行政院國家科學委員會專題研究計畫 成果報告

代謝及DNA修補基因多形性與農藥暴露之果農的DNA傷害危險

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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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摘要

農藥 (pesticide) 暴露是相關於若干的惡性腫瘤疾病以及先天畸形 (congenital malformation)，動物試驗已經指出農藥可能被肝臟細胞色素P450 3A5酵素、或是麩胺基硫轉移酶 (glutathione S-transferases) 所代謝。DNA修補基因，包括X-ray repair cross-complementing group 1 (*XRCC1*) 和xeroderma pigmentosum group D (*XPD*) 可能也參與在農藥相關的致癌過程中。因此，我們探討各種代謝與DNA修補基因型對於農藥暴露的果農之DNA傷害是否較具易感受性。彗星試驗 (Comet assay) 被執行來評估135名農藥暴露的果農與106名未暴露的對照其個人周邊血液的DNA傷害程度。代謝*CYP3A5* ($A_{-44}G$) 和*GSTP1* (*Ile*105*Val*) 基因，以及DNA修補*XRCC1* (*Arg*399*Gln*、*Arg*194*Trp*、$T_{-77}C$)、與*XPD* (*Asp*312*Asn*、與*Lys*751*Gln*) 基因之基因型是以聚合酶鏈鎖反應 (polymerase chain reaction [PCR]) 判定。以多變項迴歸模式來分析DNA尾動量，顯示年齡、高農藥暴露、低農藥暴露、*GSTP1 Ile-Ile*、和*XRCC1* 399 *Arg-Arg*基因型是相關於增加的DNA尾動量 (DNA傷害)。對於易感受性代謝*GSTP1*與*XRCC1*基因的交互作用進一步分析，顯示高農藥暴露者攜帶*GSTP1 Ile-Ile*與*XRCC1* 399 *Arg-Arg*基因型是顯著影響DNA尾動量差異 (2.49 ± 0.09 μm/cell; $P = 0.004$)，相較於攜帶*GSTP1 Ile-Val*/*Val-Val*與*XRCC1* 399 *Arg-Gln*/*Gln-Gln*基因型 (1.98 ± 0.15 μm/cell)。這些結果建議個體攜帶易感受性代謝*GSTP1*與DNA修補*XRCC1*基因，可能呈現因農藥暴露所導致DNA傷害之增加危險。

關鍵詞：農藥，*GSTP1*基因，*XRCC1*基因，DNA

Abstract

Pesticide exposure is associated with various neoplastic diseases and congenital malformations. Animal studies have indicated that pesticides may be metabolized by cytochrome P450 3A5 enzymes, or glutathione S-transferases. DNA-repair genes, including X-ray repair cross-complementing group 1 (*XRCC1*), and xeroderma pigmentosum group D (*XPD*) gene may also be implicated in the process of pesticide-related carcinogenesis. Thus, we investigated whether various metabolic and DNA repair genotypes are more susceptible to DNA damage in pesticide-exposed fruit growers. Using the Comet assay, the extent of DNA damage was evaluated in the peripheral blood of 135 pesticide-exposed fruit growers and 106 unexposed controls. The genotypes for metabolic *CYP3A5* ($A_{-44}G$) and *GSTP1* (*Ile*105*Val*) genes, and DNA repair *XRCC1* (*Arg*399*Gln*, *Arg*194*Trp*, $T_{-77}C$), and *XPD* (*Asp*312*Asn*, *Lys*751*Gln*) genes were also identified by polymerase chain reaction. Our multiple regression model for DNA tail moment showed that age, high pesticide exposure, low pesticide-exposure, *GSTP1 Ile-Ile*, and *XRCC1* 399 *Arg-Arg* genotype were found to be associated with an increased DNA tail moment (DNA damage). Further analysis of susceptible *GSTP1* and *XRCC1* genes interaction, revealed a significant difference for high pesticide-exposed subjects carrying both *GSTP1 Ile-Ile* with *XRCC1* 399 *Arg-Arg* genotypes to influence DNA tail moment (2.49 ± 0.09 μm/cell; $P = 0.004$), compared to those carrying *GSTP1 Ile-Val/Val-Val* with *XRCC1* 399 *Arg-Gln/Gln-Gln* genotypes (1.98 ± 0.15 μm/cell). These results suggest that individuals with susceptible metabolic *GSTP1* and DNA repair *XRCC1* genes may experience an increased risk of DNA damage elicited by pesticide exposure.

Key Words: pesticide, *GSTP1* gene, *XRCC1* gene, DNA damage.

前言

56種農藥已經被國際癌症研究中心 (International Agency for Research on Cancer [IARC]) 歸類為對實驗動物是具有致癌性 [2003]，統合分析 (meta-analyses) 的結果也顯示，農藥暴露的農民對於特定癌症的發生是較具危險的，包括白血病 (leukaemia) [Daniels JL. et al., 1997; Zahm SH. and Ward MH., 1997] 以及多發性骨髓癌 (multiple myeloma) [Khuder SA. and Mutgi AB., 1997]；而個人呈現癌症發展的較高危險可能是具備著特定的易感受性因子，包括遺傳代謝與DNA修補特性。

原先的研究顯示台灣最廣泛使用的有機磷農藥，主要是經由肝臟細胞色素P450 (cytochrome P450) 3A4及3A5酵素所代謝，形成具有高度活性的中間產物organophosphorus-oxon [Levi PE. and Hodgson E., 1985; Mutch E. et al., 1999]。進一步地，organophosphorus-oxon可能然後被paraoxonase (PON) 水解成diethyl phosphate以及4-nitrophenol [Costa LG. et al., 1999; Mutch E. et al., 1999]，或經由麩氨基硫轉移酶 (glutathione S-transferases [GSTs]) 的催化與麩氨基硫 (glutathione [GSH]) 接合 [Di Ilio C. et al., 1995; 1996]。一項先前在澳洲所進行的研究也顯示，*GSTP1*基因是相關於曾經暴露於農藥的帕金森症 (Parkinson disease) 病患之增加危險 [Menegon A. et al., 1998]。我們原先的研究也顯示*GSTP1*是相關於農藥暴露的果農之彗星試驗的DNA傷害增加；然而，*PON1*、*PON2*、*GSTM1*與*GSTT1*基因型明顯地並沒有影響農藥暴露之果農和對照對象的彗星試驗之DNA尾部動量。而攜帶*CYP3A5* $G_{-44}G$基因型的農藥暴露對象，在彗星試驗中也具有較高之DNA傷害；雖然，在我們先前的研究中因為較少的對象個數而限制了我們做出一個確切的結論 [Liu YJ. et al., 2006]。

除了代謝特性外，DNA 修補能力也在農藥相關的致癌機制中扮演重要角色。有幾項已知的 DNA 修補途徑，提供清楚但是重疊的保護以對抗致突變性暴露。DNA 單股斷裂 (DNA single-strand breaks) 是最常見的 DNA 損傷，是直接地傷害去氧核糖分子或者是間接地當作 DNA 鹼基切除修補 (base excision repair [BER]) 的中間產物所產生 [Beckman KB. et al., 1997; Lindahl T. et al., 1993; Xu YJ. et al., 1998]。而留下未修補的 DNA 單股斷裂，則是基因穩定性和細胞存活、加速突變比率以及染色體變異之增加程度的主要威脅 [Carrano AV. et al., 1986; Dominguez I. et al., 1998; Thompson LH. et al., 1982]。X-ray cross-complementing group 1 (*XRCC1*) 基因產物協調 DNA 聚合酶β、DNA 接合酶 III_{α}、以及 poly(ADP-ribose) polymerase、APE1、多核苷酸激酶/磷酸酶 (polynucleotide kinase/phosphatase)、和 8-oxoguanine DNA glycosylase [Caldecott KW. et al., 1996; Kubota Y. et al., 1996; Marsin S. et al., 2003; Masson M. et al., 1998; Whitehouse CJ. et al., 2001]。分子流行病學研究也已經探討 *XRCC1* 多形性與改變癌症危險間的可能相關。*Arg*399*Gln* (exon 10，鹼基 G→A) 是位於 *XRCC1* 與 poly (ADP-ribose) polymerase 之 BRCT-I 交互作用的區域，而 *Arg*194*Trp* (exon 6，鹼基 C→T) 則發生於可辨識的 PCNA 結合區域 [Fan J. et al., 2004; Shen MR. et al., 1998]。雖然，399*Gln* 和 194*Trp* 變異對偶基因的存在已經被顯示是相關於一些 DNA 修補的表現型態；例如測量 DNA 鍵結物的持續存在 [Lunn RM. et al., 1999]、增加的 *p53* 突變 [Hsieh LL. et al., 2003] 和延長細胞週期的延遲 [Hu JJ. et al., 2002]；然而，從分子流行病學研究的結果仍然是不一致的而非明確 [Goode EL. et al., 2002]。

最近，位於 *XRCC1* 啟動者區域之核苷酸-77 位置上的一個新的 T 至 C 的置換已經被辨識，並且此項取代是相關於在中國族群的食道鱗狀細胞癌危險 [Hao B. et al., 2004]。生物資訊 (Bioinformatic) 分析建議著 $T_{-77}C$ 多形性可能瓦解了 Sp1 結合位置的合理序列，暗示著此多形性可能具有改變 *XRCC1* 轉錄的潛在性。

核酸切除修補 (nucleotide excision repair [NER]) 路徑主要是移除並且修補龐大的鍵結物，但是在氧化性 DNA 傷害上的修補角色也已經被報告出來 [Arbault S. et al., 2004; Misra RR. et al., 2003]。Xeroderma pigmentosum group D (*XPD*) 蛋白，是轉錄因子 IIH 的次單位，也是個演化保留的 5'→3'解旋酶以解開在 DNA 受損區域的 DNA。*XPD* 基因的單一核苷酸多形性 (single nucleotide polymorphisms [SNP]) 已經被研究 [Goode EL. et al., 2002]，於 exon 10 的 *XPD Asp*312*Asn* 導致在 *XPD* 保留區域產生一個氨基酸置換，於 exon 23 的 *XPD Lys*751*Gln* 也引起在蛋白質的 C 終端部分產生一個氨基酸置換。*XPD* exon 10 和 exon 23 的變異對偶基因存在已經被顯示相關於較高的癌症危險 [Hou SM. et al., 2002; Zhou W. et al., 2002]，然而其他研究無法發現統計顯著相關 [Butkiewicz D. et al., 2001; David-Beabes GL. et al., 2001]。

因此，農藥暴露的個人具有遺傳易感受性代謝和DNA修補基因型可能呈現增加的DNA傷害。在本研究，我們探討代謝與DNA修補基因多形性與農藥暴露的果農在彗星試驗中之細胞DNA傷害的相關。

材料與方法

研究族群

原先，我們於台灣中部地區的東勢鎮執行一項研究以探討91名農藥暴露果農與106名未暴露的對照之DNA傷害與代謝特性的相關；此研究族群的選取條件已詳細地被描述於他處 [Liu YJ. et al., 2006]。為了獲取充分的統計檢定力以偵測DNA傷害程度於不同的遺傳代謝與DNA修補基因多形性的差異性，我們擴增了農藥暴露之果農的樣本數目。在現今的研究中，總計有135名農藥暴露之果農與106名未暴露的對照被納入。傳統上，當地的農會提供農民保險、財政資源、市場服務以及教育訓練給包含全職及業餘農民的會員。在當地的農地，整年當中農藥被常規地使用。參與我們說明會的當地農民成員，被邀請參與當作暴露對象；來自當地非農業人口中未曾暴露過農藥者也被邀請參與當作非暴露的對照。我們藉由選擇與農藥暴露對象之相同地理區域與種族的對照對象，嘗試來減少一些可能來自種族與生活方式的偏差。對照之職業包括家庭主婦、教師、銷售員、非農業之勞工、技術人員、小本生意經營者以及其他專業人員。在這些對象中，並沒有人接受過放射線治療，他們也沒有服用任何藥物。

