科技部補助專題研究計畫成果報告
期末報告

抗結核治療中肝炎之性別特異性風險及其與 PXR 受體基因多型性之關係

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 102-2629-B-002-001-
執 行 期 間 ： 102 年 08 月 01 日至 104 年 07 月 31 日
執 行 單 位 ： 國立臺灣大學醫學院檢驗醫學科

計 畫 主 持 人 ： 李麗娜
共 同 主 持 人 ： 王振源
計畫參與人員： 此計畫無其他參與人員
學士級-專任助理人員：張秀靜

報 告 附 件 ： 出席國際會議研究心得報告及發表論文

處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 104 年 08 月 31 日

中 文 摘 要 ： 從 isoniazid 及 rifampicin 在 1950 及 1970 年代開發成功後，結核病已有很有效的藥物可治療。然而目前結核病在台灣之發生率仍居高不下，其原因固然很多，但抗結核藥物引起肝炎，使得結核病患者易中斷治療，無疑是抗結核治療失敗的重要原因。主持人曾於 2007 至 2008 年執行一前瞻性之計畫，研究我國結核病患者發生治療中肝炎之危險因子。結果顯示，我國結核病病人發生治療中肝炎之百分比為 18.9%，其中 16.4%是抗結核藥物引起的藥物性肝炎，2.5%是 B 或 C 型肝炎發生急性惡化。發生藥物性肝炎之危險因子為： 1)沒有 B 型或 C 型肝炎之女性；2) N-acetyl transferase 2 (NAT2)基因之 slow acetylator； 3) 治療前 B 型肝炎病毒量高者；4) 末期腎衰竭且未作透析。發生病毒性肝炎急性惡化之危險因子為： 1) 治療前 B 或 C 型肝炎病毒量高之男性； 2)末期腎衰竭且未作透析。

我們想知道為什麼我國女性發生抗結核藥物性肝炎之危險性遠高於男性 (24% vs. 12%)？我們搜尋到一種與藥物代謝基因 CYP3A4 之表現密切相關之核受體基因 pregnane X receptor (PXR)，可能可以回答我們部分之問題。目前已知 CYP3A4 負責代謝 50%以上臨床上使用之藥物，然而 CYP3A4 基因之單核苷酸多型性(single nucleotide polymorphism，SNP) 並不常見，無法解釋為何 CYP3A4 基因之表現在個體之間差異如此大。近年來有不少研究發現，PXR 基因可正向調節(upregulate) CYP3A4 之表現，PXR 基因之多型性(SNP)有些會影響其對於 CYP3A4 之正向調節，造成對於 CYP3A4 之抑制作用，使 CYP3A4 之藥物代謝功能變差，因而可能引起藥物性肝炎。目前已知 flucloxacillin 引起之藥物性肝炎與 PXR 基因有關，且 flucloxacillin 藥物性肝炎之危險性，在女性遠高於男性，與抗結核藥物性肝炎相似。在魚類亦觀察到，雌魚與雄魚的 PXR 及 CYP3A4 之表現強度有顯著不同。

由於若要直接研究 PXR 或 CYP3A4 基因之表現，必須使用肝臟組織為研究材料，在臨床研究上極為困難。然而，PXR 基因之 SNP 研究，卻可以使用白血球作研究材料。因此我們計畫研究結核病患者及健康對照組 PXR 基因之 SNP，分析其與抗結核治療中肝炎之關係。我們的假設是：PXR 基因之多型性與抗結核藥物性肝炎之發生有關，且其多型性之分布，在男性與女性不同，使得女性發生抗結核藥物性肝炎之危險性高於男性。

我們將用三年的時間來完成此研究，預定每年收結核病患者 100 例，健康接觸者 40 例。我們將作白血球 PXR 基因 SNP 之研究，分析 PXR 基因 SNP 之分布，在男性與女性有無不同，在有發生抗結核藥物性肝炎與沒有發生肝炎之病人有無

不同，是否可以解釋女性發生抗結核藥物肝炎之危險性高於男性之現象。

中文關鍵詞： 結核病、抗結核治療中肝炎、女性、男性、藥物性肝炎、PXR基因、CYP3A4、藥物代謝、單核苷酸多型性

英文摘要： Hepatitis during anti-tuberculosis (TB) treatment (HATT) is the most important adverse event of anti-TB chemotherapy. In 2007 to 2008 we conducted a prospective study to investigate risk factors of HATT. We found that HATT developed in 18.9% of our TB patients, of whom 16.5% were due to anti-TB drugs (drug-induced HATT), and 2.4% were due to acute flare-ups of HBV/HCV hepatitis (virus-induced HATT). Multi- variate analysis showed that risk factors for drug-induced HATT were: 1) women without HBV/HCV infection； 2) N-acetyl transferase 2 (NAT2) slow acetylator； 3) High initial HBV viral load, and 4) end-stage renal disease without hemodialysis.
We want to know why women have higher risk of drug-induced HATT than men (24 vs. 12%), so we searched and found a nuclear receptor gene (pregnane X receptor, PXR) that may answer part of our questions. PXR can strongly enhance the expression of liver CYP3A4 gene. CYP3A4 metabolize > 50% of clinically prescribed drugs (including anti-TB medicine). It has been shown that PXR gene single nucleotide polymorphism (SNP) alters the expression and activity of CYP3A4, and can regulate metabolism of many drugs. Moreover, it has been demonstrated that PXR polymorphism is important in flucloxacillin-induced liver injury which is more common in women, and in fish there is also sex-specific differences in the expression of PXR and CYP3A4.
Because investigations on PXR and CYP3A4 expression need to use liver tissue, such studies are extremely difficult to perform on clinical patients. On the contrary, studies on PXR polymorphism can use peripheral blood leukocytes. Thus we decide to investigate PXR polymorphism, instead of PXR and CYP3A4 expression. Our hypothesis is that distribution of PXR SNPs might be different in

females than in males, leading to higher risk of anti-TB drug-induced hepatitis in females. We plan to complete the project in 3 years. Every year we'll enroll 100 TB patients and 40 healthy contacts. We'll perform peripheral blood PXR gene SNPs, compare the distribution of SNPs between male and female patients, patients with drug-induced HATT and those without, and analyze if PXR SNP could explain the higher risk of drug-induced HATT in females than in males.

英文關鍵詞： tuberculosis, hepatitis during anti-TB treatment (HATT), drug-induced HATT, virus-induced HATT, PXR, CYP3A4, polymorphism, female, male

Medicine®

OBSERVATIONAL STUDY

OPEN

Gender-Dimorphic Impact of *PXR* Genotype and Haplotype on Hepatotoxicity During Antituberculosis Treatment

Jann Yuan Wang, MD, PhD, Ching Hui Tsai, PhD, Yungling Leo Lee, PhD, Li Na Lee, MD, PhD, Chia Lin Hsu, MD, Hsiu Ching Chang, BS, Jong Ming Chen, MD, PhD, Cheng An Hsu, BS, Chong Jen Yu, MD, PhD, and Pan Chyr Yang, MD, PhD

Abstract: Women have a higher risk of drug-induced hepatotoxicity during antituberculosis treatment (HATT) than men. We hypothesized that single nucleotide polymorphism (SNP) genotype and derived haplotype of *pregnane X receptor* (*PXR*) gene, which could regulate the expression of phase I enzyme cytochrome P450 (CYP) 3A4, had a sex-specific influence on the risk of HATT.

