性的兒童也被觀察到，每天暴露香菸支數 0-5 支並且為 CDH1 -160 AA/CA 基因型者，有較高的氣喘發生之危險；而每天暴露香菸支數大於 5 支的兒童，其攜帶 CDH1 -160 AA/CA 基因型者，更具有 3.1 倍之氣喘發生危險，並達到統計上顯著性。這可能是兒童暴露於過敏原及香菸所導致的發炎，並且無法維持呼吸道上皮組織結構的共同後

果。

有趣的是，在本研究中我們也觀察到父母親高教育程度與兒童氣喘發生具有顯著的相關性。Martinez 等人 [61] 指出隨著個體成長而逐漸遭受外來物的暴露，可使抗原呈現細胞 (antigen-presenting cell [APC]) 漸漸成熟；當成熟的抗原呈現細胞持續受到刺激時，則可使 CD4+Th 朝 Th1 分化 [62, 63]。因此，高教育程度的父母一旦過度保護自己的子女，減少與環境的接觸，將可能影響幼兒時期 Th1 及 Th2 分化的重要因素；這也可能是兒童氣喘的貢獻因素。氣喘家族聚集性已被證實 [64]，而這也建議著具有氣喘家族史之兒童有較高的氣喘發生危險性，如同我們的結果所顯示；這可能是基因因素或共同環境因素所導致之結果。兒童大部分的時間都待在室內，因此考慮兒童因暴露到室內污染源而導致氣喘的發生之影響是重要的。然而，我們也發現家中從事紡織類工作的兒童有較高的氣喘發生危險；這可能是因為室內過敏原可附著在棉屑上，而使兒童在吸入這些過敏原後產生氣喘 [65]。其他被認為與氣喘有相關之環境危險因子，如飼養寵物等變項，發現相似之較低的氣喘發生相對危險性。而相似的現象也被觀察到，於家中燒香行為與氣喘危險之間具有一個相反的相關。在本研究中，這些指標是倚賴自我報告，因此是主觀的，可能造成暴露的錯誤分組並且減弱被觀察的相關。最後，也必須考量到在我們研究中較少的樣本數，限制了統計檢定力以偵測較小的增加危險。

總體而言，我們的結果顯示攜帶 CD14 -159、STAT6 G2964A、CDH1 -160 基因型可能增加台灣兒童氣喘的發生，特別是具有室內二手菸暴露之 CD14 -159、STAT6 G2964A、CDH1 -160 易感受性基因型的過敏兒童，可能需要更密集的醫療篩檢，特別是針對氣喘。

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成果自評：

第二型輔助T淋巴球和其他細胞激素在氣喘的致病過程中扮演著重要的角色。CD14為細菌細胞壁成分的多功能接受器，並可能調節兒童時期早期Th1-Th2的比例。信號轉導和轉錄啟動因子 6則為影響Th2細胞分化的重要轉錄因子。香菸已被顯示可減少上皮細胞的黏附以及增加分離，而細胞黏附分子上皮細胞黏附蛋白對於正常結構以及上皮組織的功能之形成與維持，扮演一個必要的角色，因此人類上皮細胞黏附蛋白基因可能相關於氣喘的發生。我們執行一個以醫院為基礎之病例對照研究來探討是否CD14、STAT6與CDH1多形性變異相關於兒童氣喘之危險。此外，二手菸暴露與CD14、STAT6與CDH1多形性變異在氣喘發展上具有交互作用的存在也被探討。總計357名兒童參與本研究，研究對象的個人特徵資料，是經由面對面的問卷訪視所收集。四種台灣常見過敏原也被執行皮膚測試加以判定；基因型則是以聚合酶鏈鎖反應判定。研究結果顯示，兒童氣喘發生顯著危險是相關於個體過敏原測試陽性以及CD14 -159、STAT6 G2964A、CDH1 -160基因型。過敏原反應呈陽性並且攜帶CD14 -159 TT/CT基因型 (RR_m = 6.3; 95% CI = 2.0-20.4)、STAT6 G2964A AA/GA基因型 (RR_m = 8.9; 95% CI = 2.8-27.9)、以及CDH1-160 AA/CA基因型 (RR_m = 19.9; 95% CI = 4.6-87.0) 的兒童分別相較於過敏原反應呈陰性且攜帶CD14 -159 CC基因型、STAT6 GG基因型、以及CDH1-160 CC基因型的兒童有較高之氣喘發病危險性。進一步地，評估室內二手菸暴露與易感受性基因型對於氣喘發生之危險。相較於過敏原測試陽性攜帶CDH1-160 CC基因型且每天暴露香菸支數0-5支的兒童，攜帶CDH1-160 AA/CA基因型且暴露香菸支數0-5支的兒童、與每天暴露香菸支數大於5支的兒童，分別呈現1.9倍 (95% C.I. = 0.9-4.2) 與3.1倍 (95% C.I. = 1.1-8.5) 的氣喘發生危險。我們的結果顯示攜帶CD14 -159、STAT6 G2964A、CDH1 -160基因型可能增加台灣兒童氣喘的發生，特別是具有室內二手菸暴露之CD14 -159、STAT6 G2964A、CDH1 -160易感受性基因型的過敏兒童。如此的結果可提供環境醫學研究對於兒童氣喘發生機制的參考依據。過程中，本研究也提供相關人員環境分子流行病學的相關訓練，與研究經歷。

表一：兒童氣喘病例及配對對照之基本特徵與 CD14、STAT6、與 CDH1 基因型頻率

變項	病例組	對照組	配對之相對危險性 [a]	
	n = 119	n = 238	RR_m	95% C.I.
性別				
男生	78 (65.5%)	156 (65.5%)	1.0	0.6-1.6
女生	41 (34.5%)	82 (34.5%)	1.0	
父母教育程度				
大學以上	60 (50.4%)	62 (26.0%)	4.2	2.0-9.2**
高中	49 (41.2%)	132 (55.5%)	1.6	0.8-3.5
高中以下	10 (8.4%)	44 (18.5%)	1.0	
氣喘家族史				
有	24 (20.2%)	16 (6.7%)	3.5	1.8-6.9**
無	95 (79.8%)	222 (93.3%)	1.0	
CD14 -159 基因型				
TT	48 (40.3%)	76 (31.9%)	2.0	1.0-3.9*
CT	56 (47.1%)	115 (48.3%)	1.5	0.8-3.0
CC	15 (12.6%)	47 (19.8%)	1.0	
CD14 -260 基因型				
TT	38 (31.9%)	73 (30.7%)	1.2	0.6-2.7
CT	62 (52.1%)	120 (50.4%)	1.2	0.7-2.3
CC	19 (16.0%)	45 (18.9%)	1.0	
STAT6 G2964A 基因型				
AA	37 (31.1%)	54 (22.7%)	2.0	1.0-3.8*
GA	60 (50.4%)	120 (50.4%)	1.5	0.8-2.6
GG	22 (18.5%)	64 (26.9%)	1.0	
CDH1 基因型				
AA	15 (12.6%)	25 (10.5%)	2.4	1.1-5.5*
CA	84 (70.6%)	131 (55.0%)	2.6	1.5-4.6**
CC	20 (16.8%)	82 (34.5%)	1.0	