流行病學資料

研究對象的個人特徵資料，是在每位研究對象提供其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、生活型態如抽菸及飲酒習慣、以及詳細工作史與疾病史。研究對象的抽菸史包括每天抽菸支數及抽菸年數；累積抽菸量是以抽菸包年計算，亦即每天的包數乘以抽菸的年數。大部分的

台灣農民已經對於飲酒所增加之肝臟傷害危險具有認知，並且對於飲酒可增加農藥中毒具有良好的理解。一般而言，在噴灑農藥期間飲酒是不允許的行為；我們考慮一旦暴露農藥的個體在此情況下被納入我們的研究中，其飲酒之盛行率將較對照為低。因此，具有飲酒行為的研究對象在資料分析中被排除。

農藥暴露評估

農藥的處理過程則包含稀釋、混合、裝載、噴灑以及使用機械的維護與清潔。在研究中，過去農藥使用的資訊包括名稱、數量、農藥使用面積、每季農藥使用次數、農藥暴露年數以及個人的防護設施，皆透過面對面的問卷訪視所獲得。果園的平均面積為1.28公頃 (範圍從0.06-4.85公頃)，果農在健康檢查前六個月期間所使用的農藥成分將近有30種不同的化合物組成，包括有機磷、氨基甲酸鹽、除蟲菊殺蟲劑、殺真菌劑、以及生長調節劑，然而有機氯是不被使用的。每位暴露者每個月平均噴灑農藥三次，並且每月平均累積噴灑時間大約為7小時 (範圍2-28小時/月)。

遺憾地，由於缺乏環境偵測的數據，對於我們研究對象的農藥暴露劑量是無法被計算；我們因此根據Scarpato等人 [1996] 原先所建議的以下標準加以修飾，將果農區分成高或低農藥暴露：(1) 對於每位噴灑農藥的研究對象，其工作噴灑農藥的總公頃數是藉由平均每年農藥使用次數 × 噴灑面積公頃，(2) 以及藉由計算 (1) 所得的數值分佈之中位數，若果農的暴露數值低或高於中位數，則分別被歸類為低或高暴露組，(3) 研究對象沒有直接處置農藥者 (例如修剪或採摘果實) 則被考慮為低暴露組。

彗星捕捉與分析

彗星試驗是依照 Singh 等人 [1988] 的研究在鹼性的條件下執行。研究對象的靜脈血被收集於含有肝素的採血管中，10 μL 的全血被懸浮在 1.5%低熔點的瓊膠 (low-melting point agarose) 內，然後在一片完全冷凍的玻片上，被包覆於 0.6%一般熔點瓊膠 (normal melting point [NMP] agarose) 及 1.5%低熔點的瓊膠之中。玻片在 4°C 下，再置入溶解液 (1% sodium sarconisate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 1% Triton X-100 以及 DMSO 10%) 中浸潤；一小時後，玻片被置於電泳緩衝液 (0.3 M NaOH, 1 mM Na_2EDTA, pH 13) 中 10 分鐘。在相同的電泳緩衝液及 300 mA 電流的條件下，電泳被執行 15 分鐘；玻片再以滅菌水中性化三次各五分鐘，並以 10% ethidium bromide 染色。對於每個研究對象，100 個隨機捕獲的彗星影像 (四個彗星玻片，每個玻片 25 細胞) 從玻片中藉由 400 倍放大之螢光顯微鏡連結黑白攝影機至影像分析系統 (Comet Assay II; Perceptive Instruments Ltd, Suttolk, United Kingdom) 來檢查。電腦影像分析系統所獲取的影像，被計算每個細胞所積分的強度，估計彗星細胞的組成，以及評估所衍生的參數範圍。未受傷的細胞有一個完整的核而未具有尾部，受傷的細胞則有明顯的彗星外形。DNA 傷害尾動量 (tail moment) 程度被評估以定量 DNA 傷害程度，尾動量是由尾部長度與 DNA 在彗星尾部的比例之乘積來計算。所有的玻片是由一名不知研究對象的狀態之操作者所計數。

基因多形性分析

基因體 DNA 是由研究對象之周邊血液所萃取出，基因型是藉由以下所描述之以聚合酶鏈鎖反應 (Polymerase chain reaction [PCR]) 為基礎的方法來分析；並且在不知研究對象之暴露狀態下完成基因型分析。嚴謹的品質控制過程在基因型分析中徹底地被要求，為避免 PCR 污染，對於 PCR 反應之試劑被小心地使用，每個試劑使用不超過三次。對於每次分析，一個陰性對照 (無 DNA 模板) 被添加來偵測 PCR 之污染；前測試驗也總被執行，以獲取最佳的限制酶消化條件。在判別每一個基因多形性之後，每一個基因型之樣本被隨機地選取 20%到 25%的樣本執行重複試驗以確定結果。

CYP3A5 $A_{-44}G$ 基因型的判定是根據 Chou 等人 [2001] 的研究來進行。簡單地說，先進行聚合酶鏈鎖反應增幅後，再以限制片段長度多形性 (restriction fragment length polymorphism [RFLP]) 分析來偵測 *Fau*I 作用點的差異。用以增幅 *CYP3A5* 基因的引發子 (primers) 序列為 5'-CAG GTG AGA GGA TAT TTA AGA GGC-3'和 5'-CAT CGC CAC TTG CCT TCT TCA AC-3'。*GSTP1-Alw*26I 基因多形性也是根據 Harries 等人 [1997] 之 PCR-RFLP 技術來加以判定。在 exon 5 (密碼子 105) 的一個 *Ile* 至 *Val* 置換被增幅以產生一段 177 bp 的未消化片段，使用的引發子序列為 5'-ACC CCA GGG CTC TAT GGG AA-3' 和 5'-TGA GGG CAC AAG AAG CCC CT-3'。*XRCC1* 於 exon 10 之基因多形性的判定是根據先前已描述的研究所執行 [Wong RH. et al., 2002]，在 exon 10 (密碼子 399) 的一個 *Arg* 至 *Gln* 置換被增幅以產生一段 242 bp 的未消化片段，使用的引發子序列為 5'-CCC CAA GTA CAG CCA GGT C-3'和 5'-TGT CCC GCT CCT CTC AGT AG-3'。對於 *XRCC1* 於 exon 6 的基因型分析是根據先前已被描述的研究加以一些修改 [Shen H. et al., 2000]，在 exon 6 (密碼子 194) 的一個 *Arg* 至 *Trp* 置換被增幅以產生一段 485 bp 的未消化片段，使用的引發子序列為 5'-GCC AGG GCC CCT CCT TCA A-3'和 5'-TAC CCT CAG ACC CAC GAG T-3'。*XRCC1 $T_{-77}C$* 基因多形性也是藉由 PCR-RFLP 偵測來判定 [Hao B. et al., 2004]，使用的引發子序列為 5'-GGG CTG GAG GAA ACG CTC-3'和 5'-TGG CCA GAA GGA TGA GGT AGA G-3'以增幅此啟動者片段。*XPD* 基因型也被藉由 PCR-RFLP 分析來加以決定 [Spitz MR. et al., 2001]，對於 *XPD* 包含多形性 *Sty*I 限制位置之 exon 10 區域的增幅，所使用的引發子序列為 5'-CTG TTG GTG GGT GCC CGT ATC TGT TGG TCT-3'和 5'-TAA TAT CGG GGC TCA CCC TGC AGC ACT TCC T-3'；對於 *XPD* Lys751Gln 基因的 PCR 引發子序列為 5'-GCC CGC TCT GGA TTA TAC G-3'和 5'-CTA TCA TCT CCT GGC CCC C-3'。

統計分析

對於研究對象的收案年齡、性別、農藥暴露年數、果園面積、現今抽菸狀況、以及抽菸包年於高與低農藥暴露及對照組間的比較，利用Student's *t* test與ANOVA來針對連續變項進行檢定，χ^2 test 則用以檢定類別變項。χ^2 test或Fisher's exact test被使用來檢定在高、低農藥暴露與對照組間*CYP3A5*、*GSTP1*、*XRCC1*、與*XPD*基因型的盛行率。後續上，根據農藥暴露與不同因子進行分層分析以評估DNA尾動量；ANOVA與Student's *t* test被使用來比較在不同農藥暴露狀態間的DNA尾動量之差異，也被使用來檢定DNA尾動量與年齡、性別、抽菸狀況和不同代謝以及DNA修補基因間的相關。這些變項與DNA尾動量的相關則進一步地以簡單直線迴歸模式 (general linear model [GLM]) 評估；最後，最小平方均

(least-squares mean) 被執行來預測攜帶易感受性基因之不同合併狀況者的調整後DNA尾動量。全部數據以SAS 9.1 (SAS Institute, Cary, NC) 分析，並且統計檢定是以雙尾檢定執行。

結果

135 名農藥暴露的研究對象與 106 名未暴露的對照被納入分析，研究對象的人口學特質整理於表一。研究對象的平均年齡為 54 歲，平均年齡 (P = 0.68，t test)、農藥暴露時程 (P = 0.40)、現今抽菸者的比例 (P = 0.55，χ^2 test)、以及抽菸包年 (P = 0.17) 在高、低農藥暴露組間並未達到統計顯著差異。性別 (P = 0.01)、以及果園平均面積 ($P < 0.01$) 在高、低農藥暴露組間達到統計顯著差異。相對地，對照組相較於農藥暴露組在年齡上是顯著較為年輕 ($P < 0.01$，ANOVA)、較多女性 ($P < 0.01$)、以及具有較少的抽菸包年 ($P < 0.01$)。

研究對象的 *CYP3A5*、*GSTP1*、*XRCC1*、以及 *XPD* 基因型比例呈現於表二。*CYP3A5* (P = 0.72，Fisher's exact test)、*GSTP1* (P = 0.32)、*XRCC1* 194 (P = 0.12)、*XRCC1* 399 (P = 0.28)、*XRCC1* $_{-77}$ (P = 0.61)、*XPD* 312 (P = 0.88)、以及 *XPD* 751 (P = 0.52) 基因型比例在高、低農藥暴露及對照組間並無顯著差異；而所有基因型的分布皆是在 Hardy-Weinberg 平衡中。在我們隨後的分析中，因為攜帶至少一個 *CYP3A5* A_{-44} 對偶基因者，原先已經被顯示相較於攜帶 *CYP3A5* G_{-44} 對偶基因者呈現較低的酵素活性 [Kuehl P. et al., 2001]，於是攜帶至少一個 *CYP3A5* A_{-44} 對偶基因者被歸類為 *CYP3A5* $A_{-44}G/A_{-44}A$ 基因型。具有至少一個 *GSTP1 Val* 對偶基因的個人相較於攜帶 *GSTP1 Ile* 對偶基因者也具有較低的酵素活性 [Zimniak P. et al., 1994]，並且因為 *GSTP1 Val-Val* 基因型者的個數很小，因此 *GSTP1 Ile-Val* 與 *Val-Val* 基因型被合併在一起。當統計檢定力被考量，因為攜帶 *XRCC1* 194 *Trp-Trp* 基因型者的個數很小，因此，個人於 *XRCC1* 密碼子 194 上呈現 *Trp-Trp* 與 *Arg-Trp* 基因型被歸類在一起。攜帶至少一個 *XRCC1* 399 *Gln* 對偶基因者被歸類為 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 基因型。攜帶至少一個 *XRCC1* C_{-77} 對偶基因者被歸類為 *XRCC1* $T_{-77}C/C_{-77}C$ 基因型。同樣地，具有 *XPD* 312*Asn-Asn* 與 751*Gln-Gln* 的人數較少，並且因為具有至少一個 *XPD* 312*Asn* 對偶基因與 *XPD* 751*Gln* 對偶基因者具有較低的 DNA 修補活性 [Spitz MR. et al., 2001]，因此，攜帶至少一個 *XPD* 312*Asn* 對偶基因者被歸類為 *XPD* 312 *Asp-Asn*/*Asn-Asn* 基因型，以及攜帶至少一個 *XPD* 751*Gln* 對偶基因者被歸類為 *XPD* 751 *Lys-Gln*/*Gln-Gln* 基因型。