Six SNPs of the *PXR* gene were sequenced. Genotypes and haplotypes of the *PXR* SNPs, and other potential risk factors for HATT were compared between pulmonary TB patients with and those without HATT. HATT was defined as an increase in serum transaminase level >3 times the upper limit of normal (ULN) with symptoms, or >5 times ULN without symptoms. We performed the study in a derivation and a validation cohort.

Among the 355 patients with pulmonary TB in the derivation cohort, 70 (19.7%) developed HATT. Logistic regression analysis revealed the risk of HATT increased in female genotype AA at rs2461823 (OR: 6.87 [2.55–18.52]) and decreased in female genotype AA at rs7643645 (OR: 0.14 [0.02–1.02]) of *PXR* gene. Haplotype analysis showed that female h001101 (OR: 2.30 [1.22–4.32]) and female h000110 (OR: 2.25 [1.08–4.69]) haplotype were associated with increased HATT risk. The identified predictors were also significantly associated with female HATT risk among the 182 patients in the validation cohort.

Two *PXR* SNP genotypes and 2 haplotypes influenced the risk of HATT only in females. The *PXR* SNP showed a sex-specific impact that contributed to an increased HATT risk in females.

Editor: Vivek Naranbhai.
Received: December 29, 2014; revised: May 3, 2015; accepted: May 15, 2015.
From the Department of Internal Medicine, National Taiwan University Hospital (JYW, CLH, CJY, PCY); Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University (CHT, YLL); and Department of Laboratory Medicine, National Taiwan University Hospital, Zhongzheng District, Taipei, Taiwan (LNL, HCC, JMC, CAH).
Correspondence: Li-Na Lee, Department of Laboratory Medicine, National Taiwan University Hospital, 7, Chung Shan South Rd., Zhongzheng District, Taipei 10002, Taiwan (e-mail: linalee@ntu.edu.tw).
JYW drafted the manuscript, and together with LNL and CJY, designed the study and interpreted results. JYW, CHT, and CHL participated in data analysis. HCC, JMC, and CAH performed the laboratory procedures. JYW, YLL, and LNL performed statistical analysis. PCY was the director responsible for general organization and instruction. JYW, LNL, CLH, CJY, and PCY all participated in patient enrollment.
This study was supported by the National Science Council of Taiwan (grant NSC 102-2629-B-002-001 and 101-2325-B-002-008), the Research Center for Biotechnology and Medicine Policy, and Liver Disease Prevention & Treatment Research Foundation.
Details of the computer code for statistical analyses are available from the corresponding author at linalee@ntu.edu.tw.
The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000000982

(*Medicine* 94(24):e982)

Abbreviations: AADAC = acrylacetamide deacetylase, ALT = alanine transaminase, AST = aspartate transaminase, CYP = cytochrome P450, HATT = hepatitis during antituberculosis treatment, HBV = hepatitis B virus, HCV = hepatitis C virus, HNF = hepatic nuclear factor, INH = isoniazid, LFT = liver function test, *NAT2* = *N*-acetyltransferase 2, OR = odds ratio, PXR = pregnane X receptor, PZA = pyrazinamide, RMP = rifampin, SNP = single nucleotide polymorphism, TB = tuberculosis, ULN = upper limit of normal.

INTRODUCTION

Tuberculosis (TB) remains a major infectious cause of deaths worldwide. To prevent transmission and future relapse, prompt and supervised anti-TB treatment for an extended period is very important.[1] However, the development of hepatotoxicity, which may be induced by anti-TB drugs or acute flare-up of concomitant viral hepatitis, is the most important adverse event leading to interruption or premature discontinuation of anti-TB treatment.[2] Among the first-line anti-TB drugs, isoniazid (INH), rifampin (RMP), and pyrazinamide (PZA) are all hepatotoxic and can increase the risk of hepatotoxicity further when used together.

For a long time, women have been reported to have a higher risk of hepatitis during antituberculosis treatment (HATT) than men. Although the definition of hepatotoxicity varies between studies, the hazard ratio of female sex ranges from 1.5 to 3.3.[3–7] A previous study in Taiwan revealed that female sex was a significant risk factor of drug-induced HATT and was independent of the *N-acetyltransferase 2* (*NAT2*) status.[6] One possible reason is the activity of cytochrome P450 (CYP) 3A4, the most abundant enzyme in the hepatic CYP family that catalyzes the phase I reaction of many drugs, is higher in women,[8] given that many adverse drug reactions are caused by the CYP dependent activation of drugs into reactive metabolites.[9]

The exact mechanism that leads to higher CYP3A4 activity in women than men is unknown. Single nucleotide polymorphisms (SNPs) in the coding region of the *CYP3A4* gene occur only rarely and cannot explain the difference in CYP3A4 activity between men and women.[10] The pregnane X receptor (PXR), a member of the nuclear receptor superfamily, is a known regulator of the *CYP3A4* gene. When bound by its ligand, PXR upregulates the expression of its target genes, including genes for phase 1 metabolizing enzymes such as CYP3A, and genes for all 3 phases of xenobiotic metabolism.[11,12] SNPs in the transcription factor binding sites of the *PXR* regulatory region (the promoter and intron1) have also

been associated with altered *PXR* and *CYP3A4* expressions,[13,14] as well as drug-induced liver injury.[15]

Given the large spectrum of PXR activating ligands and target genes, and the association between SNPs in the *PXR* regulatory region and *CYP3A4* expression, it is possible that gene variants in the *PXR* regulatory region may contribute to differences in risk of drug-induced HATT between male and female patients. We hypothesized that certain genotypes and haplotypes in *PXR* regulatory region SNPs may be risk factors for HATT, and the distribution of these genotypes and haplotypes may be different between male and female TB patients, leading to the increased risk of hepatotoxicity in females.

METHODS

Study Population and Protocols

This prospective study was conducted at National Taiwan University Hospital, a tertiary-care center in Taiwan. The hospital's Institution Review Board approved the study (NTUH REB No.: 9561707008). All of the participants provided informed written consent.

From March 2007 to February 2010, adult patients (>16 years) with culture-confirmed pulmonary TB were enrolled as the derivation cohort. Mycobacterial culture and drug susceptibility testing were performed as previously described.[16] Subjects were excluded if they were pregnant, had a life expectancy <6 months, had abnormal baseline liver function test (LFT), or had *Mycobacterium tuberculosis* (MTB) isolates resistant to INH, RMP, or both. From March 2010 to February 2013, TB patients fulfilling these criteria were enrolled as the validation cohort.

Complete medical data and radiologic imaging were recorded. Alcohol abuse was defined as a daily consumption of ≥60 g of alcohol.[17] Malnutrition was defined as either serum albumin level <3.5 g/dL[6] or body-mass index <18.5 kg/m^2.[18]

For TB patients, LFT, including aspartate transaminase (AST) and alanine transaminase (ALT), direct and total bilirubin levels, were determined before the start of anti-TB treatment. Serologic tests for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus, serum albumin, creatinine, and complete hemogram were also performed. The LFT was checked at 2, 4, 6, 8, 12, and 16 weeks after the start of anti-TB treatment or whenever symptoms of hepatitis developed during the initial 6 months of anti-TB treatment.[19] If there was elevated AST or ALT, the LFT was repeated and assessed weekly. For patients with concomitant HBV or HCV infection, serum HBV or HCV viral load was determined by quantitative PCR (Cobas Amplicor HBV and HCV monitor v2.0; Roche Diagnostics, Pleasanton, CA) simultaneously with LFT to document acute flare-up of viral hepatitis.

All TB patients received standard anti-TB treatment of daily INH, RMP, ethambutol (EMB), and PZA in the first 2 months, and daily INH and RMP for the next 4 months. The daily dosage of each drug was calculated by weight.[20] The regimen was modified by the primary care physician if necessary, for example, when there were adverse drug effects.