[a] 健康對照是以病例之性別及年齡所配對。$^{**}P < 0.01$，$^{*}0.01 < P < 0.05$。

表二：兒童氣喘病例相較於配對對照之環境因子的配對相對危險性與 95%信賴區間

變項	病例組	對照組	配對的相對危險性[a]	
	n = 119	n = 238	RR_m	95% C.I.
父母親抽菸狀況				
有	32 (26.9%)	71 (29.8%)	0.8	0.4-1.3
不在家中抽菸	23 (19.3%)	60 (25.2%)	0.6	0.4-1.1
無	64 (53.8%)	107 (45.0%)	1.0	
二手菸暴露狀況[b]				
> 5 支/天	29 (24.4%)	53 (22.3%)	1.1	0.6-1.8
1-5 支/天	3 (2.5%)	17 (7.2%)	0.3	0.1-1.2
0 支/天	87 (73.1%)	167 (70.5%)	1.0	
家中燒香				
有	40 (33.6%)	151 (63.4%)	0.3	0.2-0.5**
無	79 (66.4%)	87 (36.6%)	1.0	
在家中從事紡織類工作				
有	16 (13.4%)	13 (5.5%)	2.7	1.2-5.8*
無	103 (86.6%)	225 (94.5%)	1.0	
飼養寵物				
有	20 (16.8%)	50 (21.0%)	0.8	0.4-1.3
無	99 (83.2%)	188 (79.0%)	1.0	
家中潮溼度				
有	16 (13.4%)	22 (9.2%)	1.5	0.8-3.0
無	103 (86.6%)	216 (90.8%)	1.0	
寢室有無蟑螂				
有	52 (43.7%)	105 (44.1%)	1.0	0.6-1.5
無	67 (56.3%)	133 (55.9%)	1.0	
過敏原測試				
陽性	97 (81.5%)	101 (42.4%)	6.2	3.6-10.5**
陰性	22 (18.5%)	137 (57.6%)	1.0	

[a] 健康對照是以病例之性別及年齡所配對。

[b] 資料遺漏 = 1。$^{**}P < 0.01$，$^{*}0.01 < P < 0.05$。

表三：兒童氣喘病例相較於配對對照，其過敏原測試、CD14、STAT6、與 CDH1 基因型的調整後之相對危險性與 95%信賴區間

變項	過敏原測試陽性			過敏原測試陰性		
	病例組 n = 97	對照組 n = 101	調整後之 RR 值 (95% C.I.)[a]	病例組 n = 22	對照組 n = 137	調整後之 RR 值 (95% C.I.)[a]
CD14 -159 基因型						
TT/CT	82	81	6.3 (2.0-20.4)**	18	112	1.2 (0.4-4.3)
CC	15	20	5.2 (1.4-20.0)*	4	25	1.0
CD14 -260 基因型						
TT/CT	86	79	6.4 (2.0-20.3)**	18	112	1.1 (0.3-3.8)
CC	11	22	3.0 (0.8-11.4)	4	25	1.0
STAT6 G2964A 基因型						
AA/GA	79	73	8.9 (2.8-27.9)**	18	101	1.6 (0.5-5.3)
GG	18	28	4.1 (1.2-14.6)*	4	36	1.0
CDH1 -160 基因型						
AA/CA	79	63	19.9 (4.6-87.0)**	20	93	3.9 (0.9-17.9)
CC	18	38	8.3 (1.7-39.0)**	2	44	1.0

[a] 相對危險性是調整氣喘家族史、父母親教育程度、家中燒香、家中從事紡織類工作等變項；並且對照是以病例之性別及年齡所配對。

**$P < 0.01$，*$0.01 < P < 0.05$。

表四：具不同過敏原反應的兒童其室內二手菸暴露程度與 CD14、STAT6、與 CDH1 基因型對於氣喘發生之危險對比值

變項	過敏原測試陽性						過敏原測試陰性					
	暴露香菸支數 0-5 支/天			暴露香菸支數 >5 支/天			暴露香菸支數 0-5 支/天			暴露香菸支數 >5 支/天		
	病例	對照	RR_m (95% C.I.)[a]	病例	對照	RR_m (95% C.I.)[a]	病例	對照[b]	RR_m (95% C.I.)[a]	病例	對照	RR_m (95% C.I.)[a]
CD14 -159 基因型												
TT/CT	65	62	1.5 (0.6-3.9)	17	19	1.7 (0.6-5.0)	13	84	1.3 (0.5-6.1)	5	28	2.1 (0.4-12.3)
CC	9	17	1.0	6	3	2.5 (0.5-13.4)	3	22	1.0	1	3	2.4 (0.1-41.9)
CD14 -260 基因型												
TT/CT	66	60	2.2 (0.9-7.9)	20	19	2.6 (0.9-7.9)	13	85	1.2 (0.3-6.1)	5	27	2.0 (0.4-11.2)
CC	8	19	1.0	3	3	1.4 (0.2-10.0)	3	21	1.0	1	4	2.1 (0.1-33.4)
STAT6 G2964A 基因型												
AA/GA	58	58	1.8 (0.8-3.9)	21	15	2.6 (0.9-7.1)	14	79	2.9 (0.6-15.6)	4	22	4.2 (0.6-30.2)
GG	16	21	1.0	2	7	0.6 (0.1-3.5)	2	27	1.0	2	9	4.4 (0.4-46.4)
CDH1 -160 基因型												
AA/CA	59	50	1.9 (0.9-4.2)	20	13	3.1 (1.1-8.5)*	15	72	5.0 (0.6-41.0)	5	20	8.5 (0.9-85.4)
CC	15	29	1.0	3	9	0.6 (0.1-2.8)	1	33	1.0	1	11	3.3 (0.2-60.8)