表三呈現出測試對象之各個因子與尾動量之間的初步相關性。經歷高農藥暴露的個人顯示出最大的尾動量 (2.31 μm/cell)，接續為低農藥暴露的個人 (2.03 μm/cell) 以及對照 (1.33 μm/cell；$P < 0.01$，ANOVA)。相同地，年齡大於 54 歲者 (全體研究對象之平均年齡) 也顯示出較大的尾動量，特別是在高農藥暴露組中 (2.44 vs. 2.11 μm/cell；$P < 0.01$，t test)。現今抽菸者在高農藥暴露組中相較於過去抽菸者與非抽菸者具有較低的尾動量 ($P < 0.01$)。然而，較大的尾動量在男性以及吸菸包年數較多者中並未被發現。進一步地，較大的 DNA 尾動量在高農藥暴露組並且攜帶 *GSTP1 Ile-Ile* 基因型的個人中被發現 (vs. *Ile-Val*/*Val-Va*l，P = 0.03)。有趣的是，在攜帶 *XRCC1* 399 *Arg-Arg* 基因型的高農藥暴露組相較於攜帶 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 之高農藥暴露組也被觀察到具有較大的 DNA 尾動量 (P = 0.03)。在低農藥暴露組且攜帶 *XPD* 312 *Asp-Asp* 基因型的個人相較於攜帶

XPD 312 *Asp-Asn*/*Asn-Asn* 也具有較大的 DNA 尾動量 ($P < 0.01$)。然而，並沒有明顯的統計相關性在尾動量與 *CYP3A5* 基因型、*XRCC1* 194 基因型、*XRCC1* $_{-77}$ 基因型、以及 *XPD* 751 基因型間被發現。

以 DNA 尾動量為應變項，年齡、性別、抽菸習慣、農藥暴露、以及 *CYP3A5*、*GSTP1*、*XRCC1* 194、*XRCC1* 399、*XRCC1* $_{-77}$、*XPD* 312、以及 *XPD* 751 基因型為自變項的多變項直線迴歸模式 (GLM) 被呈現於表四。當多重比較 (Bonferroni correction) 被考量，DNA 尾動量是正向相關於增加的年齡 ($P < 0.01$)、高農藥暴露 ($P < 0.01$) 與低農藥暴露 ($P < 0.01$)。有趣的是，尾動量較大的差異在個人呈現 *GSTP1 Ile-Ile* 基因型 ($P = 0.04$) 與 *XRCC1* 399 *Arg-Arg* 基因型 ($P = 0.058$) 中被觀察到。

隨後，最小平方均 (least-squares mean) 被進一步地執行以評估在調整年齡、性別、抽菸習慣的效應後，*GSTP1* 以及 *XRCC1* 399 基因多形型在不同農藥暴露狀態對於 DNA 傷害的聯合效應 (表五)。當攜帶 *GSTP1 Ile-Val*/*Val-Val* 與 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 基因型的對照被選擇為參考組 (1.26 ± 0.06 μm/cell)，攜帶 *GSTP1 Ile-Ile* 及 *XRCC1* 399 *Arg-Arg* 基因型的對照在 DNA 尾動量上顯現出 0.11 μm/cell 的增加 (1.37 ± 0.06 μm/cell)。特別地，在攜帶 *GSTP1 Ile-Val*/*Val-Val* 與 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 基因型之高農藥暴露者被選擇為參考組 (1.98 ± 0.15 μm/cell)，則同時攜帶 *GSTP1 Ile-Ile* 及 *XRCC1* 399 *Arg-Arg* 基因型的高農藥暴露者具有最高的 DNA 尾動量 (2.49 ± 0.09 μm/cell；vs. 參考組，$P = 0.004$)，同時攜帶 *GSTP1 Ile-Ile* 及 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 基因型之高農藥暴露者 (2.28 μm/cell，$P = 0.09$)，與同時攜帶 *GSTP1 Ile-Val*/*Val-Val* 與 *XRCC1* 399 *Arg-Arg* 基因型者 (2.25 μm/cell，$P = 0.15$) 則有中度增加的 DNA 傷害程度。

討論

辨識可修飾個體暴露於致癌物質的反應之潛在基因-易感受性因子是重要的。在本研究中，我們觀察到代謝 *GSTP1* 與 DNA 修補 *XRCC1* 399 基因型以及農藥暴露是顯著相關於增加的 DNA 尾動量。

在我們的研究中，*CYP3A5 G*$_{-44}$ 對偶基因頻率 (26.6%) 是一致於對於台灣族群所執行的一項先前研究之結果 [28.2%；Chou FC. et al., 2001]；於本研究中之 *GSTP1* 105*Val* 對偶基因盛行率 (22.4%) 則相當明顯地與一項原先針對台灣人所執行的研究相似 [18%；Watson MA. et al., 1998]。*XRCC1* 194*Trp* 對偶基因 (28.4%) 和 399*Gln* 對偶基因 (23.4%) 呈現頻率也與先前針對台灣人所報告的一項研究類似 [194*Trp* 對偶基因 27%，399*Gln* 對偶基因 26%；Lunn RM. et al., 1999]；我們研究對象的 *XRCC1 C*$_{-77}$ 對偶基因盛行率 (11.2%) 則是接近於關於中國族群所報告的一項研究 [10%；Hao B. et al., 2004]。此外，*XPD* 312*Asn* 對偶基因頻率 (4.4%) 與 *XPD* 751*Gln* 對偶基因頻率 (6.0%) 也是相似於對於華人的一項原先研究 [312*Asn* 對偶基因 6.5%，751*Gln* 對偶基因 8.7%；Liang G. et al., 2003]；這些結果證實我們基因型技術的實行和成果。

彗星試驗對於評估 DNA 傷害是一項敏感的方法。彗星試驗在本研究所呈現的結果，與部份先前的研究 [Garaj-Vrhovac V. and Zeljezic D., 2000; Lebailly P. et

al., 1998]，已經顯示出暴露到農藥多重混合物的個體其周邊血液之增加的 DNA 傷害。進一步地，對於代謝農業化學物或修補隨後的 DNA 損傷之酵素其基因個別變異也可能參與在此過程中；當這些酵素在去毒性與 DNA 修補上無法作用時，代謝性產物就會累積並且 DNA 傷害持續，而貢獻至致癌過程中。

過去的研究顯示，如有機磷類的農藥主要是經由肝臟細胞色素 P450 酵素代謝成具有高度活化的中間產物 organophosphorus-oxon [Levi PE. and Hodgson E., 1985; Mutch E. et al., 1999]，而此中間產物然後被 PON 水解成 diethyl phosphate 以及 4-nitrophenol [Costa LG. et al., 1999; Mutch E. et al., 1999]，或經由 GSTs 的催化與 GSH 接合 [Di Ilio C. et al., 1995; 1996]。然而，我們先前的研究中並無法證實在彗星試驗中 *PON1*、*PON2*、*GSTM1* 與 *GSTT1* 基因型和 DNA 傷害之間的相關性；因此，*PON1*、*PON2*、*GSTM1* 與 *GSTT1* 基因型並沒有被納入現今的研究中。我們再次於農藥暴露中評估 *CYP3A5* 基因型對於 DNA 傷害之效應，然而，無法觀察到統計顯著。攜帶易感受性 *CYP3A5* $G_{-44}G$ 基因型的個體數目依然相當少，因此，這似乎可能是在我們的研究對象中，我們無法觀察到 *CYP3A5* 基因多形性與 DNA 傷害之間有顯著相關性的原因。重要的是，農藥暴露的果農攜帶有 *GSTP1 Ile-Ile* 基因型相較於未攜帶有此基因型者反映出較高的 DNA 傷害。一項先前的報告也觀察到，*GSTP1 Ile-Ile* 攜帶者相較於 *GSTP1 Ile-Val* 與 *Val-Val* 攜帶者較容易形成 benzo(a)pyrene diolepoxide (BPDE)-DNA 鍵結物 [Watson MA. et al, 1998]。農藥暴露的果農攜帶 *GSTP1 Ile-Ile* 基因型反映出較低代謝活性，相較於攜帶 *GSTP1* Ile-Val 與 Val-Val 基因型者，因此前者是較可能經歷較高的 DNA 傷害程度。

已知在保護以對抗基因突變與癌症起始上，DNA 修補是一項非常重要的機制。DNA 傷害可能被環境致癌物質如農藥和/或藉由易感受性代謝過程所誘發，如果沒有修補，如此傷害便會導致基因突變和基因體的不穩定性。在我們的研究，我們進一步地探討，在 BER 與單股斷裂修補中為一項關鍵角色的蛋白 XRCC1，與參與在 NER 與基礎轉錄之解旋酶 *XPD* 之基因多形性，在農藥暴露的個體是否能夠對於發展 DNA 傷害具有增加危險。

先前的研究已經觀察到，具有無功能性的*XRCC1*蛋白之突變老鼠或是中國倉鼠 (Chinese hamster) 卵巢細胞 (EM9和EM-C11) 對於致烷基化物質 (alkylating agents)、反應性氧化性物種 (reactive oxygen species) 或是游離輻射所導致的廣泛性DNA傷害具有高度敏感性 [Tebbs RS. et al., 1999; Thompson LH. et al., 1982; 1990]。有趣的是，我們發現在我們的農藥暴露族群中*XRCC1* 399 *Arg-Arg*基因型是相關於增加的DNA尾動量；並沒有顯著相關在*XRCC1* 194基因型、*XRCC1* $_{-77}$基因型與增加的DNA尾動量於我們的研究中被發現。關於*XPD*基因，此研究族群無論是*Asp*312*Asn*或者是*Lys*751*Gln*多形性皆無影響DNA傷害程度。XRCC1蛋白對於DNA BER、單股斷裂修補與基因穩定度之維持是有其獨特的需求性 [Fan J. et al., 2004; Shen MR. et al., 1998]；但是對於*XRCC1*基因多形性的功能性影響仍然不清楚。實驗研究已經觀察到*XRCC1* 399*Gln*對偶基因是相關於增加的DNA鍵結物量 [Lunn RM. et al., 1999]、增加的*p53*突變 [Hsieh LL. et al., 2003] 和延長細胞週期的延遲 [Hu JJ. et al., 2002]；而並無先前研究對於DNA修補基因多形性與由農藥暴露所導致的DNA傷害之間的潛在相關進行探討。然而，我們的結果發現減少的DNA傷害危險存在於攜帶*XRCC1* 399*Gln*對偶基因之農藥暴露者，建議著

細胞凋亡 (apoptosis) 的增加為一個可能之機制。在細胞凋亡中XRCC1的角色較未被考量，但是未修補的BER中間產物一是具基因斷裂特性，並且可能會扮演細胞凋亡路徑上之強烈促使者 [Kaina B. et al., 2001]。一項動物研究已經顯示冷凍腦部傷害之細胞凋亡的引發是緊密相關於XRCC1的表現減少 [Fujimura M. et al., 2000]。因此， XRCC1蛋白的減少功效 (399*Gln*對偶基因的結果) 可能會減少修補DNA傷害的能力，並且如此細胞可能較會趨向細胞凋亡。另一項可能的解釋為*XRCC1*變異在DNA修補能力上之效應可能會由於DNA傷害暴露之型態與強度而有所不同；未來的研究是需要來驗證這些假說。