Definition and Etiology of Hepatitis During Antituberculosis Treatment (HATT)

HATT was defined as increased serum AST and/or ALT >3 times the upper limit of normal (ULN) in symptomatic patients, or >5 times the ULN in asymptomatic patients.[2] Once HATT occurred, potentially hepatotoxic drugs (INH, RMP, and PZA) were stopped. Anti-TB drugs were reintroduced after serum levels of AST and/or ALT returned to <3 times the ULN and the clinical symptoms of hepatitis resolved. As in a previous study,[6] the diagnosis of INH- or RMP-induced hepatitis required a positive rechallenge test (at least doubling of serum AST or ALT levels and recurrence of clinical symptoms of hepatitis after rechallenge), whereas PZA-induced hepatitis was diagnosed either by a positive rechallenge test or by exclusion. Virus-induced HATT was diagnosed if the rise in serum AST and ALT was associated with a concomitant rise in viral load.

Genotyping for *PXR* SNPs

Genotyping for the *PXR* and *NAT2* genes for TB patients was performed on genomic DNA extracted from peripheral white blood cells. Laboratory technicians were blinded to the status of the participants during the entire process of *PXR* and *NAT2* genotyping.

The *PXR* gene, also known as the nuclear receptor subfamily 1, group I, member 2 (*NR1I2*) gene, is located in chromosome 3. We first obtained a list of *PXR* SNPs based on predicted regulatory function or known association with diseases.[13,21–23] All SNPs were used as input files for the Haploview v4.1 (http://www.broadinstitute.org/mpg/haploview) to search for tag SNPs in the genomic region of *PXR* that encompasses 36 kb and contains 33 polymorphic sites, using squared correlation (r^2) cutoff ≥0.95 and minor allele frequency (MAF) ≥0.1. We selected 6 tag SNPs in the regulatory region of *PXR* gene to be investigated: rs3814055 (located in the 5′ untranslated region), rs12488820, rs2461823, rs7643645 (all located in intron 1), rs6785049 (located in intron 5), and rs3814057 (located in the 3′ untranslated region) (Supplementary Figure S1, http://links.lww.com/MD/A303).

SNP genotyping using the Sequenom MassARRAY system (iPLEX GOLD) (Sequenom, San Diego, CA) was performed according to the manufacturer's recommendations (Sequenom) (see Supplement File for details, http://links.lww.com/MD/A303).[24] Call rates for individual polymorphisms were >98%. Concordance of duplicates was 100%.

Genotyping for *NAT2* Gene

Genotyping for *NAT2* was performed by direct sequencing.[6] Four *NAT2* variants, that is, 191G>A (rs1801279, R64Q or *NAT2**14 allele), 341T>C (rs180128012, I114T or *NAT2* *5 allele), 590G>A (rs1799930, R197Q or *NAT2* *6 allele), and 857G>A (rs1799931, G286E or *NAT2* *7 allele), result in amino acid substitution and are associated with slow acetylator phenotype. The presence of 2 of these variants in a patient was defined as slow acetylator genotype.

Data Analysis and Statistical Analysis

All SNPs were tested for Hardy-Weinberg equilibrium.[25] Double data entry were performed to ensure data quality. Differences between groups were analyzed by independent sampled *t* test for continuous variables and by chi-square test or Fisher exact test for categorical variables. Linkage disequilibrium analysis was performed using Haploview.[26] Haplotype frequencies were calculated from *PXR* genotype data and analyzed using the EM algorithm by TagSNPs.[27] We collapsed rare haplotypes (frequency <0.05) into a category in final haplotype analyses.

The association between drug-induced HATT and clinical factors, *NAT2* and *PXR* genotype frequencies, *PXR* allele frequencies, and *PXR* haplotype frequencies were analyzed

TABLE 1. Clinical, Laboratory Characteristics, and *NAT2* Genotype of Tuberculosis (TB) Patients With Drug-Induced Hepatitis During Antituberculous Treatment (HATT) (n = 70) and the TB Patients Without (n = 285)

	All Patients (n = 355)	TB Patients With HATT (n = 70)	TB Patients Without HATT (n = 285)	*p* Value
Age, years	57.6 ± 19.4	58.8 ± 19.1	57.3 ± 19.5	0.551
Female	122 (34.4%)	31 (44.3%)	91 (31.9%)	0.051
Current smoker	70 (19.7%)	10 (14.3%)	60 (21.1%)	0.202
Alcohol abuse*	6 (1.7%)	0 (0%)	6 (2.1%)	0.603
Prior history of TB	38 (10.7%)	7 (10.0%)	31 (10.9%)	0.832
Malnutrition†	70 (19.7%)	16 (22.9%)	54 (18.9%)	0.461
Comorbidity	159 (44.8%)	31 (44.3%)	128 (44.9%)	0.925
Cancer	80 (22.5%)	16 (22.9%)	64 (22.5%)	0.943
Diabetes mellitus	69 (19.4%)	10 (14.3%)	59 (20.7%)	0.224
End-stage renal disease	25 (7.0%)	6 (8.6%)	19 (6.7%)	0.602
Autoimmune disease	13 (3.7%)	5 (7.1%)	8 (2.8%)	0.145
HIV infection	4 (1.1%)	2 (2.9%)	2 (0.7%)	0.176
Transplant	3 (0.8%)	0 (0%)	3 (1.1%)	>0.999
Liver cirrhosis	2 (0.6%)	1 (1.4%)	1 (0.4%)	0.356
Initial sputum smear-positive	147 (41.4%)	31 (44.3%)	116 (40.7%)	0.585
Sputum culture-positive after 2-month treatment	17 (4.8%)	0 (0%)	17 (11.1%)	0.014
Cavitation on CXR	50 (14.1%)	5 (7.1%)	45 (15.8%)	0.062
Miliary lesions on CXR	11 (3.1%)	2 (2.9%)	9 (3.2%)	>0.999
Hemoglobin <12 g/dL	167 (47.0%)	35 (58.3%)	132 (55.2%)	0.665
Platelet <140,000/μL	52 (17.4%)	11 (18.3%)	41 (17.2%)	0.830
Leukocyte <4000/μL	3 (0.8%)	0 (0%)	3 (1.3%)	>0.999
HBV or HCV infection	56 (15.8%)	11 (15.7%)	45 (15.8%)	>0.999
High HBV or HCV viral load	24 (6.8%)	6 (8.6%)	18 (6.3%)	0.594
NAT2 slow acetylator genotype	85 (23.9%)	23 (32.9%)	62 (21.8%)	0.051

Data are either mean ± standard deviation or No. (%) unless otherwise mentioned. CXR = chest radiography, *NAT2* = *N*-acetyltransferase 2.
* Alcohol abuse was defined as daily consumption of ≥60 g of alcohol.[17]
† Malnutrition was defined as either serum albumin level <3.5 g/dL[6] or body-mass index <18.5 kg/m^2.[18]

using chi-square method, univariate, and multivariate logistic regression model. In the multivariate logistic regression analysis, an interaction variable between sex and *PXR* genotypes and haplotypes was also included. The identified predictors of drug-induced HATT were then validated using a validation cohort. A 2-sided $p < 0.05$ was considered significant. All analyses were performed using the SAS (Version 9.2, SAS Institute Inc., Cary, NC).