[a] 相對危險性是調整氣喘家族史、父母親教育程度、家中燒香、家中從事紡織類工作等變項；並且對照是以病例之性別及年齡所配對。

[b] 資料遺漏＝1。*$0.01 < P < 0.05$。

CD14 and STAT6 genetic polymorphisms and susceptibility to childhood asthma

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Abstract

Type 2 T helper lymphocytes (Th2 cells) and their cytokine products are important in the pathogenesis of asthma. CD14 functions as a multifunctional receptor for bacterial cell wall components and is likely to play a role in the modulation of Th1-Th2 response during early childhood. Signal transducer and activator of transcription factor (Stat) 6 is a transcription factor essential for Th2 cell differentiation. In the present study, the hypothesis that polymorphisms in the CD14/-159, CD14/260, and STAT6 G2964A genes are associated with childhood asthma were examined under a hospital-based case-control study. A total of 70 asthmatic children and 138 potential controls were recruited into our study. Allergen test was performed by an intracutaneous skin test or by MAST (Multiple Antigen Simultaneous Test) with Taiwan common aeroallergens. The genotypes of CD14 C-159T, C-260T, and STAT6 G2964A were identified by polymerase chain reaction (PCR). Our results revealed

that allergen test-positive children with the CD14/-159 TT genotype (RR_m = 6.4; 95% CI = 1.0-42.7, P = 0.05), the CD14/-260 TT genotype (RR_m = 7.5; 95% CI = 1.0-54.57, P = 0.05), and the STAT6 G2964A AA (RR_m = 10.0; 95% CI = 2.3-4.5, P < 0.01) had a higher risk of asthma development than did allergen test-negative children with the CD14/-159 CC genotype, the CD14/-260 CC genotype, and STAT6 GG genotype, respectively. Further analysis revealed that allergen-test positive children with more susceptible genotypes of CD14/-159 TT, CD14/-260 TT, and STAT6 AA, were more likely to develop asthma. These results suggested that susceptible CD14 and STAT6 genotypes may modulate the asthma development in allergen-exposed children.

KEY WORDS: CD14 gene; STAT6 gene; childhood asthma; Allergen.

Introduction

Asthma is a chronic disease characterized by variable airway obstruction, airway hyperresponsiveness (AHR), airway inflammation and remodeling [1]. Importantly, prevalence of asthma has been increasing to 8.2% in Taiwanese children in 2001 [2]. Changes in environmental factors more likely play an important role on asthma development, such as life style, diet, air pollution, allergen exposure, and microbial environment [3-5], while children are more likely to develop asthma than adults, stemming from a combination of genetic and environmental causes.

The two major subsets of $CD4^+$ T helper cells, termed Th1 and Th2, secrete mutually distinct profiles of cytokines and thereby coordinate different classes of immune response [6, 7]. Th1 cells secrete interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10, and

IL-13. Polarization of an immune response toward a Th2 phenotype may prove harmful if directed against an otherwise innocuous environmental antigen, as occurs in the pathogenesis of allergic diseases like asthma. The Th2 cytokines control all the major components that characterize an inflammatory asthmatic response, including immunoglobulin E (IgE) isotype switching, mucus production, and the recruitment and activation of eosinophils.

Studies have suggested that bacterial infections in infancy may protect against the development of allergy [8]. It was also hypothesized that bacterial signals play a functional role in the maturation of the Th1-type immune response, thereby suppressing the Th2-type response, which may produce an atopic phenotype [9]. Microbial products, such as lipopolysaccharides (LPS), can provide activation signals for Th1-maturation. An important high-affinity receptor for LPS and other bacterial wall components is CD14 [10], a 55-kD glycosylphosphatidylinositol-anchored protein localized on monocytes, macrophages, and polymorphonuclear cells [11]. Binding of LPS to CD14 is facilitated by lipopolysaccharide-binding protein.

The cytokines IL-12 and IL-4 direct the differentiation of Th1 and Th2 cells, respectively, from naive T helper cells [6, 7]. In addition, previous studies have revealed that, like most other cytokines, IL-4 and IL-12 activate the Janus kinase-signal transducer and activator transcription factor (Jak-Stat) signaling cascade [12-14]. In this signaling pathway, binding of a cytokine to its receptor leads to the activation of members of the JAK family of receptor-associated kinases. These kinases subsequently activate, via tyrosine phosphorylation, preexistent cytoplasmic factors termed Stats. Tyrosine phosphorylation allows the Stat proteins to dimerize and translocate to the nucleus, where they mediate changes in gene expression by binding specific DNA elements.

The IL-4 stimulates Jak1 and Jak3 to activate Stat6 [14]. In contrast, IL-12 leads to the activation of Jak2 and Tyk2 and the subsequent phosphorylation of Stat4. Several groups have also investigated the expression and activation of STAT6 in asthmatic individuals. Peripheral blood lymphocytes from asthmatic and allergic patients do not display significant differences in the level of STAT6 activity relative to healthy controls [15], but these patients do have a higher density of STAT6-expressing cells in their airways [16], intriguingly, the density of these STAT6-expressing cells is significantly higher in atopic than in nonatopic asthmatics. One recent study of subjects with severe asthma confirms that such patients show significantly elevated airway levels of STAT6 and also identifies the major STAT6-expressing cell type in this tissue as the bronchial epithelial cell [17].

The human CD14 is localized on chromosome 5q31.1 [18]. The polymorphism in the CD14 gene promoter is located near the Spl recognition sequence factor, which is necessary for CD14 expression [19]. In the promoter region of the CD14 gene, a C-to-T transition was identified at position -159 upstream from the major transcription site, and was also reported to be associated with atopy [20]. In addition, Hubacek et al. [21] indicated that healthy volunteers without acute illness showed that the C-to-T transition at position -260 upstream affects the level of CD14 gene expression, and thus we suppose that this increased density is permanent and genetically determined. Thus, the genetic variation in the CD14 gene could explain the accumulation of childhood asthma. Moreover, the human STAT6 gene maps to chromosome 12q13.3-q14.1 [22]. Interestingly, the allele frequencies for STAT6 G2964A in the Japanese (G allele 76%, A allele 24%) and the British populations (G allele 33%, A allele 67%) are quite different [23], this may suggest the “genetic heterogeneity” among and within ethnic groups.

Therefore, we designed a hospital-based case-control study to evaluate the effects of susceptibly genetic polymorphisms of CD14 and STAT6 on the risk of childhood asthma, and that might provide insights into the asthma development.