另一方面，我們並無法在我們農藥暴露者中觀察到 *XPD* 基因多形性與 DNA 傷害之間的任何相關。如此的結果可能暗示著針對龐大的 DNA 傷害之修補的主要路徑－NER 系統，可能並不參與由農藥所引起的 DNA 傷害之修補。此外，必須要謹慎地去解釋這些結果，因為已知有超過 20 種基因參與在 NER 路徑中，並且每一個人都可能為在一個基因中是野生型但在另一個基因中是變異的。

更進一步地，在我們的彗星試驗中農藥暴露的個體攜帶更多 *GSTP1 Ile-Ile* 和 *XRCC1* 399 *Arg-Arg* 易感受性基因型是較有可能貢獻於 DNA 傷害的增加程度。*GSTP1* 對於農藥反應性代謝產物扮演去毒性酵素 [Di Ilio C. et al., 1995; 1996]，而 *XRCC1* 則參與在隨後的 DNA 修補 (或細胞凋亡) 過程中。這些結果顯示出每種易感受性基因型可能會對 DNA 傷害產生中度的危險；然而，當它們被合併在一起，一個更明顯的危險可能發展出。

我們藉由選取與農藥暴露對象相同地理環境與種族之對照，嘗試減少由生活型態與種族所產生的一些可能誤差。然而，在我們研究區域中現今從事的農民族群大多數由較年長者所構成；年輕者多數對於從事農業工作的意願低落。此外，我們的對照對象並無與暴露農藥者在年齡上進行配對。因此，在我們的研究中，對照組在年齡上是顯著地較為年輕。如同預期的，年長的抽菸農民相較於年輕者也具有較多的抽菸包年。原先的報告也顯示，年齡是相關於增加的 DNA 傷害 [Moller P. et al., 2000; Singh NP. et al., 1991]；在現今的農藥暴露之果農研究中，年齡較大者也是顯著相關於較大的 DNA 尾動量，在年長者中較大的 DNA 尾動量反映出年齡、農藥或者是與其他未知的致癌物或致突變物的累積，會增加對於 DNA 傷害的易感受性。此外，性別在我們的研究中並未相關於增加的 DNA 傷害，並且也沒有醫學文獻的資料顯示實質上的性別差異。原先報告顯示抽菸相關於 DNA 傷害 [Moller P. et al., 2000]，然而現今的研究沒有發現抽菸與 DNA 傷害具有任何正向相關。這可能是因為相較於其他研究的參與者之抽菸量 [Garaj-Vrhovac V and Zeljezic D., 2000]，現今研究的抽菸數量是相當小。

重建個人原先的農藥暴露史，包括在處置農藥時的個人保護程度，是困難的工作。在本研究中，可獲得的過去暴露資料不足，並且缺乏對於累積暴露量的定量估計。而個人暴露資料是在未知健康狀態下所獲得，因此，暴露的錯誤分組可以被假設是沒有方向的，並且假如存在，將傾向於低估 DNA 傷害的危險性。

總體而言，結果顯示易感受性代謝 *GSTP1* 與 DNA 修補 *XRCC1* 基因可能修飾農藥暴露的果農的 DNA 傷害。未來需要更進一步地探討代謝與 DNA 修補基因型與農藥暴露所導致之癌症間的相關。

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計畫成果自評

農藥可能被肝臟細胞色素 P450 3A5 酵素、或是麩胺基硫轉移酶所代謝。DNA 修補基因可能也參與在農藥相關的致癌過程中。因此，我們探討各種代謝與 DNA

修補基因型對於農藥暴露的果農之 DNA 傷害是否較具易感受性。彗星試驗被執行來評估 135 名農藥暴露的果農與 106 名未暴露的對照其個人周邊血液的 DNA 傷害程度。代謝 *CYP3A5* ($A_{-44}G$) 和 *GSTP1* (*Ile*105*Val*) 基因，以及 DNA 修補 *XRCC1* (*Arg*399*Gln*、*Arg*194*Trp*、$T_{-77}C$)、與 *XPD* (*Asp*312*Asn*、與 *Lys*751*Gln*) 基因之基因型是以聚合酶鏈鎖反應判定。以多變項迴歸模式來分析 DNA 尾動量，顯示年齡、高農藥暴露、低農藥暴露、*GSTP1 Ile-Ile*、和 *XRCC1* 399 *Arg-Arg* 基因型是相關於增加的 DNA 尾動量 (DNA 傷害)。對於易感受性代謝 *GSTP1* 與 *XRCC1* 基因的交互作用進一步分析，顯示高農藥暴露者攜帶 *GSTP1 Ile-Ile* 與 *XRCC1* 399 *Arg-Arg* 基因型是顯著影響 DNA 尾動量差異，相較於攜帶 *GSTP1 Ile-Val*/*Val-Val* 與 *XRCC1* 399 *Arg-Gln*/*Gln-Gln* 基因型。總體而言，我們的結果建議著個體攜帶易感受性代謝 *GSTP1* 與 DNA 修補 *XRCC1* 基因，可能呈現因農藥暴露所導致 DNA 傷害之增加危險。如此的結果可提供環境醫學研究對於農藥危害的參考依據。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：根據不同暴露強度分組之農藥暴露的果農與對照的人口學特質

變項	對照	農藥暴露	
		低	高
個數	106[*]	62	73
年齡 (歲)	48.9 ± 1.1[*]	57.5 ± 1.2	56.8 ± 1.2[†]
性別：男性 (%)	38 (35.8%)	30 (48.4%)	51 (69.9%)[†,‡]
農藥暴露年數 (年)	0	30.0 ± 2.1	32.3 ± 1.8
果園面積 (公頃)	0	0.8 ± 0.1	1.7 ± 0.1[‡]
抽菸狀態			
現今抽菸者 (%)	14 (13.2%)	11 (17.7%)	16 (21.9%)
抽菸包年	2.1 ± 0.6	5.7 ± 1.8	9.9 ± 2.5[†]

[*]數據以個人之個數或連續變項之平均值 ± 標準誤呈現。

[†] $P < 0.01$；對照組顯著不同於高及低農藥暴露組。

[‡] $P < 0.01$，相較於低農藥暴露組。

表二：根據農藥暴露分組之農藥暴露果農與對照間之*CYP3A5*、*GSTP1*、*XRCC1*、以及*XPD*基因型之盛行率

基因型		對照	農藥暴露	
基因	對偶基因		低	高
個數		106	62	73
CYP3A5	$A_{-44}A$*	55 (51.9%)	35 (56.5%)	40 (54.8%)
	$A_{-44}G$	41 (38.7%)	23 (37.1%)	30 (41.1%)
	$G_{-44}G$	10 (9.4%)	4 (6.4%)	3 (4.1%)
GSTP1	*Ile-Ile*	56 (52.8%)	40 (64.5%)	49 (67.1%)
	Ile-Val	43 (40.6%)	19 (30.7%)	22 (30.1%)
	Val-Val	7 (6.6%)	3 (4.8%)	2 (2.8%)
XRCC1 194	*Arg-Arg*	55 (51.9%)	22 (35.5%)	42 (57.5%)
	Arg-Trp	44 (41.5%)	35 (56.5%)	28 (38.4%)
	Trp-Trp	7 (6.6%)	5 (8.0%)	3 (4.1%)
XRCC1 399	*Arg-Arg*	66 (62.3%)	38 (61.3%)	40 (54.8%)
	Arg-Gln	35 (33.0%)	22 (35.5%)	24 (32.9%)
	Gln-Gln	5 (4.7%)	2 (3.2%)	9 (12.3%)
$XRCC1_{-77}$	$T_{-77}T$	79 (74.6%)	50 (80.6%)	60 (82.2%)
	$T_{-77}C$	26 (24.5%)	12 (19.4%)	12 (16.4%)
	$C_{-77}C$	1 (0.9%)	0 (0.0%)	1 (1.4%)
XPD 312	*Asp-Asp*	98 (92.5%)	56 (90.3%)	67 (91.8%)
	Asp-Asn	7 (6.6%)	6 (9.7%)	6 (8.2%)
	Asn-Asn	1 (0.9%)	0 (0.0%)	0 (0.0%)
XPD 751	*Lys-Lys*	96 (90.6%)	55 (88.7%)	62 (84.9%)
	Lys-Gln	9 (8.5%)	7 (11.3%)	11 (15.1%)
	Gln-Gln	1 (0.9%)	0 (0.0%)	0 (0.0%)

* 個數（百分比%)

表三：於農藥暴露狀態及各種因子間之每個細胞的平均尾動量 (μm)

變項	對照		農藥暴露 低		農藥暴露 高	
	個數	平均值±標準誤	個數	平均值±標準誤	個數	平均值±標準誤
全部	106	1.33 ± 0.03	62	2.03 ± 0.05	73	2.31 ± 0.06[*,†]
年齡 (歲)						
≥ 54	31	1.38 ± 0.07	38	2.07 ± 0.07	44	2.44 ± 0.07[†]
< 54	75	1.30 ± 0.03	24	1.97 ± 0.08	29	2.11 ± 0.11
性別						
男性	38	1.33 ± 0.04	30	2.02 ± 0.08	51	2.29 ± 0.07
女性	68	1.32 ± 0.04	32	2.04 ± 0.07	22	2.36 ± 0.10
抽菸狀態						
現今抽菸者	14	1.31 ± 0.05	11	2.01 ± 0.14	16	1.96 ± 0.08[†]
過去抽菸者	4	1.31 ± 0.13	4	1.95 ± 0.29	8	2.39 ± 0.12
非抽菸者	88	1.33 ± 0.03	47	2.04 ± 0.06	49	2.41 ± 0.07
累積抽菸量						
> 10 包年	7	1.36 ± 0.11	9	1.95 ± 0.14	18	2.19 ± 0.08
≤ 10 包年	99	1.32 ± 0.03	53	2.04 ± 0.07	55	2.35 ± 0.07
CYP3A5						
$A_{-44}A$	55	1.30 ± 0.02	35	1.98 ± 0.07	40	2.34 ± 0.08
$A_{-44}G$	41	1.33 ± 0.05	23	2.07 ± 0.09	30	2.29 ± 0.08
$G_{-44}G$	10	1.44 ± 0.12	4	2.13 ± 0.11	3	2.11 ± 0.34
GSTP1						
Ile-Ile	56	1.37 ± 0.05	40	2.03 ± 0.07	49	2.39 ± 0.06[‡]
Ile-Val	43	1.27 ± 0.02	19	1.98 ± 0.07	22	2.17 ± 0.10
Val-Val	7	1.30 ± 0.05	3	2.35 ± 0.31	2	1.77 ± 0.02
XRCC1 194						
Arg-Arg	55	1.33 ± 0.04	22	2.00 ± 0.08	42	2.24 ± 0.08
Arg-Trp	44	1.32 ± 0.04	35	2.01 ± 0.06	28	2.39 ± 0.08
Trp-Trp	7	1.33 ± 0.08	5	2.29 ± 0.33	3	2.51 ± 0.11
XRCC1 399						
Arg-Arg	66	1.34 ± 0.04	38	2.06 ± 0.07	40	2.42 ± 0.07[‡]
Arg-Gln	35	1.31 ± 0.03	22	1.99 ± 0.07	24	2.10 ± 0.09
Gln-Gln	5	1.29 ± 0.10	2	1.91 ± 0.23	9	2.39 ± 0.16
XRCC1 $_{-77}$						
$T_{-77}T$	79	1.31 ± 0.03	50	2.03 ± 0.06	60	2.35 ± 0.06
$T_{-77}C$	26	1.37 ± 0.07	12	2.01 ± 0.09	12	2.13 ± 0.12
$C_{-77}C$	1	1.33	0	—	1	1.92
XPD 312						
Asp-Asp	98	1.33 ± 0.03	56	2.07 ± 0.05[‡]	67	2.31 ± 0.06
Asp-Asn	7	1.30 ± 0.06	6	1.69 ± 0.05	6	2.34 ± 0.19
Asn-Asn	1	1.14	0	—	0	—
XPD 751						
Lys-Lys	96	1.33 ± 0.03	55	2.06 ± 0.06	62	2.31 ± 0.06
Lys-Gln	9	1.26 ± 0.04	7	1.83 ± 0.12	11	2.30 ± 0.13
Gln-Gln	1	1.14	0	—	0	—