RESULTS

Derivation Cohort Case Enrollment

From March 2007 to February 2010 a total of 964 cases of culture-confirmed pulmonary TB were identified. Of the 964 cases, 222 cases were excluded due to the following reasons: 21 cases with multidrug-resistant MTB isolates which were resistant to both INH and RMP, 104 cases with INH-resistant MTB isolates, 3 cases with RMP-resistant MTB isolates, 51 cases with a life expectancy <6 months, 10 cases with abnormal baseline LFT (7 due to congestive heart failure and 3 due to excessive alcohol consumption), and 33 cases withdrew their consent later. Of the remaining 742 TB patients, 355 (36.8% of 964) agreed to participate, completed the study and formed the derivation cohort. Their mean age was 57.6 ± 19.4 years and 233 (65.6%) were male. The overall follow-up duration was 2004.5 person-months (5.6 months/patient).

Characteristics of Patients With HATT

During the 6-month follow-up, 70 (19.7%) patients developed drug-induced HATT, including 31 women (25.4% of 122 women) and 39 men (16.7% of 233 men). Sixty (16.9% of 355) patients were symptomatic with transaminases >3 times ULN and the other 10 (2.8% of 355) were asymptomatic with transaminases >5 times ULN. Of the 70 patients with drug-induced HATT, 13 (19% of 70 patients) had serum total bilirubin >2.0 mg/dL (mean 3.3 ± 1.9, range 2.0–7.4 mg/dL). None was complicated with hepatic failure. The responsible anti-TB drug was INH in 8 (11%), RMP in 18 (26%), and PZA in 44 (63%). Thirty three (47%) developed drug-induced hepatitis within the first month of the treatment and 32 (46%) in the second month.

Table 1 shows that when compared to patients without drug-induced HATT, those who developed drug-induced HATT showed a tendency of higher percentage of female gender (44.3% vs 31.9%, $p = 0.051$), *NAT2* slow acetylator genotype (32.9% vs 21.8%, $p = 0.051$, power = 0.489), lower percentage of cavitation on initial chest radiography (7.1% vs 15.8%, $P = 0.062$), and significantly lower probability of positive sputum culture for *M. tuberculosis* after 2 months of anti-TB

TABLE 2. Influence of Genotype at Each *PXR* SNP Site on Drug-Induced Hepatitis During Antituberculous Treatment (HATT) in Males and Females Using Chi-Square Analysis

				All patients			Males			Females		
SNP	Location	*p* Value for HWE	Genotype	No.	% With HATT	*p* Value	No.	% With HATT	*p* Value	No.	% With HATT	*p* Value
rs3814055	5′ UTR	0.075	CC	238	19	0.742	156	16	0.916	82	24	0.761
			CT	99	22		60	18		39	28	
			TT	18	17		17	18		1	0	
rs12488820[†]	Intron 1	<0.001	CC	337	20	0.747	216	17	>0.999	121	26	>0.999
			TT	16	13		15	13		1	0	
rs2461823[*,‡]	Intron 1b	0.699	GG	128	18	0.052	89	19	0.729	39	15	0.007
			AG	172	17		114	15		58	22	
			AA	53	32		29	17		24	50	
rs7643645[*]	Intron 1b	0.528	AA	95	13	0.142	65	17	0.368	30	3	0.004
			AG	171	22		117	19		54	30	
			GG	88	22		50	10		38	37	
rs6785049	Intron 5	0.123	GG	119	18	0.284	72	15	0.883	47	21	0.091
			AG	185	23		129	18		56	34	
			AA	51	14		32	16		19	11	
rs3814057[†]	3′ UTR	0.002	AA	73	15	0.431	50	18	0.636	23	9	0.117
			AC	205	22		139	18		66	30	
			CC	75	19		42	12		33	27	

HWE = Hardy-Weinberg equilibrium, *PXR* = *pregnane X receptor*, SNP = single nucleotide polymorphism.
[*] SNP genotype was unknown in 1 man.
[†] SNP genotype was unknown in 2 men.
[‡] SNP genotype was unknown in 1 woman.

treatment (0% vs 11.1%, $p = 0.014$). None of the 4 *NAT2* variants (191G>A, 341T>C, 590G>A, and 857G>A) or 7 alleles (*4, *5, *6, *7, *11, *12, *13) were significantly associated with overall HATT (Supplementary Table S1, http://links.lww.com/MD/A303)

Genotype of *PXR* SNPs and Association With Overall Drug-Induced HATT

All of the participants had *PXR* SNPs successfully genotyped, except for 1 man and 1 woman in rs2461823, 1 man in rs7643645, and 2 men in rs12488820 and rs3814057. All *PXR* polymorphisms met the Hardy-Weinberg equilibrium ($p > 0.05$) except for rs12488820 ($p < 0.001$) and rs3814057 ($p = 0.002$). Table 2 shows the risk of drug-induced hepatitis among TB patients with different SNP genotypes in *PXR* gene (see also Supplementary Figure S2, http://links.lww.com/MD/A303). In male TB patients, none of the 6 SNPs in the *PXR* gene was associated with the development of overall drug-induced HATT, with similar genotype frequencies between male patients with hepatitis and those without. In female TB patients, 2 *PXR* variants were significantly associated with the risk of overall drug-induced hepatitis. The frequency of drug-induced HATT in female patients with AA genotype at rs2461823 site (50%) was significantly higher compared to female patients carrying other genotypes (AG, 22%; GG, 15%, $p = 0.007$). The higher risk of AA genotype at rs2461823 in females was also shown in the recessive model (odds ratio [OR] and 95% confidence interval [CI]: 0.24 [0.10–0.63]) and additive model (OR: 2.4 [1.30–4.44]) (Supplementary Table S2, http://links.lww.com/MD/A303). On the other hand, the frequency of HATT in female patients with AA genotype at the rs7643645 site (3%) was significantly lower compared to female patients with other genotypes (AG 30%, GG 37%, $p = 0.004$). The protective effect of AA genotype at rs7643645 in females was also evident in the dominant model (OR: 14.0 [1.82–108]) and additive model (OR: 2.56 [1.37–4.76]) (Supplementary Table S2, http://links.lww.com/MD/A303). Both SNPs were located at intron 1 (Table 2), near the promoter of the *PXR* gene.

Haplotype Frequency and Association With Overall Drug-Induced HATT

There were 7 haplotypes with a frequency ≥0.05 (Supplementary Table S3, http://links.lww.com/MD/A303). In male TB patients, none of the 7 haplotypes were associated with the development of overall drug hepatitis (Table 3). However, in female TB patients, 3 haplotypes, h001101 (OR: 3.08 [1.24–7.61]), h001110 (OR: 3.04 [1.29–7.16]), and h000110 (OR: 2.59 [1.12–5.98]), were significantly associated with increased risk of drug-induced HATT.