Materials and methods

Identification for cases and controls

This study was approved by the ethic committee of Chung-Shan Medical University. Study subjects aged 5-12 years old had been recruited from Chung-Shan Medical University Hospital in central Taiwan, and were accessible to patients from all socioeconomic classes. Cases were selected from the pediatric asthma outpatient clinic of Chung-Shan Medical University Hospital, and they were fulfilled the American Thoracic Society's criteria for reversible airway disease (i.e., bronchia asthma) as determined by a pediatrician [24]. In addition, children who have no previous diagnosis of asthma, no asthma symptoms such as wheezing and cough were defined as control in this study. A 1: 2 ratio of cases to controls was used in this study. Healthy controls were matched to cases on age (± 5 years), gender, and parental education status. All subjects must be able to give clinical history and informed consent. In total, 70 cases and 138 controls were included in the analysis conducted herein.

Interview

Independent interviews with parents by our well-trained research staffs with a semi-structured clinical questionnaire during the study period. The semi-structured questionnaire contained questions which covered demographic characteristics, life styles including habits of family members' cigarette-smoking, incense burning at

home, textile work at home, pet feeding, home dampness, or observed cockroaches in bedroom during the past year, as well as family history of asthma. The smoking history of subject's family members included the number of cigarettes smoked daily, and the duration the child was exposed to environmental tobacco smoke. Home dampness was defined as the presence of any one of the following during the past year: visible mold or mildew growth on surfaces inside the home, standing water, within the home, water damage, or leakage of after into the building. Family history of asthma was defined as asthma within the first-degree relatives of the test subjects.

Allergen test

Allergen test was performed by an intracutaneous skin test or by MAST (Multiple Antigen Simultaneous Test) with Taiwan common aeroallergens, including house dust, American cockroach, and dust mite (standardized mite Dermatophagoides farinae, standardized mite Dermatophagoides pteronyssinus) as Lee et al. [25] suggested previously. A positive skin test was considered to be present if the largest wheal diameter was ≥ 5 mm.

Genotyping of polymorphic CD14 and STAT6 genes

Briefly, for the CD14/-159 gene analysis, restriction fragment length polymorphism (RFLP) was detected by differences in AvaII sites in the promoter region following polymerase chain reaction (PCR) amplification, using method described in 2001 by Koppelman et al. [26]. Venous blood was collected in heparinized tube from all subjects then further to extract DNA. Primers used for the amplification of CD14/-159 gene were 5'- GTG CCA ACA GAT GAG GTT CAC -3' and 5'- GCC TCT GAC AGT TTA TGT AAT C -3'. One half microliter of DNA was

added to a PCR buffer containing 200 ng of primers, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH = 8.3) and 0.1% of BSA in a final volume of 50 μl. Amplification was carried out under conditions that the denaturing step was conducted at 96°C for 40 sec, annealing at 56°C for 40 sec, and extension at 72°C for 50 sec. The PCR products were digested with AvaII. Homozygous CC individuals exhibited a product fragment of 497-bp, whereas homozygous TT individuals revealed a 353-bp and a 144-bp fragment, and heterozygous CT individuals demonstrated all three fragments (Fig. I). The CD14/-260 polymorphism was determined by a modification of the methods described in 2001 by Zee et al. [27]. The sequences of CD14 -260 primers were 5'- TGA GGA TCA TCC TTT TCC CAC AC -3' and 5'- CAG GCT TCA CAA TTG TGA ACT CTT -3'. Amplification was carried out under conditions similar to those described for the CD14/-159, except denaturing step was conducted at 94°C for 30 sec, annealing at 60°C for 45 sec, and extension at 74°C for 1 min. The PCR products were digested with HaeⅢ. Homozygous TT individuals exhibited a product fragment of 318-bp, whereas homozygous CC individuals revealed a 172-bp and a 146-bp fragment, and heterozygous CT individuals demonstrated all three fragments (Fig. II).

Similar to the CD14 gene analysis, the STAT6 G2964-BsaHI polymorphism was determined by a modification of the methods developed by Amoli et al. [28]. Primers used for the amplification of STAT6 gene are 5'- GAA GTT CAG GCT CTG AGA GAC -3' and 5'- CCA TCA CCC TCA GAG AGC -3'. The cycling parameters consist of an initial incubation of 3 min at 95°C, following by 35 cycles of 1 min at 95°C, 1 min at 57°C, and 1 min at 72°C. The reaction was terminated after a final extension of 5 min at 72°C. The PCR products were also digested with BsaHI. The digested products were visualized on 4.0% agarose gels stained with ethidium bromide.

Homozygous GG individuals exhibited a product fragment of 93-bp, whereas homozygous AA individuals revealed a 74-bp and a 19-bp fragment, and heterozygous AG individuals demonstrated all three fragments (Fig. III)

Statistical analysis

Matched relative risk (RR_m) and corresponding 95% confidence interval (CI) on childhood asthma were evaluated for the CD14/-159 genotype, the CD14/-260 genotype, STAT6 G2964A genotype, family history of asthma, passive smoking exposure status, home dampness, pet feeding, incense burning, or cockroaches during the past year, and allergen test using a conditional logistic regression model. Additionally, allergen test result and susceptible CD14/-159, CD14/-260, STAT6 G2964A genotypes together were also taken into multiple conditional logistic regression model to determine their association with asthma development. All *P* values were calculated from two-tailed statistical tests.

Results

In total, 208 children (128 boys and 80 girls) were involved in this study. Their ages ranged from 5 to 12 years old (mean, 9.9 years). Basic characteristics and genotype frequencies of CD14/-159, CD14/-260, and STAT6 G2964A of the study subjects are shown in Table I. 66.3% of the parents of study subjects had achieved greater than a senior high school education. A significantly higher proportion of family history of asthma was found in our cases than controls (RR_m = 3.6, 95%C.I. = 1.4-8.9; P = 0.01). The frequencies for STAT6 G2964A AA genotype among the cases was significantly higher from that among controls (38.6% vs. 25.4%), and those carrying STAT6 AA genotype experienced a 2.2-fold risk of asthma development

compared to those carrying STAT6 GG genotype (95% C.I. = 1.0-5.1, $P = 0.06$). However, there were no significant differences in the distribution of CD14/-159, and CD14/-260 between both asthma case and matched control groups.