[*] 不同農藥暴露狀態分組之比較，是以 ANOVA 所執行，並且對於不同年齡、抽菸狀態、以及基因型分組間之比較，是分別執行 *t* test 與 ANOVA。

[†] $P < 0.01$。

[‡] $0.01 < P < 0.05$。

表四：每個細胞的DNA尾動量之多變項迴歸模式

變項	迴歸係數	標準誤	*P* 值
截距	0.62	0.16	< 0.01
年齡：每增加一歲	0.008	0.002	< 0.01
性別：男性 vs. 女性	0.02	0.06	0.80
抽菸狀態			
現今抽菸者 vs. 非抽菸者	-0.16	0.07	0.04
過去抽菸者 vs. 非抽菸者	-0.07	0.11	0.53
農藥暴露			
高暴露 vs. 對照	0.93	0.06	< 0.01
低暴露 vs. 對照	0.63	0.06	< 0.01
基因型			
CYP3A5: $G_{-44}G$ vs. $A_{-44}A/A_{-44}G$	0.09	0.09	0.31
GSTP1: *Ile-Ile* vs. *Ile-Val*/*Val-Val*	0.10	0.05	0.04
XRCC1 194: *Arg-Trp*/*Trp-Trp* vs. *Arg-Arg*	0.05	0.05	0.30
XRCC1 399: *Arg-Arg* vs. *Arg-Gln*/*Gln-Gln*	0.10	0.05	0.058
XRCC1 $_{-77}$: $T_{-77}T$ vs. $T_{-77}C/C_{-77}C$	0.08	0.06	0.19
XPD 312: *Asp-Asp* vs. *Asp-Asn*/*Asn-Asn*	0.12	0.13	0.35
XPD 751: *Lys-Lys* vs. *Lys-Gln*/*Gln-Gln*	0.03	0.11	0.82

表五：根據易感受性代謝*GSTP1*及DNA修補*XRCC1* 399基因型之農藥暴露狀態分組間之調整平均尾動量*

變項	對照						低農藥暴露						高農藥暴露					
GSTP1 基因型	*Ile-Val/Val-Val*			*Ile-Ile*			*Ile-Val/Val-Val*			*Ile-Ile*			*Ile-Val/Val-Val*			*Ile-Ile*		
	個數	平均 ± 標準誤	*P*	個數	平均 ± 標準誤	*P*	個數	平均 ± 標準誤	*P*	個數	平均 ± 標準誤	*P*	個數	平均 ± 標準誤	*P*	個數	平均 ± 標準誤	*P*
XRCC1 399 基因型																		
Arg-Arg	31	1.30 ± 0.05	0.63	35	1.37 ± 0.05	0.15	14	2.06 ± 0.12	0.79	24	2.05 ± 0.09	0.84	15	2.25 ± 0.11	0.15	25	2.49 ± 0.09	0.004
Arg-Gln/Gln-Gln	19	1.26 ± 0.06	Ref	21	1.34 ± 0.06	0.37	8	2.01 ± 0.16	Ref	16	1.98 ± 0.11	0.87	9	1.98 ± 0.15	Ref	24	2.28 ± 0.09	0.09

*調整年齡、性別、抽菸狀態

Polymorphisms in Metabolic *GSTP1* and DNA Repair *XRCC1* Genes with an Increased Risk of DNA Damage in Pesticide-Exposed Fruit Growers[1]

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Running Title: Pesticide induced-DNA damage modulated by *GSTP1*, and *XRCC1*.

Key Words: DNA damage, *GSTP1* gene, pesticide, *XPD* gene, *XRCC1* gene

Footnotes:

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[3] The abbreviations used are: *GSTP1*, glutathione S-transferase P1; *PON1*, paraoxonase 1; *PON2*, paraoxonase 2; *GSTM1*, glutathione S-transferase M1; *GSTT1*, glutathione S-transferase T1; *CYP3A5*, cytochrome P450 3A5; BER, base excision repair; *XRCC1*, X-ray cross-complementing group 1, NER, nucleotide excision repair; *XPD*, xeroderma pigmentosum group D; GLM, general linear model.

Abstract

Pesticide exposure is associated with various neoplastic diseases and congenital malformations. Animal studies have indicated that pesticides may be metabolized by cytochrome P450 3A5 or glutathione S-transferases. DNA-repair genes, including X-ray repair cross-complementing group 1 (*XRCC1*) and xeroderma pigmentosum group D (*XPD*), may also be implicated in the process of pesticide-related carcinogenesis. Thus, we investigated whether various metabolic and DNA repair genotypes increase the risk of DNA damage in pesticide-exposed fruit growers. Using the comet assay, the extent of DNA damage was evaluated in the peripheral blood of 135 pesticide-exposed fruit growers and 106 unexposed controls. The metabolic genotypes *CYP3A5* ($A_{-44}G$) and *GSTP1* (*Ile*105*Val*) and DNA repair genotypes *XRCC1* (*Arg*399*Gln*, *Arg*194*Trp*, $T_{-77}C$) and *XPD* (*Asp*312*Asn*, *Lys*751*Gln*) were identified by polymerase chain reaction. Our multiple regression model for DNA tail moment showed that age, high pesticide exposure, low pesticide-exposure, *GSTP1 Ile-Ile*, and *XRCC1* 399 *Arg-Arg* genotype were associated with increased DNA tail moment (DNA damage). Further analysis of interaction between *GSTP1* and *XRCC1* genes that increase susceptibility revealed a significant difference in DNA tail moment for high pesticide-exposed subjects carrying both *GSTP1 Ile-Ile* with *XRCC1* 399 *Arg-Arg* genotypes (2.49 ± 0.09 μm/cell; $P = 0.004$), compared to those carrying *GSTP1 Ile-Val/Val-Val* with *XRCC1* 399 *Arg-Gln/Gln-Gln* genotypes (1.98 ± 0.15 μm/cell). These results suggest that individuals with susceptible metabolic *GSTP1* and DNA repair *XRCC1* genotypes may be at increased risk of DNA damage due to pesticide exposure.

Introduction

Fifty-six pesticides have been classified as carcinogenic to laboratory animals by the International Agency for Research on Cancer (1). Meta-analyses also showed that pesticide-exposed farmers are at risk for specific tumors including leukemia (2, 3) and multiple myeloma (4). Those individuals at greater risk of developing cancers may possess certain susceptibility factors including inherited metabolic and DNA-repair traits.

Previous studies revealed that organophosphate pesticides, which are most extensively used in Taiwan, are primarily metabolized by hepatic cytochrome P450 3A4 and 3A5 to become an active intermediate organophosphorus-oxon (5, 6). Furthermore, organophosphorus-oxon may then be hydrolyzed by paraoxonase (PON) to diethyl phosphate and 4-nitrophenol (6, 7), or conjugated to glutathione (GSH), with subsequent catalysis by glutathione S-transferases (GSTs) (8, 9). A previous study performed in Australia also showed that the *GSTP1* gene is associated with an increased risk of Parkinson disease among patients who have been exposed to pesticides (10). Our previous study has also revealed that *GSTP1* (but not *PON1*, *PON2*, *GSTM1*, and *GSTT1* genotypes) in pesticide-exposed fruit growers is associated with increased DNA damage measured by the comet assay (11). Although DNA damage (comet assay) was also higher in pesticide exposed subjects with *CYP3A5* $G_{-44}G$ genotype, the small number of subjects in our previous study has precluded us from drawing a firm conclusion in this regard.

In addition to metabolic traits, DNA repair capacity also plays an important role in pesticide-related carcinogenesis. Several DNA repair pathways are known to provide distinct but overlapping protection against mutagenic exposures. DNA single-strand breaks are among the most frequent DNA lesions, arising directly from damage to the deoxyribose moieties or indirectly as intermediates of DNA base excision repair (BER) (12-14). Left unrepaired, DNA single-strand breaks are a major threat to genetic stability and cell survival,

accelerating mutation rates and increasing levels of chromosomal aberrations (15-17). The X-ray cross-complementing group 1 (*XRCC1*) gene product coordinates the actions of DNA polymerase β, DNA ligase III_{α}, and poly (ADP-ribose) polymerase, APE1, polynucleotide kinase/phosphatase, and 8-oxoguanine DNA glycosylase (18-22). Molecular epidemiological studies have also investigated the possible associations between *XRCC1* polymorphisms and altered cancer risk. The *Arg*399*Gln* (exon 10, base G→A) is located in the region of the BRCT-I interaction domain of *XRCC1* with poly (ADP-ribose) polymerase, and the *Arg*194*Trp* (exon 6, base C→T) occurs in the identified proliferating cell nuclear antigen (PCNA) binding region (23, 24). Although measurement of persistence of DNA adducts (25), increased *p53* mutations (26), and prolonged cell cycle delay (27) has been used to show the association of 399*Gln* and 194*Trp* variant alleles with some DNA repair phenotypes, the results from molecular epidemiological studies are still conflicting and rather inconclusive (28). Recently, a novel *T*-to-*C* transition located at nucleotide -77 in the promoter region of *XRCC1* has been identified, and this substitution was associated with risk of esophageal squamous cell carcinoma in a Chinese population (29). Bioinformatic analysis suggests that this $T_{-77}C$ polymorphism might disrupt a consensus sequence for Sp1-binding site, implying that this polymorphism could alter *XRCC1* transcription.

The nucleotide excision repair (NER) pathway not only removes and repairs bulky adducts but also may play a role in repair of oxidative DNA damage (30, 31). The xeroderma pigmentosum group D (*XPD*) protein, a subunit of transcription factor IIH, is an evolutionarily conserved 5'→3' helicase that unwinds the DNA in the region of DNA damage. Single nucleotide polymorphisms (SNP) in the *XPD* gene have been studied (28). *XPD Asp*321*Asn* in exon 10 causes an amino acid substitution in a conserved region of *XPD*. *XPD Lys*751*Gln* in exon 23 also causes an amino acid substitution in the C-terminal part of the protein. The presences of the variant allele *XPD* exon 10 and exon 23 have been associated

with relatively high cancer risk in some studies (32, 33). Other studies fail to find statistically significant associations (34, 35).

Therefore, pesticide exposed individuals with inherited, susceptibility-associated, metabolic and DNA repair genotypes may have increased risk of DNA damage. In this study, we investigated the association of metabolic and DNA repair genetic polymorphisms in pesticide-exposed fruit growers with cellular DNA damage as measured by the comet assay.

Materials and Methods

Study Population. Previously, we conducted a study to explore the association between DNA damage and metabolic traits among 91 pesticide exposed fruit growers and 106 non-exposed controls in Tungshin Town, which is located in central Taiwan. Criteria for selection of the study population were described in detail elsewhere (11). In current study, sample size was increased to 135 pesticide exposed fruit growers and 106 non-exposed controls to acquire sufficient statistical discriminatory power to detect a difference in the level of DNA damage. Traditionally, local farmer associations provide farmer insurance, financial support, marketing services, and educational training for their members, who include commercial and hobby farmers. On these farms, pesticides are regularly applied all year. Local farmers who were exposed to pesticides and unexposed controls from the local non-farm population were invited to attend our orientation and participate in our study. We tried to minimize biases due to differences in ethnicity and lifestyle by selecting control subjects who were from the same geographic area and of the same ethnicity as the pesticide-exposed subjects. Control occupations included housewives, teachers, clerks, non-farm laborers, skilled workers, small-business persons, and professionals. Among these individuals, none had received any therapeutic irradiation. They were also not taking any medications.