Multivariate Analyses for Risk Factors of Overall Drug-Induced, INH-, RIF-, and PZA-Induced HATT

Multivariate logistic regression analysis, including all the variables listed in Table 1, *NAT2* variants, *PXR* genotypes, allele numbers, haplotypes, and sex interaction, was performed to identify independent predictors of overall and individual drug-induced HATT. Table 4 shows that the only independent predictors of overall drug-induced HATT were *PXR* variants genotypes, allele numbers, and haplotypes. Genotype analysis revealed that the female genotype AA at rs2461823 site (OR 4.64 [1.96–11.0]), a risk genotype, and the female genotype AA at rs7643645 site (OR: 0.14 [0.02–1.02]), a protective genotype, were both independently associated with overall drug-induced HATT. Allele number analysis showed that the number

TABLE 3. Risk of Drug-Induced Hepatitis During Antituberculous Treatment (HATT) in Males and Females With Different Haplotypes of the *PXR* Gene

	All		Males		Females	
Haplotype*	**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
h001101: yes vs No	1.32	0.78–2.27	0.71	0.35–1.45	**3.08**	**1.24–7.61**
h000010: yes vs No	1.20	0.70–2.04	1.30	0.64–2.64	1.17	0.51–2.68
h100001: yes vs No	1.13	0.59–2.19	1.11	0.45–2.74	0.87	0.44–1.71
h001110: yes vs No	1.34	0.78–2.33	0.78	0.36–1.67	**3.04**	**1.29–7.16**
h000110: yes vs No	1.23	0.72–2.12	0.74	0.35–1.55	**2.59**	**1.12–5.98**
h000000: yes vs No	1.08	0.63–1.85	1.14	0.56–2.32	1.22	0.50–2.95
h000001: yes vs No	0.95	0.56–1.61	0.95	0.47–1.92	0.96	0.42–2.17
Others: yes vs No	1.36	0.63–2.93	1.15	0.42–3.20	1.83	0.57–5.88

Bold value signifies the statistical significance. CI = confidence interval, OR = odds ratio, *PXR = pregnane X receptor*.
* 0: common allele and 1: minor allele, by the order of rs3814055: C → T; rs12488820: C → T; rs2461823: G → A; rs7643645: A → G; rs6785049: G → A; rs3814057: A → C.

of G allele at rs7643645 was significantly associated with increased risk of drug-induced HATT in females (OR: 1.91 [1.35–2.71]). Haplotype analysis revealed that 2 haplotypes (both carrying G allele at rs7643645 site), h001101 (OR 2.30 [1.22–4.32]) and h000110 (OR 2.25 [1.08–4.69]), were associated with increased risk of overall drug-induced HATT in females.

As for INH-induced hepatitis, 1 *NAT2* variant (857G>A, corresponding to *NAT2* *7, a slow acetylator genotype) was associated with increased risk, and the wild type allele *NAT2* *4, a rapid acetylator genotype was associated with decreased risk of INH-induced HATT (Supplementary Table S1, http://links.lww.com/MD/A303). In multivariate logistic regression analysis, *NAT2* and malnutrition were independent risk factors for INH-induced hepatitis in both male and female patients, but genotype AA at rs2461823 site (OR: 10.5 [1.91–58.1]) and number of A allele at rs6785049 site (OR: 11.7 [1.06–129]) were independent risk factors only in females (Supplementary Table S4, http://links.lww.com/MD/A303). None of the *PXR* haplotypes were significantly associated with INH-induced hepatitis.

For RMP-induced hepatitis (Supplementary Table S5, http://links.lww.com/MD/A303), multivariate logistic regression analysis revealed that end-stage renal disease, number of A allele at rs6785049, and h000010 haplotype were independent risk factors in both male and female patients, but genotype AG at rs6785049 (OR: 3.09 [1.09–8.81]) and h001101 haplotype (OR: 5.51 [1.68–18.1]) were independent risk factors only in females.

For PZA-induced hepatitis (Supplementary Table S6, http://links.lww.com/MD/A303), multivariate logistic regression analysis revealed that genotype AG at rs7643645 (OR: 2.85 [1.33–6.11]) was an independent risk factor for both male and female patients, but genotype AA at rs2461823 (OR: 7.29 [2.54–20.9]), number of G allele at rs7643645 (OR: 1.84 [1.19–2.85]), and h000110 haplotype (OR: 5.10 [1.92–13.5]) were independent risk factors only in females.

Validation for Risk Factors of Overall Drug-Induced HATT

A total of 182 TB patients were enrolled into the validation cohort. Their mean age was 58.3 ± 37.5 years and 114 (62.6%) were male. During follow-up, 18 (26%) of the 68 female patients and 17 (15%) of the 114 male patients developed HATT.

TABLE 4. Factors Associated With all Drug-Induced Hepatotoxicity During Antituberculous Treatment, by Multivariate Logistic Regression Analysis

Variables	***p***	**OR (95% CI)**
Factors including genotypes		
AA at rs2461823 in females (risk genotype)	<0.001	4.64 (1.96–11.0)
AA at rs7643645 in females (protective genotype)	0.052	0.14 (0.02–1.02)
Factors including allele numbers		
No. of G allele at rs7643645 in female (risk allele)	<0.001	1.91 (1.35–2.71)
Factors including haplotypes		
h001101* in females (risk haplotype)	0.010	2.30 (1.22–4.32)
h000110* in females (risk haplotype)	0.030	2.25 (1.08–4.69)

CI = confidence interval; OR = odds ratio.
* 0: common allele and 1: minor allele, by the order of rs3814055: C → T; rs12488820: C → T; rs2461823: G → A; rs7643645: A → G; rs6785049: G → A; rs3814057: A → C.

TABLE 5. Influence of Genotype at Each *PXR* SNP Site on Drug-Induced Hepatitis During Antituberculous Treatment (HATT) in Males and Females Among Validation Cohort (n = 182) Using Chi-Square Analysis

			All Patients			Males			Females		
SNP	*p* Value for HWE	Genotype	No.	% With HATT	*p* Value	No.	% With HATT	*p* Value	No.	% With HATT	*p* Value
rs3814055	0.123	CC	104	20	0.927	63	14	0.976	41	29	0.519
		CT	72	18		45	16		27	22	
		TT	6	17		6	17				
rs12488820	<0.001	CC	176	19	>0.999	108	15	0.901	68	26	-
		TT	6	17		6	17				
rs2461823	0.232	GG	67	19	0.892	43	19	0.252	24	21	0.103
		AG	93	18		58	16		35	23	
		AA	22	23		13	0		9	56	
rs7643645	0.614	AA	55	20	0.772	33	21	0.439	22	18	0.417
		AG	87	17		54	11		33	27	
		GG	40	23		27	15		13	39	
rs6785049	0.411	GG	55	22	0.547	40	13	0.572	15	47	0.125
		AG	95	20		54	19		41	22	
		AA	32	13		20	10		12	17	
rs3814057	0.362	AA	46	9	0.096	27	7	0.434	19	11	0.078
		AC	97	22		61	18		36	28	
		CC	39	26		26	15		13	46	

HWE = Hardy-Weinberg equilibrium, *PXR* = *pregnane X receptor*, SNP = single nucleotide polymorphism.

Table 5 shows the influence of genotypes at each *PXR* SNP site on the risk of drug-induced HATT. Although none of the SNP genotypes at rs7643645 or rs2461823 were significantly associated with HATT by chi-square test, risk of HATT was higher in female patients with AA genotype at rs2461823 (56%) and GG genotype at rs7643645 (39%). Table 6 compares risk of drug-induced HATT in patients with or without risk predictors. It is evident that the risk of drug-induced HATT was significantly different between the 3 subgroups with different combination of genotypes at rs7643645 and rs2461823 ($p = 0.018$), borderline different in the 3 subgroups with different number of G allele at rs7643645 ($p = 0.057$), and significantly different in the 4 subgroups with different haplotypes ($p = 0.008$). Although the *P* values in the female patients alone did not reach statistical significance, probably owing to the small sample size of the validation group, these findings suggested that the risk of drug-induced HATT was influenced by above-mentioned *PXR* variants and haplotypes only in females.

DISCUSSION

By prospective observation and genotyping in 355 culture-confirmed pulmonary TB patients and validation in another 182 cases, this is the first study to show that the SNPs of the *PXR* gene are independent risk factors of overall drug-induced hepatitis in TB patients, and that the association occurs only in women. Risk factors of hepatitis for individual first-line anti-TB drugs vary, but *PXR* SNP genotypes and haplotypes remain significant risk factors and show gender difference.