Furthermore, the matched RRs for asthma development based on various environmental factors are shown in Table II. Children whose parents smoked had a decreased asthma risk than did children whose parents did not smoke ($RR_m = 0.5$; 95% CI = 0.2-0.9, $P = 0.04$). Similarly, inverse associations were also found between incense burning at home ($RR_m = 0.2$; 95% CI = 0.1-0.3, $P < 0.01$), pet feeding ($RR_m = 0.4$; 95% CI = 0.2-1.0, $P = 0.06$), and observed cockroaches in bedroom ($RR_m = 0.6$; 95% CI = 0.3-1.0, $P = 0.07$) with childhood asthma development. Increase risk in childhood asthma was also associated with allergy test positive ($RR_m = 6.5$; 95% CI = 3.2-13.2) compared to subjects with allergen test negative. However, no significant association was found between cigarette smoke exposed status, textile work at home, and home dampness and the risk of developing childhood asthma in our study children.

The adjusted RRs for childhood asthma were calculated to investigate the joint effect of allergen test results and metabolic genotype and are shown in Table III. After adjusting for the effects of family history of asthma, parental smoking status, incense burning at home, pet feeding, and observed cockroaches in bedroom, using the allergen test-negative with the CD14/-159 CC genotype as a reference ($RR_m = 1.0$), an obvious risk of asthma development was observed for those individuals having experienced allergic sensitization and possessing the CD14/-159 TT genotype ($RR_m = 6.4$; 95% CI = 1.0-42.7, $P = 0.05$). In the allergen test-negative group, those with CD14/-159 TT genotype had also a higher risk of asthma ($RR_m = 2.1$; 95% CI = 0.3-14.8), although it was not statistically significant. When CD14/-159 was replaced by CD14/-260 in the statistical analysis, individuals with

allergen test-positive and the CD14/-260 TT genotype had a greater risk of developing asthma than those with allergen test-negative and the CD14/-260 CC genotype (RR_m = 7.5; 95% CI = 1.0-54.57, P = 0.05). Children with allergen test-positive and the STAT6 G2964A AA genotype also had elevated risk of developing asthma compared to those with allergen test-negative and STAT6 GG genotype (RR_m = 10.0; 95% CI = 2.3-4.5, $P < 0.01$).

Subsequently, we also performed a model analysis to evaluate the combined effects of CD14/-159 and STAT6 G2964A genotypes adjusted for family history of asthma, parental smoking status, incense burning at home, pet feeding and observed cockroaches in bedroom (Fig. IV). When children with allergen test-negative and carried none or one of susceptible genotype were used as a reference, those with 2 susceptible genotypes (CD14/-159 TT and STAT6 AA) had higher risk of asthma development, especially allergen test-positive children (RR_m = 13.2, 95%C.I. = 2.4-72.4, $P < 0.01$). When CD14/-159 was replaced by CD14/-260 in our statistical analysis, similar result was also be found that children with 2 susceptible genotypes (CD14/-260 TT and STAT6 AA) had higher risk of asthma development, especially allergen test-positive children (RR_m = 33.8, 95%C.I. = 3.4-337.9, $P < 0.01$) when those with allergen test-negative and carried none or one of susceptible genotype were used as a reference.

Discussion

Asthma is a complex multifactorial disease with an obvious genetic predisposition, immunological aberration, and involvement of noxious environmental factors [3-5]. Atopy is the immune disorder of hypersensitivity to some agents such as house dust, dust mites, and cockroach [24]. Atopy is also the leading cause of childhood asthma [29]. In our study, allergen-test positive children also experienced a significantly

higher risk of asthma development. A crucial control mechanism lies in the character of the helper T lymphocytes and their associated cytokine products [6, 7]. Thl lymphocyte development is primed by IL-12 and is associated with IFN-γ and IL-2 cytokine production; these Thl cytokines play a crucial role in the development of cell-mediated immunity. Th2 lymphocyte development is primed by IL-4, is associated with IL-5 and IL-13 cytokine production and promotes the secretion of IgE, and the recruitment of eosinophil. These Th2 mechanisms are central to the development of hypersensitivity to environmental antigens in atopy.

CD14 is a receptor that has specificity for lipopolysaccharides (LPS) and other bacterial wall-derived components [10]. Engagement of CD14 by these bacterial components is associated with strong IL-12 responses by antigen-presenting cells [30]. It is well established that IgE responses are regulated by inhibitory signals derived from Th1-type cells and by stimulatory signals provided by Th2-type cells [6]. In the promoter region of the CD14 gene, a C-to-T transition was identified at position -159 upstream from the major transcription site, and was also reported to be associated with atopy [26]. Among Caucasian children, those with homozygous TT genotype had higher serum levels of sCD14 than those with homozygous CC genotype. In addition, among skin test-positive children, homozygotes with the TT genotype had lower levels of serum total IgE and a lower number of positive skin prick tests, when compared with the pooled group of subjects carrying CC and CT [26]. However, a recent study also indicated that -159 T allele was more common among patients with nonatopic asthma and food allergy than among control subjects [31]. In addition, a C-to-T transition at position -260 upstream was also identified; such genetic variant affects the level of CD14 gene expression. In our study, allergen test-positive children with the CD14/-159 TT genotype, and the CD14/-260 TT genotype had a higher risk

of asthma development than did allergen test-negative children with the CD14/-159 CC genotype, or the CD14/-260 CC genotype, respectively. Our findings suggested that CD14 genetic variation might alter expression of CD14, and this might regulate the proportion of Th2- to Th1-type cells responding to environmental allergen stimuli, thus increasing subsequent asthma development.

In addition to CD14, in our study, allergen test-positive children with the STAT6 G2964A AA genotype also experienced a higher risk of asthma development. STAT6 is suggested to be involved in IL-4 and IL-13 signaling pathway [32, 33]. IL-4 induces the proliferation of T-lymphocytes and is important for the differentiation of Th2-cells [6]. It is well known that elevated numbers of activated Th2-cells, mast cells and eosinophils, both in the bronchial mucosa and in bronchoalveolar lavage fluid, are constant features of asthma [34] as well as increased serum IgE levels for the atopic form. Although, Duetsch et al. [35] identified 13 single-nucleotide polymorphisms (SNPs) in STAT6 and tested them for association with asthma in 108 Caucasian sib-pairs, neither the SNPs nor a GT repeat in exon 1 showed linkage/association to asthma, two groups of investigators found associations of G2964A genotype in the 3' untranslated region of the STAT6 gene with asthma and mild-type atopy [28] and nut allergy [36]. An animal study also indicated that the initial aerosol antigen challenge allows limited numbers of specific Th2 cells to enter the lung in a Stat6-independent process [34]. Once in the lung these Th2 cells secrete cytokines, such as IL-4 and IL-13, that activate resident pulmonary cells in a Stat6-dependent manner to secrete chemokines active on Th2 cells and eosinophils. These chemokines then amplify the Th2 response by attracting large numbers of Th2 cell and eosinophils. Recruited Th2 cells then induce mucus production and airway hyperresponsiveness in a Stat6-dependent manner. However, our data regarding the association between a