Epidemiologic Information. After giving their informed consent, subjects responded to interviewer-administered questionnaires, giving information pertaining to demographic characteristics and lifestyles (including habits of cigarette smoking and alcohol drinking), and detailed occupational and medical histories. The smoking history included the number of cigarettes smoked daily and duration of the smoker's habit. A variable termed "pack-years" was coined as an indicator of cumulative smoking dose and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. Most Taiwanese farmers have been alerted to the risk of alcohol induced liver damage and have a good understanding that alcohol ingestion aggravates pesticide poisoning. In general, drinking alcohol during the period of pesticide application is not permissible. We therefore were concerned that prevalence of alcohol drinking would be lower in our pesticide-exposed subjects than in our controls. Therefore, all subjects who drank alcohol were excluded.

Assessment of Pesticide Exposure. Since exposure to pesticides occurs during diluting, mixing, loading, spraying, maintaining, and cleaning used equipment, information on past pesticide use by name, amount, area of pesticide application, numbers of treatments per season, years of agrochemical exposure, and use of personal protection equipment was obtained via interviewer-administered questionnaire. The mean orchard size was 1.28 hectares (range, 0.06-4.85 ha). The pesticides used by the fruit growers during the preceding 6 months before the medical examination consisted of almost 40 different compounds, including organophosphates, carbamates, pyrethroid insecticides, fungicides, and growth regulators. Application of organochlorines was negligible. On average, each exposed person reported about three pesticide applications per month with an average cumulative spraying duration of about 7 h/mo (range, 2–28 h/mo).

Unfortunately, levels of pesticide exposure could not be calculated for the study subjects owing to the lack of environmental monitoring data. Thus, we will categorize pesticide exposure as high or low by a modification of the criteria developed by Scarpato et al. (36): (a) For each subject spraying pesticides, the number of hectares treated was determined and pesticide exposure was calculated by multiplying the average number of treatments × the number of hectares sprayed; (b) the median value of the distribution obtained in (a) was determined, and fruit growers with exposure values less than or greater than the median were assigned to the low or high exposure group, respectively; and (c) subjects who did not directly handle pesticides (e.g., those who cut or harvested fruits) were assigned to the low exposure group.

Comet Capture and Analysis. The comet assay was conducted under alkali conditions according to Singh et al. (37). Venous blood was collected in heparinized tubes. Whole blood (10 μL) was suspended in 1.5% low-melting point agarose and sandwiched between a layer of 0.6% normal-melting agarose and a top layer of 1.5% low-melting point agarose on fully frosted slides. Slides were immersed in lysis solution (1% sodium sarconisate, 2.5 mol/L NaCl, 100 mmol/L Na_2EDTA, 10 mmol/L Tris-HCl, 1% Triton X-100 and DMSO 10%) at 4°C. After 1 hour, slides were placed in electrophoresis buffer (0.3 mmol/L NaOH, 1 mmol/L Na_2EDTA [pH 13]) for 10 minutes. Electrophoresis was conducted in the same buffer for 15 minutes at 300 mA. The slides were neutralized with sterilized H_2O thrice for 5 minutes and stained with 10% ethidium bromide. For each subject, 100 randomly captured comets from slides (25 cells on each of four comet slides) were examined at x400 magnification using an epifluorescence microscope connected through a black and white camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, Haverhill, Suffolk, United Kingdom). The computerized image analysis system acquired images, computed the integrated intensity

profiles for each cell, estimated the comet cell components, and evaluated the range of derived parameters. Undamaged cells have an intact nucleus without a tail, and damaged cells have the appearance of a comet. To quantify DNA damage, the tail moment was calculated as the product of the tail length and the fraction of DNA in the comet tail. All slides were scored by one reader who was blind to the status of the subjects.

Polymorphic Analysis. Genomic DNA was extracted from peripheral blood of subjects. Genotyping were analyzed using polymerase chain reaction (PCR)-based methods as described below. Genotyping was also accomplished with blinding to exposure status of subjects. Rigorous quality control procedures were applied throughout the genotyping process. To avoid PCR contamination, reagents for PCR reaction were carefully aliquoted, and each aliquot was used no more than three times. For each assay, a negative control (no DNA template) was added to monitor PCR contamination. Pilot experiments were always conducted to optimize the restriction digestion conditions. After genotyping each genetic polymorphism, ~ 20% to 25% of the samples in each genotype group were randomly selected for repeated assays to validate the results.

The determination of *CYP3A5* $A_{-44}G$ genotypes was done according to Chou et al. (38). Briefly, for *CYP3A5* gene analysis, any restriction fragment length polymorphism (RFLP) was detected by differences in *Fau*I sites following PCR amplification. Primers used for the amplification of the *CYP3A5* gene were 5'-CAG GTG AGA GGA TAT TTA AGA GGC-3' and 5'-CAT CGC CAC TTG CCT TCT TCA AC-3'. *GSTP1-Alw*26I polymorphism was also determined using a PCR-RFLP technique of Harries et al. (39). An *Ile* to *Val* substitution in exon 5 (codon 105) was amplified to form an undigested fragment of 177 bp using the primer pair 5'-ACC CCA GGG CTC TAT GGG AA-3' and 5'-TGA GGG CAC AAG AAG CCC CT-3'. The determination of *XRCC1* polymorphism in exon 10 was done as previously

described (40). An *Arg* to *Gln* substitution in exon 10 (codon 399) was amplified to form an undigested fragment of 242 bp using the primer pair 5'-CCC CAA GTA CAG CCA GGT C-3' and 5'-TGT CCC GCT CCT CTC AGT AG-3'. The genotyping assay for the *XRCC1* in exon 6 has been described previously with some modifications (41). An *Arg* to *Trp* substitution in exon 6 (codon 194) was amplified to form an undigested fragment of 485 bp using the primer pair 5'-GCC AGG GCC CCT CCT TCA A-3' and 5'-TAC CCT CAG ACC CAC GAG T-3'. The *XRCC1* $T_{-77}C$ polymorphism was also detected using the PCR-RFLP assay (29) and the primers 5'-GGG CTG GAG GAA ACG CTC-3' and 5'-TGG CCA GAA GGA TGA GGT AGA G-3' to amplify this promoter fragment. The *XPD* genotypes were also determined by PCR-RFLP analysis (42). For amplification of the exon 10 region of *XPD*, which contains the polymorphic *Sty*I restriction site, the oligonucleotide primers used were 5'-CTG TTG GTG GGT GCC CGT ATC TGT TGG TCT-3' and 5'-TAA TAT CGG GGC TCA CCC TGC AGC ACT TCC T-3'. The PCR primers for the *XPD Lys*751*Gln* gene were 5'-GCC CGC TCT GGA TTA TAC G-3' and 5'-CTA TCA TCT CCT GGC CCC C-3'.

Statistical Analysis. Low and high pesticide exposure subjects and control subjects were compared with respect to age at recruitment, gender, duration of pesticide exposure, size of orchard, current smoking status, and pack-years of smoking using the Student *t* test and ANOVA for continuous variables and the χ^2 test for discrete variables. A χ^2 test or Fisher exact test was used to test the prevalence of genotypes of *CYP3A5*, *GSTP1*, *XRCC1*, and *XPD* among low and high pesticide exposure groups and controls. Subsequently, the crude DNA tail moment was evaluated using an analysis stratified by pesticide exposure and different factors. ANOVA and the Student *t* test was used to compare difference in DNA tail moment by different pesticide exposure status, and to test the association between the DNA tail moment and age, gender, smoking status, and metabolic and DNA repair traits. The

association of these variables with the DNA tail moment was further assessed using a general linear model (GLM). Finally, a least-squares mean was performed to predict the adjusted DNA tail moment for individuals with different combinations of susceptible genotypes. All data were analyzed using SAS 9.1 (SAS Institute, Cary, NC, USA) and statistical tests were two-sided.

Results

In total, 135 pesticide-exposed subjects and 106 unexposed controls were included in the analysis. Their demographic characteristics are summarized in Table 1. The mean age was 54 years. Gender ($P = 0.01$, χ^2 test) and mean size of orchard ($P < 0.01$, *t* test) but not mean age ($P = 0.68$), duration of pesticide exposure ($P = 0.40$), proportion of current smokers ($P = 0.55$), and cigarette pack-years ($P = 0.17$), differed significantly between the high and low pesticide groups. In contrast, the control group was significantly younger ($P < 0.01$, ANOVA), included more females ($P < 0.01$), and had fewer pack-years of smoking ($P < 0.01$) than the pesticide-exposed groups.

The genotypic prevalence of *CYP3A5*, *GSTP1*, *XRCC1*, and *XPD* is shown in Table 2. The prevalence of *CYP3A5* ($P = 0.72$, Fisher exact test), *GSTP1* ($P = 0.32$), *XRCC1* 194 ($P = 0.12$), *XRCC1* 399 ($P = 0.28$), $XRCC1_{-77}$ ($P = 0.61$), *XPD* 312 ($P = 0.88$), and *XPD* 751 ($P = 0.52$) genotypes among the low and high pesticide exposure and control groups did not differ significantly. The distributions of all genotypes were in Hardy-Weinberg equilibrium. In our subsequent analysis, since enzyme activity level is lower in individuals with at least one *CYP3A5* A_{-44} allele than individuals with the *CYP3A5* G_{-44} allele (43), we combined those with at least one *CYP3A5* A_{-44} allele into a *CYP3A5* $A_{-44}G/A_{-44}A$ genotypes group. Additionally, since enzyme activity is lower in individuals with at least one *GSTP1 Val* allele than those with the *GSTP1 Ile* allele (44) and since few had the *GSTP1 Val-Val* genotype,

those with the *GSTP1 Ile*-*Val* and *Val*-*Val* genotypes were combined. As statistical power was considered and the few had *XRCC1* genotypes, subjects possessing *Trp*-*Trp* and *Arg*-*Trp* genotypes in *XRCC1* 194 were combined. Those with at least one *Gln* allele in *XRCC1* 399 were merged into a *XRCC1* 399 *Arg*-*Gln*/*Gln*-*Gln* genotypes group and those with at least one *XRCC1* C_{-77} allele were combined into a *XRCC1* $T_{-77}C/C_{-77}C$ genotypes group. Similarly, because few people had *XPD* 312*Asn*-*Asn* and 751*Gln*-*Gln* and because those with at least one *XPD* 321*Asn* allele and *XPD* 751*Gln* allele have a lower capacity for DNA repair (42), those with at least one *XPD* 321*Asn* allele were placed in a *XPD* 312 *Asp*-*Asn*/*Asn*-*Asn* genotypes group and those with at least one *XPD* 751*Gln* allele in a *XPD* 751 *Lys*-*Gln*/*Gln*-*Gln* genotypes group.

Table 3 summarizes the crude association of tail moment with various factors. Individuals exposed to high levels of pesticide had the highest tail moment (2.31 μm/cell) followed by those exposed to low levels (2.03 μm/cell) and controls (1.33 μm/cell; $P < 0.01$, ANOVA). Similarly, individuals older than 54 years (mean age of all subjects) also showed a higher tail moment, especially in the high exposure group (2.44 vs. 2.11 μm/cell; $P < 0.01$, *t* test). Current smokers in the high exposure group had a lower tail moment compared with former and never smokers ($P < 0.01$). However, tail moment was higher for high exposure individuals with *GSTP1 Ile*-*Ile* genotype (vs. *Ile*-*Val*/*Val*-*Val*, $P = 0.03$) but not in males or those with higher packyears of smoking. Interestingly, tail moment was higher in the high exposure group with *XRCC1* 399 *Arg*-*Arg* genotype than the high exposure group with *XRCC1* 399 *Arg*-*Gln*/*Gln*-*Gln* ($P = 0.03$) and higher in the low exposure group with *XPD* 312 *Asp*-*Asp* genotype than in the low exposure group with *XPD* 312 *Asp*-*Asn/Asn*-*Asn* ($P < 0.01$). However, tail moment was not associated with the *CYP3A5*, *XRCC1* 194, *XRCC1* $_{-77}$, and *XPD* 751 genotypes.