Both rs7643645 and rs2461823 were located at intron 1b of the regulatory region close to the *PXR* promoter. Rs7643645 is located in the binding site of the transcription factor, hepatic nuclear factor (HNF)4α, and a change from the wild A allele to the mutant G allele leads to a loss of the HNF4α binding site.[13] Holloway et al[28] found that in a mouse model, HNF4α exerted both a positive and a negative regulatory effect on many hepatic genes. With loss of the HNF4α, 82% of the 4994 HNF4α-dependent genes were suppressed in males. In contrast, only 56% of HNF4α-dependent genes were suppressed, while some of the HNF4α-dependent genes were upregulated upon loss of HNF4α in females.[28] Such findings suggest that *PXR* gene expression (and its regulation on target xenobiotic metabolism genes) in response to HNF4α loss is different between the 2 sexes.

As for rs2461823, there has been no transcription factor binding sequence identified in this site to date. Nonetheless, it is in strong linkage disequilibrium with several other SNP sites in the *PXR* promoter region, including rs2472677, which is located within a hepatocyte nuclear factor 3-β (HNF3β) binding site. Its CC genotype is associated with high level *PXR* induction by RMP.[29] In mice, the HNF3β binding site has been associated with female predominant expression of the *CYP2b9* gene.[30] Thus, the rs2461823 AA genotype may occur in strong linkage disequilibrium with certain genotypes of other SNPs (such as rs2472677) that bear a transcription factor binding sequence, and this may become associated with different risk of HATT between males and females.

A previous study found that the rs7643645 GG and rs2461823 AA genotypes were associated with increased risk and/or severity of nonalcoholic fatty liver disease, but without any sex difference.[21] Another study found that the rs2461823 GG genotype was associated with susceptibility to intrahepatic cholestasis of pregnancy. In that study all of the subjects were females.[29] These reports, together with the current findings, suggest that these 2 *PXR* SNPs affect detoxification and elimination pathways of drugs like INH and PZA, either in females alone or in both sexes. The underlying mechanisms of these observations may be a loss of *PXR* transcription factor binding site.

TABLE 6. Risk of Drug-Induced Hepatitis During Antituberculous Treatment (HATT) in Validation Cohort (n = 182)

						Women (n = 68)			Men (n = 114)	
	Presence or Absence of First Predictor	Presence or Absence of Second Predictor	Total No.	No. (%) With HATT	p^*	Total No.	No. (%) With HATT	p^*	Total No.	No. (%) With HATT
	AA at rs7643645 in female (protective)	AA at rs2461823 in female (risk)								
Genotype analysis	Yes	No	22	4 (18%)	0.018	22	4 (18%)	0.092		
	No	No	151	26 (17%)		37	9 (24%)		114	17 (15%)
	No	Yes	9	5 (56%)		9	5 (56%)			
Allele number analysis	No. of G allele (risk) at rs7643645 in female									
	0		136	21 (15%)	0.057	22	4 (18%)	0.417	114	17 (15%)
	1		33	9 (27%)		33	9 (27%)			
	2		13	5 (39%)		13	5 (39%)			
Haplotype analysis	h001101† in female (risk)	h000110† in female (risk)								
	No	No	137	21 (15%)	0.008	23	4 (17%)	0.086	114	17 (15%)
	No	Yes	14	2 (14%)		14	2 (14%)			
	Yes	No	16	8 (50%)		16	8 (50%)			
	Yes	Yes	15	4 (27%)		15	4 (27%)			

* Calculated by chi-square test.

† 0: common allele and 1: minor allele, by the order of rs3814055: C → T; rs12488820: C → T; rs2461823: G → A; rs7643645: A → G; rs6785049: G → A; rs3814057: A → C.

The findings that SNP in rs2461823 of the *PXR* gene is associated with both INH- and PZA-induced hepatitis have never been reported. The hepatotoxin that leads to INH-induced hepatitis has been proposed to be hydrazine and other hydrazine metabolites that can generate free radicals. The conversion from INH to hydrazine involves either a classical P450 oxidase that can be induced by PXR ligands like RMP, phenobarbital or dexamethasone,[31] or an amidase which can also catalyze the reaction from monoacetylhydrazine to hydrazine (Supplementary Figure S3, http://links.lww.com/MD/A303).[32] Monoacetylhydrazine can be converted to free radical hepatotoxins by a P450-dependent CYP2E1. Therefore, PXR could increase the production of hydrazine, free radical hepatotoxins, and thus the risk of INH-induced hepatitis, through P450 oxidase and CYP2E1. Whether PXR could influence amidase is unknown. In addition, a recent study has demonstrated that cotreatment with INH and RMP causes liver injury through PXR-mediated alteration of the heme biosynthesis pathway.[33]

As for PZA-induced hepatitis, recently PZA metabolites pyrazinoic acid and 5-hydroxypyrazinoic acid have been reported to correlate with PZA-induced hepatitis.[34] The conversion of PZA to pyrazinoic acid, and that of 5-hydroxy-PZA to 5-hydroxypyrazinoic acid both involve an amidase (Supplementary Figure S4, http://links.lww.com/MD/A303).[35] The other source of 5-hydroxypyrazinoic acid production is from pyrazinoic acid via xanthine oxidase. There has been no report regarding to the effect of PXR on the activity of amidase or xanthine oxidase. Since both INH-induced and PZA-induced hepatitis were associated with rs2461823 variant, it was also possible that PXR's effect was through its regulation on amidase, which metabolizes both INH and PZA. Further studies are needed to clarify these hypotheses.

The mechanism of hepatotoxicity due to rifampicin and its derivatives remains unknown. It is hydrolyzed in liver by acrylacetamide deacetylase (AADAC; for rodents, Aadac), a member of the carboxylesterase-5 family, which catalyze the hydrolysis of many ester- and amide-containing chemicals.[36] A previous study in mice revealed that Aadac mRNA was highly expressed in mouse livers and was suppressed by 2 PXR ligands—pregnenolone-16α-carbonitrile and dexamethasone.[37] However, whether the Aadac mRNA expression was altered by the SNP polymorphisms of the *PXR* gene was not studied. The finding that the 1 genotype and 2 haplotypes of the *PXR* gene were significantly associated with RMP-induced hepatitis in the study suggests that PXR may regulate the metabolism of RMP through AADAC and alter the risk of hepatitis. Further studies are needed to confirm these finding and investigate the underlying mechanisms.

From the results of genotype distribution we calculated the risk of anti-TB drug-induced HATT associated with haplotypes, and found that 2 haplotypes were also associated with the risk of HATT by multivariate analysis. We observed that all the 3 *PXR* SNPs (rs2461823, rs7643645, and rs6785049) contributed to the association between haplotypes and HATT.

We also observed that NAT2 slow acetylator and malnutrition were independent risk factors for INH-induced hepatitis both in males and females, consistent with previous reports.[6,38] Malnutrition has been reported to reduce the activity of hepatic glutathione S-transferase and increase vulnerability to oxidative injury,[39] leading to increased risk of INH-induced hepatotoxicity in TB patients.

The finding that end-stage renal disease is associated with RMP-induced hepatotoxicity is unexpected since 60% to 65%

of RMP dose appears in feces, and it does not accumulate in patients with impaired renal function (Rifadin package insert, Marion Merrell Dow, Ohio, US). Because uremic toxins can alter the hepatic clearance of many drugs, either by down-regulation of specific isoforms of CYP via affecting promoter[40] or by impaired hepatic uptake mediated by uptake transporters,[41] it is possible that the increased risk of RMP-induced hepatotoxicity in patients with end-stage renal disease was associated with the accumulation of uremic toxin.