common genetic variant of STAT6 with more asthma, may be relevant to the complex relationship between allergen stimuli and asthma and allergy. Many studies have reported positive relationships between allergen exposure and asthma, but have not taken into account pertinent molecular genetic variants. Interestingly, our further analysis revealed that allergen-test positive children with more susceptible genotypes of CD14/-159 TT, CD14/-260 TT, and STAT6 AA, were more likely to develop asthma. Take together, such findings suggest that important element may be a relationship, through STAT6 genetic variation in Th-2 immune signaling, in which those with heavy allergen burdens with CD14 expression are more likely to manifest asthma. Future epidemiological studies into the relationship between allergen exposure and asthma/allergy therefore need to take into account genetic variants in Th-2 signaling.

Familial aggregation of asthma has frequently been noted [37], suggesting that a positive family history might be used to identify children at risk as our study, and that familial risk of asthma could be due to genetic factors or shared environmental factors. Children spend their most of their time indoors. It is, therefore, important to consider the effects that exposure to indoor air pollutants may have on children's asthma development. In our study, 44.3% of asthmatic cases and 60.1% of matched controls were exposed to environmental tobacco smoke. Compare to controls, environmental tobacco smoke exposure in our cases were less, and this maybe reflect a change from parents' behavior due to the awareness of children's health. Thus Taiwanese asthmatic children may not have regular chances to be exposed to tobacco smoke from their family members, the effects of passive smoking on asthma in children are less likely to appear in our case-control study. Similar phenomenon was also observed an inversely association between suspected environmental factors including incense

burning at home, pet feeding and observed cockroaches in bedroom with asthma risk. In addition, in our study, these indicators were self-reported and, therefore, were subjective, and could have resulted in misclassification of exposure and reduce the observed associations. Lastly, there is concern about that the small sample size in our study limits statistical power to detect a small increase in risk.

In conclusion, we report the association of the susceptible CD14 and STAT6 genotypes with asthma in the Taiwanese children. Those allergic children, particularly those with susceptible CD14 and STAT6, may need intensive medical screening, particularly for asthma.

Acknowledgements

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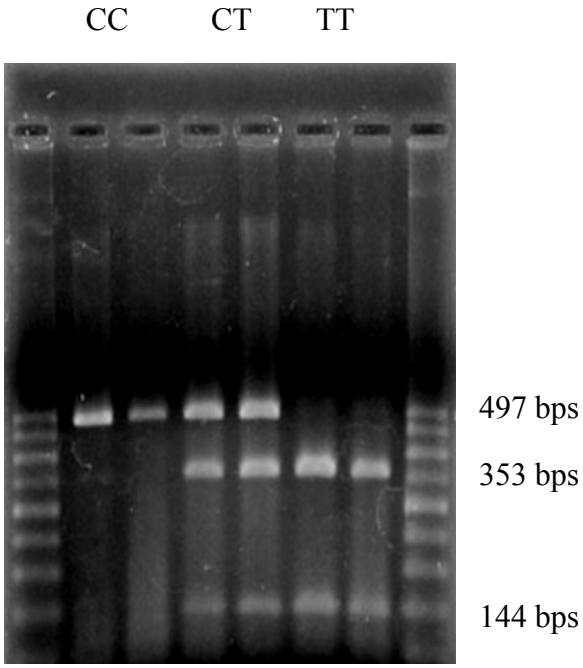

Fig. I. PCR-RFLP analysis for CD14/-159 genotypes. Extracted genomic DNAs were subjected to PCR amplification specific for a CD14 region followed by a AvaII digestion and then electrophoresis on an agarose gel. The presence of a band (497 bps), 3 bands (497, 353, and 144 bps), and 2 bands (353 and 144 bps) indicated homozygous CC, heterozygous CT and homozygous TT, respectively.

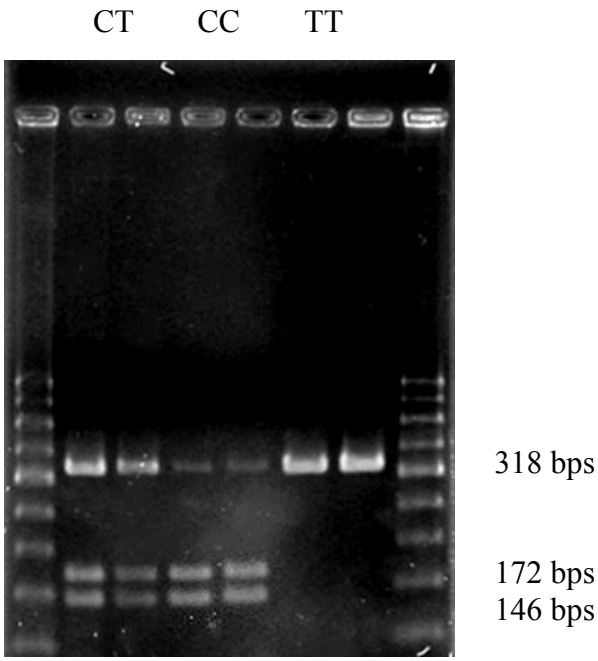

Fig. II. PCR-RFLP analysis for CD14/-260 genotypes. Extracted genomic DNAs were subjected to PCR amplification specific for a CD14 region followed by a HaeⅢ digestion and then electrophoresis on an agarose gel. The presence of a band (318 bps), 3 bands (318, 172, and 146 bps), and 2 bands (172 and 146 bps) indicated homozygous TT, heterozygous CT and homozygous CC, respectively.