A multiple linear regression model for the relationship between DNA tail moment and

age, gender, smoking habit, pesticide exposure, and genotypes of *CYP3A5*, *GSTP1*, *XRCC1* 194, *XRCC1* 399, *XRCC1*$_{-77}$, *XPD* 312, and *XPD* 751 is shown in Table 4. When multiple testing (Bonferroni correction) was taken into consideration, the DNA tail moment was positively associated with increased age ($P < 0.01$), high exposure ($P < 0.01$), and low exposure ($P < 0.01$). Interestingly, greater differences in tail moment were observed among individuals with *GSTP1 Ile-Ile* genotype ($P = 0.04$), and those with *XRCC1* 399 *Arg-Arg* genotype ($P = 0.058$).

Subsequently, a least-squares mean analysis was performed to assess the joint effect on DNA damage of the *GSTP1* and *XRCC1* 399 polymorphisms and pesticide exposure after adjustment for the effects of age, gender, and smoking status (Table 5). Compared to controls with *GSTP1 Ile-Val/Val-Val* and *XRCC1* 399 *Arg-Gln/Gln-Gln* genotypes (1.26 ± 0.06 μm/cell), controls with *GSTP1 Ile-Ile* and *XRCC1* 399 *Arg-Arg* genotypes had a greater DNA tail moment (1.37 ± 0.06 μm/cell; the increase was 0.11 μm/cell). Notably, relative to the high exposure group with *GSTP1 Ile-Val/Val-Val* and *XRCC1* 399 *Arg-Gln/Gln-Gln* genotypes (1.98 ± 0.15 μm/cell), the high exposure group with both *GSTP1 Ile-Ile* and *XRCC1* 399 *Arg-Arg* had the highest DNA tail moment (2.49 ± 0.09 μm/cell; vs. reference group, $P = 0.004$), while the high exposure group with both *GSTP1 Ile-Ile* and *XRCC1* 399 *Arg-Gln/Gln-Gln* (2.28 ± 0.09 μm/cell, $P = 0.09$) and the one with both *GSTP1 Ile-Val/Val-Val* and *XRCC1* 399 *Arg-Arg* (2.25 ± 0.11 μm/cell, $P = 0.15$) had a moderately increased level of DNA damage.

Discussion

It is important to identify the potential genetic susceptibility factors affecting individual responses to carcinogen exposure. In this study, the metabolic *GSTP1* genotype and DNA

repair *XRCC1* 399 genotype, as well as pesticide exposure, were significantly associated with an increased DNA tail moment.

In our study, the frequency of the *CYP3A5* G_{-44} allele (26.6%) was consistent with the results of a previous study in Taiwanese adults (28.2%; Ref. 38). The prevalence of the *GSTP1* 105*Val* allele (22.4%) in our study appears to be quite similar to that previously reported for Taiwanese populations (18%; Ref. 45). The frequency of the *XRCC1* 194*Trp* allele (28.4%) and 399*Gln* allele (23.4%) was also comparable to that previously reported for those of Taiwanese descent (194*Trp* allele 27%, 399*Gln* allele 26%; Ref. 25). The prevalence of the *XRCC1* C_{-77} allele (11.2%) in our sample was close to that reported for those of Chinese descent (10%; Ref. 29). In addition, the frequency of the *XPD* 321*Asn* allele (4.4%) and *XPD* 751*Gln* allele (6.0%) was also similar to that reported for those of Chinese descent (321*Asn* allele 6.5%, 751*Gln* allele 8.7%; Ref. 46). These findings, to some extent, validate the practice and results of our genotyping technique.

The comet assay is a sensitive method of assessing DNA damage. The comet assay of peripheral blood samples in our study and several previous studies has revealed an increase in DNA damage in individuals exposed to complex mixtures of pesticides (47, 48). Furthermore, genetic variability in the enzymes that metabolize agricultural chemicals or repair DNA damage may also be involved in this process. When detoxification and DNA repair are inefficient, metabolic products accumulate and DNA damage persists, contributing to the carcinogenic process.

Previous studies revealed that pesticide-like organophosphates are primarily metabolized by hepatic cytochrome P450 enzymes to active intermediate organophosphorus-oxon (5, 6), which may then be hydrolyzed by PON to diethyl phosphate and 4-nitrophenol (6, 7), or be conjugated to GSH *via* catalysis by GSTs (8, 9). However, our previous study was unable to demonstrate any association of *PON1*, *PON2*, *GSTM1*, and *GSTT1* genotypes with DNA

damage as measured by the comet assay. Thus, *PON1*, *PON2*, *GSTM1*, and *GSTT1* were not included in current study. A re-evaluation of the effect of *CYP3A5* genotype on DNA damage in pesticide-exposed subjects failed to show a statistically significant association. The most likely reason for this failure was that the number of subjects who carry the $G_{-44}G$ genotype of *CYP3A5* (which is associated with increased susceptibility) is relatively small. Importantly, DNA damage was higher in pesticide-exposed fruit growers with the *GSTP1 Ile-Ile* genotype than in those without this genotype. A previous report also observed that level of benzo(a)pyrene diolepoxide (BPDE)-DNA adducts was higher in *GSTP1 Ile-Ile* carriers than *GSTP1 Ile-Val* and *Val-Val* carriers (45). Elevated level of DNA damage in the former may reflect their lower level of metabolic activity.

DNA repair is a very important mechanism in protection against gene mutation and cancer initiation. DNA damage could be induced by environmental carcinogens like pesticides and/or through metabolic processes that increase susceptibility. If not repaired, such damage can be converted into gene mutations and genomic instability. In our study, we further investigated whether genetic polymorphisms in *XRCC1* (a protein that plays a central role in BER and single strand break repair) and in *XPD* (a helicase involved in NER and basal transcription) could increase the risk of DNA damage in pesticide exposed-subjects.

Previous studies have observed that mutant mouse or Chinese hamster ovary cells (EM9 and EM-C11) with no functional *XRCC1* protein are hypersensitive to a broad range of DNA damage induced by alkylating agents, reactive oxygen species, or ionizing radiation (49-51). Interestingly, we found that the *XRCC1* 399 *Arg-Arg* genotype was associated with elevated risk of DNA damage in our pesticide-exposed population. In our study, *XRCC1* 194 genotype and *XRCC1* $_{-77}$ genotype were not significantly associated with increased DNA damage and, with regard to the *XPD* gene, neither *Asp*312*Asn* nor *Lys*751*Gln* polymorphisms influenced DNA damage level. XRCC1 protein is exclusively required for DNA BER, strand-break

repair, and maintenance of genetic stability (23, 24). The functional consequences of the *XRCC1* polymorphisms are still not known. *XRCC1* 399*Gln* allele has been associated with increased DNA adducts level (25), increased *p53* mutations (26), and prolonged cell cycle delay (27). No previous studies have examined the potential relationships between DNA repair gene polymorphisms and DNA damage caused by pesticide exposure. However, our finding of decreased risk of DNA damage among pesticide-exposed subjects with the *XRCC1* 399*Gln* allele suggests the enhancement of apoptosis as a possible mechanism. Little is known concerning the role of XRCC1 in apoptosis, but unrepaired BER intermediates are clastogenic and may be able to act as a strong trigger of the apoptotic pathway (52). An animal study has shown that induction of apoptosis following cold brain injury is tightly linked to reduction in XRCC1 expression (53). Therefore, reduced efficiency of the XRCC1 protein (a consequence of the 399*Gln* allele) may result in the impaired ability to repair DNA damage, and such cells may be more likely to undergo apoptosis. Another possible explanation is that the effect of the *XRCC1* variant on DNA repair capacity may differ with type and strength of the DNA damaging exposures. Further studies would be required to test these hypotheses.

On the other hand, we failed to observe any association between the genetic polymorphisms in the *XPD* gene with DNA damage in our pesticide-exposed subjects. Thus, the NER system, a major pathway for repair of bulky DNA damage, might not be involved in repair of DNA damage produced by pesticide. In addition, these findings should be interpreted with caution since it is well known that more than 20 genes are involved in the NER pathway and different combinations of the wild type of one gene and variant of another are possible.

Furthermore, the level of DNA damage was more likely to be increased in our pesticide-exposed individuals with *GSTP1 Ile-Ile* and *XRCC1* 399 *Arg-Arg* (genotypes that

increase susceptibility). *GSTP1* encodes enzymes that are detoxifying for the reactive metabolites of pesticides (8, 9), whereas *XRCC1* is involved in the subsequent DNA-repair (or apoptosis) process. This indicates that each susceptibility-associated genotype may generate a moderate risk for DNA damage and combinations of these may further increase the risk.

We tried to minimize possible bias due to lifestyle and ethnicity by selecting control subjects from the same geographic area and of the same ethnicity as our pesticide-exposed subjects. However, the active farm population consisted largely of older people in our study area. Most of the younger residents have a low regard for agricultural work. Thus, our control and test subjects were not matched for age and the former were significantly younger. As expected, older smoking farmers also had more pack-years of smoking than younger. Previous reports also showed that age is associated with increased DNA damage (54, 55). In the present study, older age was also associated with higher DNA tail moment. The higher DNA tail moment in older subjects indicates either an increased susceptibility to damage with older age or a greater accumulation of pesticide or unidentified carcinogens or mutagens with age. In addition, gender was not associated with a higher DNA damage in our study, and no data in the medical literature indicates substantial gender differences. Previous reports, but not the present study, showed that smoking is associated with DNA damage (54). This is probably due to the fact that the quantity of cigarettes smoked was smaller in the current study than in other studies (47).

It is often difficult to reconstruct an individual's previous pesticide exposure history, including the degree of personal protection used during handing pesticides. In this study, the available historical exposure data were too sparse and lacking in detail to estimate cumulative exposure. Data pertaining to individual exposure were obtained without the knowledge of health outcome. Consequently, exposure misclassification is assumed to be non-differential

and, if apparent, lead to an underestimation of the risk of DNA damage.

In summary, the results revealed that both metabolic *GSTP1* and DNA repair *XRCC1* genes can modulate DNA damage in pesticide-exposed fruit growers. Further study to determine the relationship of metabolic and DNA repair genes with cancers caused by pesticide exposure is warranted.

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Table 1. Demographic characteristics of pesticide exposed fruit growers and controls stratified by different intensity of exposure

Variables	Controls	Pesticide exposure	
		Low	High
No. of subjects	106[*]	62	73
Age (years)	48.9 ± 1.1[*]	57.5 ± 1.2	56.8 ± 1.2[†]
Gender: male (%)	38 (35.8%)	30 (48.4%)	51 (69.9%)[†,‡]
Duration of pesticide exposure (years)	0	30.0 ± 2.1	32.3 ± 1.8
Size of orchard (ha)	0	0.8 ± 0.1	1.7 ± 0.1[‡]
Smoking habit			
Current smoker (%)	14 (13.2%)	11 (17.7%)	16 (21.9%)
Pack-years	2.1 ± 0.6	5.7 ± 1.8	9.9 ± 2.5[†]

[*] Data represent numbers of individuals or means ± SE for continuous variables.

[†] $P < 0.01$; control group significantly different from the high and low pesticide exposure groups.

[‡] $P < 0.01$, compared with the low pesticide exposure group.