The present study has limitations regarding the interpretation of its findings. First, the mechanisms of INH-, RMP-, or PZA-induced HATT are likely to be different, and mixing together all patients with drug-induced HATT may mask unknown risk factors. Yet we analyzed risk factors for individual drugs and found that risk factors for individual drugs also included *PXR* variants. Second, drug-metabolizing enzymes other than PXR and NAT2 were not genotyped. Third, serum levels of drugs and toxic metabolites were not measured. Nonetheless, even if serum levels of metabolites have been shown to be highly predictive for some of HATT, routine measurement is not practical. Fourth, the validation cohort is small in sample size and did not directly replicate the results of the derivation cohort. Yet when we further analyzed patients with the presence or absence of the risk factors that were identified in the derivation cohort, the influence of *PXR* variants and haplotypes was still evident in the female patients of the validation cohort. Lastly, the study was conducted in a medical center and nearly half of the TB patients had underlying comorbidities that might influence laboratory results and radiographic findings.

CONCLUSIONS

This study is the first to show that 2 *PXR* SNP genotypes and 2 haplotypes influenced the risk of HATT only in females. The *PXR* gene variants have sex-dimorphic impact that contributes to the increased risk of drug-induced HATT in females.

ACKNOWLEDGMENTS

The authors thank the staff of the Eighth Core Lab of the Department of Medical Research of National Taiwan University Hospital for their technical support.

REFERENCES

1. Albert RK, Iseman M, Sbarbaro JA, et al. Monitoring patients with tuberculosis for failure during and after treatment. *Am Rev Respir Dis.* 1976;114:1051–1060.
2. American Thoracic Society, C.D.C., Infectious Diseases Society of America. Treatment of tuberculosis. *MMWR Recomm Rep.* 2003;52:1–77.
3. Yee D, Valiquette C, Pelletier M, et al. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *Am J Respir Crit Care Med.* 2003;167:1472–1477.
4. van Hest R, Baars H, Kik S, et al. Hepatotoxicity of rifampin-pyrazinamide and isoniazid preventive therapy and tuberculosis treatment. *Clin Infect Dis.* 2004;39:488–496.
5. Shu CC, Lee CH, Lee MC, et al. Hepatotoxicity due to first-line anti-tuberculosis drugs: a five-year experience in a Taiwan medical centre. *Int J Tuberc Lung Dis.* 2013;17:934–939.
6. Wang JY, Liu CH, Hu FC, et al. Risk factors of hepatitis during anti-tuberculous treatment and implications of hepatitis virus load. *J Infect.* 2011;62:448–455.
7. Teleman MD, Chee CB, Earnest A, et al. Hepatotoxicity of tuberculosis chemotherapy under general programme conditions in Singapore. *Int J Tuberc Lung Dis.* 2002;6:699–705.
8. Hunt CM, Westerkam WR, Stave GM. Effect of age and gender on the activity of human hepatic CYP3A. *Biochem Pharmacol.* 1992;44:275–283.
9. Vignati L, Turlizzi E, Monaci S, et al. An in vitro approach to detect metabolite toxicity due to CYP3A4-dependent bioactivation of xenobiotics. *Toxicology.* 2005;216:154–167.
10. Lamba JK, Lin YS, Schuetz EG, et al. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev.* 2002;54:1271–1294.
11. Handschin C, Meyer UA. Induction of drug metabolism: the role of nuclear receptors. *Pharmacol Rev.* 2003;55:649–673.
12. Maglich JM, Stoltz CM, Goodwin B, et al. Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol Pharmacol.* 2002;62:638–646.
13. Lamba J, Lamba V, Strom S, et al. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. *Drug Metab Dispos.* 2008;36:169–181.
14. Ramappa V, Aithal GP. Hepatotoxicity related to anti-tuberculosis drugs: mechanisms and management. *J Clin Exp Hepatol.* 2013;3:37–49.
15. Andrews E, Armstrong M, Tugwood J, et al. A role for the pregnane X receptor in flucloxacillin-induced liver injury. *Hepatology.* 2010;51:1656–1664.
16. Ruan SY, Chuang YC, Wang JY, et al. Revisiting tuberculous pleurisy: pleural fluid characteristics and diagnostic yield of mycobacterial culture in an endemic area. *Thorax.* 2012;67:822–827.
17. Scotet V, Merour MC, Mercier AY, et al. Hereditary hemochromatosis: effect of excessive alcohol consumption on disease expression in patients homozygous for the C282Y mutation. *Am J Epidemiol.* 2003;158:129–134.
18. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* 2004;363:157–163.
19. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA.* 1999;281:1014–1018.
20. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med.* 2003;167:603–662.
21. Sookoian S, Castano GO, Burgueno AL, et al. The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. *Pharmacogenet Genomics.* 2010;20:1–8.
22. Hustert E, Zibat A, Presecan-Siedel E, et al. Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos.* 2001;29:1454–1459.
23. Zhang J, Kuehl P, Green ED, et al. The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics.* 2001;11:555–572.
24. Al-Eitan LN, Jaradat SA, Hulse GK, et al. Custom genotyping for substance addiction susceptibility genes in Jordanians of Arab descent. *BMC Res Notes.* 2012;5:497.
25. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet.* 2005;76:887–893.
26. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263–265.

27. Stram DO, Leigh Pearce C, Bretsky P, et al. Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case-control study of unrelated individuals. *Hum Hered.* 2003;55:179–190.
28. Holloway MG, Miles GD, Dombkowski AA, et al. Liver-specific hepatocyte nuclear factor-4alpha deficiency: greater impact on gene expression in male than in female mouse liver. *Mol Endocrinol.* 2008;22:1274–1286.
29. Castano G, Burgueno A, Fernandez Gianotti T, et al. The influence of common gene variants of the xenobiotic receptor (PXR) in genetic susceptibility to intrahepatic cholestasis of pregnancy. *Aliment Pharmacol Ther.* 2010;31:583–592.
30. Hashita T, Sakuma T, Akada M, et al. Forkhead box A2-mediated regulation of female-predominant expression of the mouse Cyp2b9 gene. *Drug Metab Dispos.* 2008;36:1080–1087.
31. Ono Y, Wu X, Noda A, et al. Participation of P450-dependent oxidation of isoniazid in isonicotinic acid formation in rat liver. *Biol Pharm Bull.* 1998;21:421–425.
32. Sarma GR, Immanuel C, Kailasam S, et al. Rifampin-induced release of hydrazine from isoniazid. A possible cause of hepatitis during treatment of tuberculosis with regimens containing isoniazid and rifampin. *Am Rev Respir Dis.* 1986;133:1072–1075.
33. Li F, Lu J, Cheng J, et al. Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid co-therapy. *Nat Med.* 2013;19:418–420.
34. Shih TY, Pai CY, Yang P, et al. A novel mechanism underlies the hepatotoxicity of pyrazinamide. *Antimicrob Agents Chemother.* 2013;57:1685–1690.
35. Lacroix C, Hoang TP, Nouveau J, et al. Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects. *Eur J Clin Pharmacol.* 1989;36:395–400.
36. Satoh T, Hosokawa M. Structure, function and regulation of carboxylesterases. *Chem Biol Interact.* 2006;162:195–211.
37. Zhang Y, Cheng X, Aleksunes L, et al. Transcription factor-mediated regulation of carboxylesterase enzymes in livers of mice. *Drug Metab Dispos.* 2012;40:1191–1197.
38. Shakya R, Rao BS, Shrestha B. Incidence of hepatotoxicity due to antitubercular medicines and assessment of risk factors. *Ann Pharmacother.* 2004;38:1074–1079.
39. Pascual P, Pedrajas JR, Toribio F, et al. Effect of food deprivation on oxidative stress biomarkers in fish (Sparus aurata). *Chem Biol Interact.* 2003;145:191–199.
40. Guevin C, Michaud J, Naud J, et al. Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators. *Br J Pharmacol.* 2002;137:1039–1046.
41. Sun H, Huang Y, Frassetto L, et al. Effects of uremic toxins on hepatic uptake and metabolism of erythromycin. *Drug Metab Dispos.* 2004;32:1239–1246.