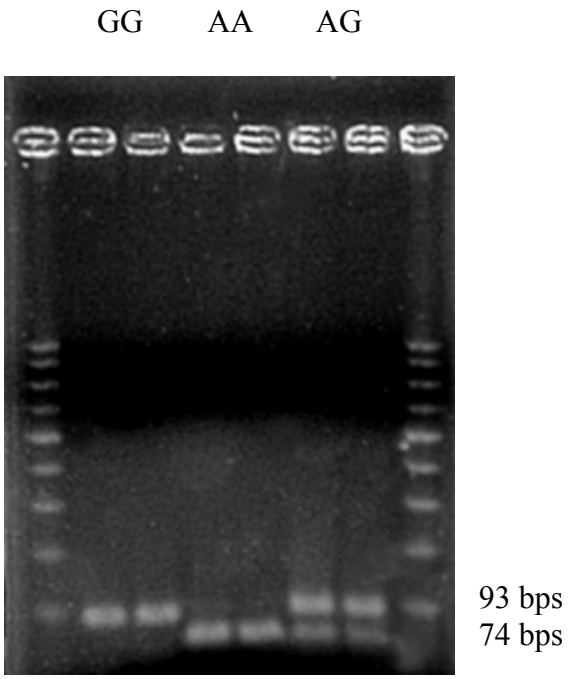

Fig. III. PCR-RFLP analysis for STAT6 G2964A genotypes. Extracted genomic DNAs were subjected to PCR amplification specific for a STAT6 region followed by a BsaHI digestion and then electrophoresis on an agarose gel. The presence of a band (93 bps), 3 bands (93, 74, and 19 bps), and 2 bands (74 and 19 bps) indicated homozygous GG, heterozygous AG and homozygous AA, respectively.

Table I. Basic characteristics and genotype frequencies of CD14/-159, CD14/-260, and STAT6 G2964A among childhood asthma cases and matched controls.

Variables	Cases	Controls	Matched Risk Ratio[a]	
	n = 70	n = 138	RR_m	95% C.I.
Gender				
Boys	43 (61.4%)	85 (61.6%)		
Girls	27 (38.6%)	53 (38.4%)		
Parental education				
Below junior high school	24 (34.3%)	46 (33.3%)		
High school	43 (61.4%)	86 (62.3%)		
Above college	3 (4.3%)	6 (4.4%)		
Family history of asthma				
Yes	13 (18.6%)	8 (5.8%)	3.6	1.4-8.9**
No	57 (81.4%)	130 (94.2%)	1.0	
CD14/-159 genotype				
TT	21 (30.0%)	46 (33.3%)	0.9	0.4-2.0
CT	35 (50.0%)	66 (47.8%)	1.0	0.5-2.1
CC	14 (20.0%)	26 (18.9%)	1.0	
CD14/-260 genotype				
TT	25 (35.7%)	46 (33.3%)	1.0	0.4-2.3
CT	31 (44.3%)	66 (47.8%)	0.9	0.4-1.9
CC	14 (20.0%)	26 (18.9%)	1.0	
STAT6 G2964A genotype				
AA	27 (38.6%)	35 (25.4%)	2.2	1.0-5.1*
AG	31 (44.3%)	69 (50.0%)	1.3	0.6-2.8
GG	12 (17.1%)	34 (24.6%)	1.0	

[a] Controls were matched to cases on age, gender, and parental education status.

**$P < 0.01$, *$P = 0.06$

Table II. Matched relative risk (RR_m) and 95% confidence intervals (CI) of environmental factors in childhood asthma cases compared with matched controls.

Variables	Cases	Controls	Matched Relative Ratio[a]	
	n = 70	n = 138	RR_m	95% C.I.
Parental smoking status				
Yes	16	48	0.5	0.2-0.9*
With household smoking restriction	15	35	0.6	0.3-1.3
No	39	55	1.0	
Cigarette smoke exposed status				
> 20 cigarettes/day	10	25	0.4	0.1-1.9
10-20 cigarettes/day	12	26	0.6	0.2-1.8
0-9 cigarettes/day	48	87	1.0	
Incense burning at home				
Yes	20	95	0.2	0.1-0.3**
No	50	43	1.0	
Textile work at home				
Yes	7	10	1.4	0.5-3.9
No	63	128	1.0	
Pet feeding				
Yes	8	31	0.4	0.2-1.0†
No	62	107	1.0	
Home dampness				
Yes	3	16	0.3	0.1-1.2
No	67	122	1.0	
Observed cockroaches in bedroom				
Yes	26	70	0.6	0.3-1.0†
No	44	68	1.0	
Allergen test				
Positive	58	59	6.5	3.2-13.2**
Negative	12	79	1.0	

[a] Controls were matched to cases on age, gender, and parental education status.

**$P < 0.01$, *$0.01 < P < 0.05$, †$0.05 < P < 0.10$.

Table III. Adjusted relative risk and 95% confidence intervals (CI) of allergen test with CD14/-159, CD14/-260, and STAT6 G2964A genotypes in childhood asthma cases compared with matched controls.

Variables	Allergen test positive			Allergen test negative		
	Cases n = 58	Controls n = 59	Adjusted RR_m (95% C.I.)[a]	Cases n = 12	Controls n = 79	Adjusted RR_m (95% C.I.)[a]
CD14/-159 genotype						
TT	15	18	6.4 (1.0-42.7)*	6	28	2.1 (0.3-14.8)
CT	31	28	5.9 (1.0-35.4)*	4	38	0.6 (0.1-4.5)
CC	12	13	6.1 (0.9-41.5)	2	13	1.0
CD14/-260 genotype						
TT	18	17	7.5 (1.0-54.5)*	7	29	2.0 (0.3-14.6)
CT	28	26	5.2 (0.8-35.1)	3	40	0.4 (0.0-3.8)
CC	12	16	4.0 (0.5-28.7)	2	10	1.0
STAT6 G2964A genotype						
AA	24	17	10.0 (2.3-4.5)**	3	18	2.2 (0.3-14.9)
AG	25	31	7.4 (1.8-30.6)**	6	38	1.7 (0.4-8.3)
GG	9	11	10.8 (2.0-56.7)**	3	13	1.0

[a]Relative ratios were adjusted for family history of asthma, parental smoking status, incense burning at home, pet feeding and observed cockroaches in bedroom, and controls were matched to cases on age, gender, and parental education status.