Table 2. Prevalence of genotypes of *CYP3A5*, *GSTP1*, *XRCC1,* and *XPD* among pesticide-exposed fruit growers and controls stratified by pesticide exposure

Genotype		Controls	Pesticide exposure	
Gene	Alleles		Low	High
Number of subjects		106	62	73
CYP3A5	$A_{-44}A$	55 (51.9%)	35 (56.5%)	40 (54.8%)
	$A_{-44}G$	41 (38.7%)	23 (37.1%)	30 (41.1%)
	$G_{-44}G$	10 (9.4%)	4 (6.4%)	3 (4.1%)
GSTP1	*Ile-Ile*	56 (52.8%)	40 (64.5%)	49 (67.1%)
	Ile-Val	43 (40.6%)	19 (30.7%)	22 (30.1%)
	Val-Val	7 (6.6%)	3 (4.8%)	2 (2.8%)
XRCC1 194	*Arg-Arg*	55 (51.9%)	22 (35.5%)	42 (57.5%)
	Arg-Trp	44 (41.5%)	35 (56.5%)	28 (38.4%)
	Trp-Trp	7 (6.6%)	5 (8.0%)	3 (4.1%)
XRCC1 399	*Arg-Arg*	66 (62.3%)	38 (61.3%)	40 (54.8%)
	Arg-Gln	35 (33.0%)	22 (35.5%)	24 (32.9%)
	Gln-Gln	5 (4.7%)	2 (3.2%)	9 (12.3%)
$XRCC1_{-77}$	$T\text{-}_{77}T$	79 (74.6%)	50 (80.6%)	60 (82.2%)
	$T\text{-}_{77}C$	26 (24.5%)	12 (19.4%)	12 (16.4%)
	$C\text{-}_{77}C$	1 (0.9%)	0 (0.0%)	1 (1.4%)
XPD 312	*Asp-Asp*	98 (92.5%)	56 (90.3%)	67 (91.8%)
	Asp-Asn	7 (6.6%)	6 (9.7%)	6 (8.2%)
	Asn-Asn	1 (0.9%)	0 (0.0%)	0 (0.0%)
XPD 751	*Lys-Lys*	96 (90.6%)	55 (88.7%)	62 (84.9%)
	Lys-Gln	9 (8.5%)	7 (11.3%)	11 (15.1%)
	Gln-Gln	1 (0.9%)	0 (0.0%)	0 (0.0%)

Data represent the numbers of subjects (with percentage in parentheses, where shown).

Table 3. Dependence of average tail moment per cell (μm) stratified by pesticide-exposure status on various factors

Variables			Pesticide exposure			
	Controls		Low		High	
	n	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
All	106	1.33 ± 0.03	62	2.03 ± 0.05	73	2.31 ± 0.06*,†
Age (y)						
≥ 54	31	1.38 ± 0.07	38	2.07 ± 0.07	44	2.44 ± 0.07†
< 54	75	1.30 ± 0.03	24	1.97 ± 0.08	29	2.11 ± 0.11
Gender						
Males	38	1.33 ± 0.04	30	2.02 ± 0.08	51	2.29 ± 0.07
Females	68	1.32 ± 0.04	32	2.04 ± 0.07	22	2.36 ± 0.10
Smoking status						
Current smokers	14	1.31 ± 0.05	11	2.01 ± 0.14	16	1.96 ± 0.08†
Past smokers	4	1.31 ± 0.13	4	1.95 ± 0.29	8	2.39 ± 0.12
Never smokers	88	1.33 ± 0.03	47	2.04 ± 0.06	49	2.41 ± 0.07
Cumulative smoking dose						
> 10 pack-years	7	1.36 ± 0.11	9	1.95 ± 0.14	18	2.19 ± 0.08
≤ 10 pack-years	99	1.32 ± 0.03	53	2.04 ± 0.07	55	2.35 ± 0.07
CYP3A5						
$A_{-44}A$	55	1.30 ± 0.02	35	1.98 ± 0.07	40	2.34 ± 0.08
$A_{-44}G$	41	1.33 ± 0.05	23	2.07 ± 0.09	30	2.29 ± 0.08
$G_{-44}G$	10	1.44 ± 0.12	4	2.13 ± 0.11	3	2.11 ± 0.34
GSTP1						
Ile-Ile	56	1.37 ± 0.05	40	2.03 ± 0.07	49	2.39 ± 0.06‡
Ile-Val	43	1.27 ± 0.02	19	1.98 ± 0.07	22	2.17 ± 0.10
Val-Val	7	1.30 ± 0.05	3	2.35 ± 0.31	2	1.77 ± 0.02
XRCC1 194						
Arg-Arg	55	1.33 ± 0.04	22	2.00 ± 0.08	42	2.24 ± 0.08
Arg-Trp	44	1.32 ± 0.04	35	2.01 ± 0.06	28	2.39 ± 0.08
Trp-Trp	7	1.33 ± 0.08	5	2.29 ± 0.33	3	2.51 ± 0.11
XRCC1 399						
Arg-Arg	66	1.34 ± 0.04	38	2.06 ± 0.07	40	2.42 ± 0.07‡
Arg-Gln	35	1.31 ± 0.03	22	1.99 ± 0.07	24	2.10 ± 0.09
Gln-Gln	5	1.29 ± 0.10	2	1.91 ± 0.23	9	2.39 ± 0.16
$XRCC1_{-77}$						
$T_{-77}T$	79	1.31 ± 0.03	50	2.03 ± 0.06	60	2.35 ± 0.06
$T_{-77}C$	26	1.37 ± 0.07	12	2.01 ± 0.09	12	2.13 ± 0.12
$C_{-77}C$	1	1.33	0	—	1	1.92
XPD 312						
Asp-Asp	98	1.33 ± 0.03	56	2.07 ± 0.05‡	67	2.31 ± 0.06
Asp-Asn	7	1.30 ± 0.06	6	1.69 ± 0.05	6	2.34 ± 0.19
Asn-Asn	1	1.14	0	—	0	—
XPD 751						
Lys-Lys	96	1.33 ± 0.03	55	2.06 ± 0.06	62	2.31 ± 0.06
Lys-Gln	9	1.26 ± 0.04	7	1.83 ± 0.12	11	2.30 ± 0.13
Gln-Gln	1	1.14	0	—	0	—

* Comparison amongst different pesticide-exposure status groups conducted with ANOVA, and comparison between different age, smoking status, and genotype groups conducted with *t*-test and ANOVA, respectively.

† $P < 0.01$. ‡ $0.01 < P < 0.05$.

Table 4. Multiple regression model for tail moment per cell

Variables	Regression coefficient	SE	*P*-value
Intercept	0.62	0.16	< 0.01
Age: per 1-y increment	0.008	0.002	< 0.01
Gender: male vs. female	0.02	0.06	0.80
Smoking status			
Current smokers vs. never smokers	-0.16	0.07	0.04
Past smokers vs. never smokers	-0.07	0.11	0.53
Pesticide exposure			
High vs. control	0.93	0.06	< 0.01
Low vs. control	0.63	0.06	< 0.01
Genotyping			
CYP3A5: $G_{-44}G$ vs. $A_{-44}A/A_{-44}G$	0.09	0.09	0.31
GSTP1: *Ile-Ile* vs. *Ile-Val/Val-Val*	0.10	0.05	0.04
XRCC1 194: *Arg-Trp/Trp-Trp* vs. *Arg-Arg*	0.05	0.05	0.30
XRCC1 399: *Arg-Arg* vs. *Arg-Gln/Gln-Gln*	0.10	0.05	0.058
XRCC1 $_{-77}$: $T_{-77}T$ vs. $T_{-77}C/C_{-77}C$	0.08	0.06	0.19
XPD 312: *Asp-Asp* vs. *Asp-Asn/Asn-Asn*	0.12	0.13	0.35
XPD 751: *Lys-Lys* vs. *Lys-Gln/Gln-Gln*	0.03	0.11	0.82

Table 5. Adjusted mean tail moment stratified by pesticide-exposure status according to the susceptible metabolic *GSTP1* and DNA repair *XRCC1* 399 genotypes[*]

Variables	Controls						Low pesticide exposure						High pesticide exposure					
GSTP1 genotype	*Ile-Val/Val-Val*			*Ile-Ile*			*Ile-Val/Val-Val*			*Ile-Ile*			*Ile-Val/Val-Val*			*Ile-Ile*		
	n	Mean ± SE	*P*	*n*	Mean ± SE	*P*	*n*	Mean ± SE	*P*	*n*	Mean ± SE	*P*	*n*	Mean ± SE	*P*	*n*	Mean ± SE	*P*
XRCC1 399 genotype																		
Arg-Arg	31	1.30 ± 0.05	0.63	35	1.37 ± 0.05	0.15	14	2.06 ± 0.12	0.79	24	2.05 ± 0.09	0.84	15	2.25 ± 0.11	0.15	25	2.49 ± 0.09	0.004
Arg-Gln/Gln-Gln	19	1.26 ± 0.06	Ref	21	1.34 ± 0.06	0.37	8	2.01 ± 0.16	Ref	16	1.98 ± 0.11	0.87	9	1.98 ± 0.15	Ref	24	2.28 ± 0.09	0.09

[*] Adjusted for age, gender, and smoking status.

出席國際學術會議心得報告

計畫編號	NSC 95-2314-B-040-038
計畫名稱	代謝及 DNA 修補基因多形性與農藥暴露之果農的 DNA 傷害危險
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 4 月 14 日至 4 月 18 日; 美國加州洛杉磯市
會議名稱	美國癌症研究學會年度會議
發表論文題目	代謝 *GSTP1* 及 DNA 修補 *XRCC1* 基因多形性與農藥暴露之果農的 DNA 傷害增加危險

一、參加會議經過

過去曾參加過世界頂級的癌症研討會 AACR 年會 (American Association for Cancer Research)，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 AACR 年會。2007 年美國癌症研究學會年度會議於 4 月 14 日至 4 月 18 日在美國加州洛杉磯市的 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在 4 月 15 日下午進行壁報展示，向與會學者介紹我們的研究成果。

二、與會心得

我們今年於 AACR 年會所發表的研究題目是代謝 *GSTP1* 及 DNA 修補 *XRCC1* 基因多形性與農藥暴露之果農的 DNA 傷害增加危險。農藥暴露是相關於若干的惡性腫瘤疾病以及先天畸形，動物試驗已經指出農藥可能被肝臟細胞色素 P450 3A5 酵素、或是麩胺基硫轉移酶所代謝。DNA 修補基因，包括 X-ray repair cross-complementing group 1 (*XRCC1*) 和 xeroderma pigmentosum group D (*XPD*) 可能也參與在農藥相關的致癌過程

中。而我們的結果顯示年齡、高農藥暴露、低農藥暴露、*GSTP1 Ile-Ile*、和 *XRCC1* 399 *Arg-Arg* 基因型是相關於增加的 DNA 尾動量 (DNA 傷害)。這些結果建議個體攜帶易感受性代謝 *GSTP1* 與 DNA 修補 *XRCC1* 基因，可能呈現因農藥暴露所導致 DNA 傷害之增加危險。

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人類基因相關研究計畫計劃和新技術對生命科學研究的貢獻。由於對基因體的了解，現在可以進行一些以前做不到的事，如分析癌症相關之tyrosine kinase，或許是尋找新的致癌基因之途徑，並可找出有些癌症有基因體不穩定的現象，代表有一未知的抑癌基因。此外，如以siRNA破壞ubiquitin ligase，或許可以找出一些signal transduction的途徑；並且以retrovirus tagging 的方式可以找出致癌基因的互補作用。而癌症治療逐漸邁向標的治療，越來越多的研究顯示唯有尋找專一性針對腫瘤細胞進行毒殺的ligands才能避免正常組織之傷害，如此一來對於藥物的劑量可以提高避免低劑量藥物誘發癌細胞產生抗藥性，當然癌症治療絕不是單一療法，而是要合併數種不同的方式來多管其下，如此未來對於癌症問題的解決將更有幫助。因為不論單一種的治療效果多好，癌細胞總有辦法逃過追殺，最後讓治療失敗。也因此幾乎所有的癌症治療策略都傾向合併治療，也唯有如此才能減少癌細胞逃過追殺的機會。 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: 會議論文摘要及全文光碟）