科技部補助專題研究計畫項下：

日期：2015 年 6 月 11 日

出國人員 姓名	李麗娜	服務機構 及職稱	台大醫學院附設 醫院檢驗醫學部
會議時間	2015 年 5 月 15 日至 2015 年 5 月 20 日	會議地點	美國科羅拉多州丹佛市
會議名稱	(中文)2015 年美國胸腔學會國際會議 (英文)2015 American Thoracic Society International Conference		
發表論文 題目	(中文) Pregnane X receptor 基因變異與結核病之易感性有相關 (英文) Pregnane X receptor genetic variants are associated with susceptibility to tuberculosis		

一、參加會議經過

美國胸腔協會每年五月舉辦之國際會議為全世界最大之胸腔醫學大會，每年都有來自全世界之胸腔科醫師、科學家、衛生行政專家、呼吸治療師、護理人員等熱烈參與，參與人數超過一萬人，發表超過5000 篇之新論文，是全世界胸腔醫學領域之專業人員最重視之會議。今年(2015)之年會在美國科羅拉多州丹佛市舉行。丹佛市是美國胸腔醫學的重鎮，在肺結核、肺癌、慢性阻塞性肺疾、氣喘病方面的研究，在全美國及全世界均居於極重要的領導地位。今年大會之研討主題包括: 肺癌之最新標靶治療、免疫療法及基因研究，肺結核菌之基因研究，結核病之分子免疫學研究，非結核性分枝桿菌感染，肺部之細菌、黴菌、病毒感染(包括中東呼吸道冠狀病毒)，流感、禽流感、慢性阻塞性肺疾、氣喘病、職業性肺部疾病、急性呼吸窘迫症候群、呼吸衰竭及機械式呼吸、長期呼吸照護、睡眠呼吸終止症候群、肺泡蛋白沉著症、類肉瘤、肺動脈高壓、間質性肺炎、肺臟移植等。

職參加此次會議主要目的有三:

一. 發表論文；

二. 汲取胸腔醫學最新之觀念、最新的研究成果、最尖端的研究技術；

三. 與各國學者討論、切磋，汲取他人寶貴的經驗，作為改進我們研究方法、設計將來研究主題之參考。

職在本次大會中,發表壁報論文一篇,題目為: Pregnane X receptor genetic variants are associated with susceptibility to tuberculosis (Pregnane X receptor 基因變異與結核病之易感性有相關)。

職在研討會中，得以接觸到與結核菌及結核病有關之最新基因體研究，這些知識對於職的研究方向、研究方法以及研究結果之判讀均有莫大的幫助。同時職亦感到職本身在 PXR (Pregnane X receptor)基因之研究方面，也有其獨特之處，值得更深入研究下去。

除了發表論文演講外，職亦參與多場討論會，汲取多國學者在結核病及其他胸腔疾病領域之最新研究成果，並交換意見。各國學者對於我們的研究，深感興趣，亦給予我們許多寶貴的意見。職參與此次年會在結核病領域之尖端知識方面深感收獲甚豐,對日後職之研究方向與實驗設計有很大的幫助。

二、與會心得

此次參加 2015 年美國胸腔學會國際會議，不僅能將我們過去數年之研究成果發表，讓世界各國的學者都認識到，我們在結核病之基礎、臨床及流行病學方面之研究，也讓我們得知目前各國正進行之最新有關結核病及其他胸腔疾病之研究，研習有關這些疾病之新觀念、

新知識、新發現、新的研究方法與技術，並與各國學者討論、切磋，向他們請教我們研究上遇到的困難與瓶頸，也向他們提供我們在研究結核病及其他胸腔疾病之經驗，對我們的研究工作，不論是在實驗室的技術、臨床上的處理、統計學上的方法、將來研究的方向等，均有極大的幫助。

三、考察參觀活動(無是項活動者略)

四、建議

感謝科技部提供經費，讓職出國發表論文，參加國際會議，增廣見聞及專業知識，拓展視野，研習最尖端的醫學科技，職受益良多。懇請科技部能繼續支持我們的研究，也希望我們明年也有新的研究成果在國際上發表。

五、攜回資料名稱及內容

ATS 2015 International Conference Final Program

六、其他

科技部補助計畫衍生研發成果推廣資料表

日期:2015/08/30

科技部補助計畫	計畫名稱: 抗結核治療中肝炎之性別特異性風險及其與PXR受體基因多型性之關係
	計畫主持人: 李麗娜
	計畫編號: 102-2629-B-002-001- 學門領域: 性別主流科技計畫

無研發成果推廣資料

102 年度專題研究計畫研究成果彙整表

計畫主持人：李麗娜	計畫編號：102-2629-B-002-001-
計畫名稱：抗結核治療中肝炎之性別特異性風險及其與 PXR 受體基因多型性之關係	

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

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	本計畫之研究結果曾應美國胸腔學會 2014 年國際年會之邀請，於 2014 年 5 月 18 日在’’ Tuberculosis Genome Symposium’’ 中作演講。

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

科技部補助專題研究計畫成果報告自評表

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

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：（以 100 字為限）

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

從 isoniazid (INH)及 rifampicin (RIF)在 1950 及 1970 年代開發成功後，結核病已有很有效的藥物可治療。然而目前結核病在台灣之發生率仍居高不下，其原因固然很多，但抗結核藥物引起肝炎，使得結核病患者易中斷治療，無疑是治療失敗的重要原因。主持人曾於 2007 至 2008 年執行一前瞻性之計畫，研究我國結核病患者發生治療中肝炎之危險因子。結果顯示，我國結核病病人發生治療中肝炎之百分比為 18.9%，其中 16.4%是抗結核藥物引起的藥物性肝炎。發生藥物性肝炎之危險因子為： 1)女性；2) N-acetyl transferase 2 (NAT2)基因之 slow acetylator； 3) 有 B 型肝炎且病毒量高者；4) 末期腎衰竭且未作透析。

為了瞭解為什麼我國女性發生抗結核藥物性肝炎之危險性遠高於男性 (24% vs. 12%)，主持人遂執行本計畫，以研究 pregnane X receptor (PXR) 基因調控區之單核苷酸多型性(SNP)與抗結核藥物性肝炎之間的關係。結果發現，PXR 基因調控區之 rs2461823，其基因型若為 AA，則在女性其發生抗結核藥物性肝炎之風險為其他基因型之 6.87 倍，但在男性則無此現象。PXR 基因調控區之 rs7643645，其基因型若為 AA，則在女性其發生抗結核藥物性肝炎之風險為其他基因型之 7 分之 1，但在男性則無此現象。過去研究抗結核藥物性

肝炎之學者均未發現 PXR 基因之重要性，本研究是全球首先發現 PXR 基因與女性發生抗結核藥物性肝炎之風險有關者。