**$P < 0.01$, *$0.01 < P < 0.05$.

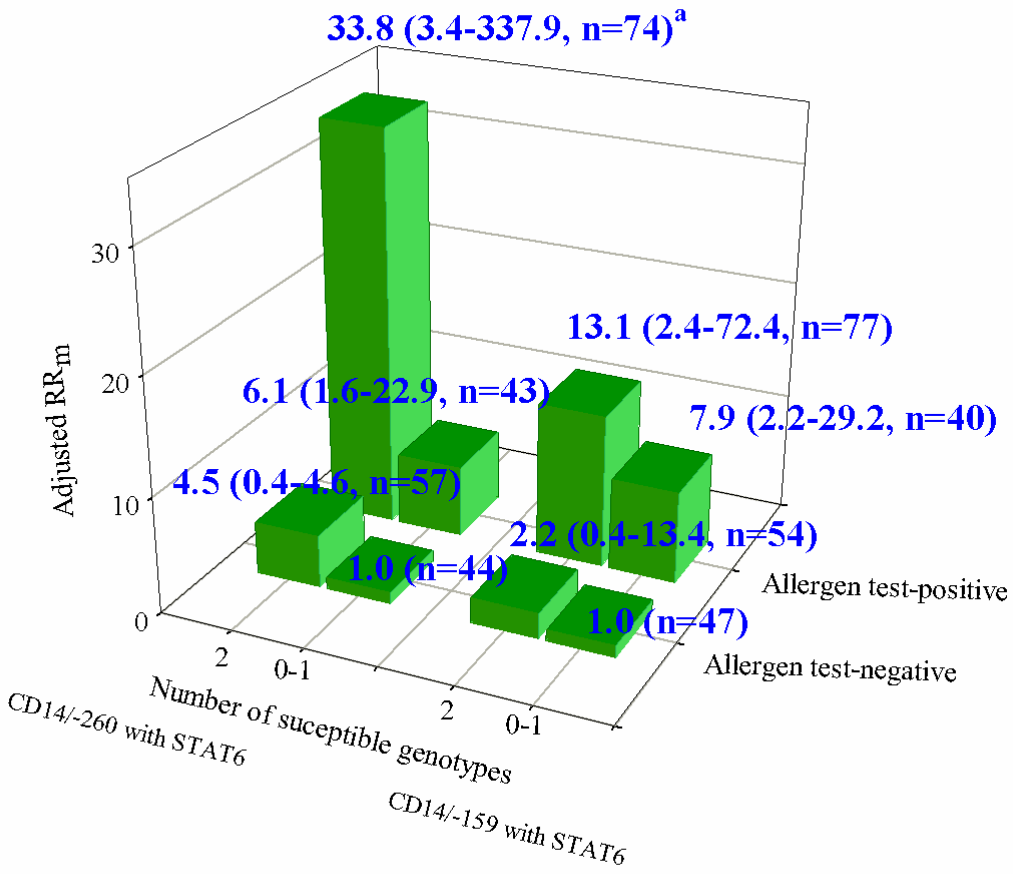

Fig. IV. Adjusted relative risk (RR) of asthma development by allergen test with susceptible CD14 and STAT6 genotypes after adjusting for potential confounding variables. [a] RR_m (95% confidence interval, and sample number).

出席國際學術會議心得報告

計畫編號	NSC 94-2314-B-040-033
計畫名稱	CD14, STAT6 及人類 E-cadherin 基因多形性在兒童氣喘之易感受性角色
出國人員姓名 服務機關及職稱	翁瑞宏 中山醫學大學　　副教授
會議時間地點	2007 年 6 月 9 日至 6 月 13 日; 瑞典歌德堡市 (Goteborg)
會議名稱	歐洲過敏與臨床免疫學會年度會議
發表論文題目	CD14 及 STAT6 易感受基因型與兒童氣喘

一、參加會議經過

過去曾參加過世界頂級的學術研討會，由於在會議中所學到的不僅對於研究上邏輯的思考及實驗上實驗技巧的修正都有極大的收穫，於是又再一次提出申請參加 2007 年歐洲過敏與臨床免疫學會 (European academy of allergology and clinical immunology) 年會。2007 年歐洲過敏與臨床免疫學會 (European academy of allergology and clinical immunology EAACI) 第二十八屆大會於 6 月 9 日至 6 月 13 日在瑞典歌德堡市 (Goteborg) 的 Convention Center 舉行。會議的進行包括口頭報告，壁報展示和廠商展示三部分，而身為僅有的台灣代表，我們也而 6 月 12 日上午進行壁報展示，向與會學者介紹我們的研究成果。

二、與會心得

我們今年於EAACI年會所發表的研究題目是CD14及STAT6易感受基因型與兒童氣喘。第二型輔助T淋巴球 (Th2細胞) 和其他細胞激素在氣喘的致病過程中扮演著重要的角色。CD14為細菌細胞壁成分的多功能接受器，並可能調節兒童時期早期Th1-Th2的比例。信號轉導和轉錄啟動因子(Stat) 6則為影響Th2細胞分化的重要轉錄因子。在此篇研究中，我們以一個醫院為基礎的病例對照研究檢視 CD14/-159、CD14/260以及STAT6 G2964A基因多形性與兒童氣喘相關的假說。而我們的結果顯示，過敏原反應呈陽性並且攜帶CD14/-159 TT基因型、CD14/-260 TT基因型以及STAT6 G2964A AA基因型的兒童分別相較於過敏原反應呈陰性且攜帶CD14/-159 CC基因型、CD14/-260 CC基因型，以及STAT6 GG基因型的兒童有較高之氣喘發病危險性。進一步的分析顯示，帶有較多易感受基因型 (CD14/-159 TT基因型、CD14/-260 TT基因型，以及STAT6 AA基因型) 的過敏原反應陽性兒童有較高之氣喘發病危險性。以上的結果表示，CD14和STAT6 易感受基因型可能調控過敏原反應呈陽性兒童之氣喘發病歷程。

本次與會，聽取各研究單位的研究成果，最大的收獲是感受到相關研究計劃和新技術對生命科學研究的貢獻。由於全球現今對於過敏與免疫疾病患者的人數眾多，極需尋找新的解決之途徑。而首要的是，要提高民眾對於過敏性疾病的認識，過敏性疾病的發病率極高，流行病學資料顯示約有三分之一以上的人在一生中曾罹患過過敏性疾病。過敏性疾病可嚴重至影響患者的生活品質。並且，隨著生活水準的提高，過敏性疾病的發病率越高，比如近年来金金屬飾物過敏、藥物过敏、堅果等食物過敏的發病率也明顯升高，很顯然地與生活水準的的提高有着密切關係。此外，某些過敏性疾病尚有流行性的特點，過敏原的播散可在同一時段造成群體發病，如有些地區的花粉症患病率可達10%以上。然而，流行病學研究也顯示，不同國家地區間免疫疾病的發病率存在很大差異。因此，針對不同地區環境與遺傳因素進行瞭解，也可能對於過敏性疾病尋找出制定預防策略的依據。

這次的會議行程豐富、內容精采，對於本人在研究思路的拓廣、研究的進行及實驗之計畫都有莫大助益。最後建議，希望國科會或教育部能多鼓勵研究學者參加大型國際學術會議，不

僅能吸收新知更能拓展國際觀。（攜回資料: 會議論文摘要）