科技部補助專題研究計畫成果報告
期末報告

Isoniazid 藥物性肝炎之性別特異性風險與粒線體NADH去氫酶基因多型性之關聯性

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中 文 摘 要 ： 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。

然而抗結核藥物性肝炎最終之傷害，究竟發生在肝細胞之哪個分子機轉，至今尚不清楚，女性發生肝細胞最終傷害之風險是否較高亦不清楚。近年來由於粒線體科學之發展，科學家發現，抗結核藥物INH或其代謝物hydrazine，會抑制粒線體複合體II，但通常不會引起明顯的粒線體功能異常，因為粒線體的儲備功能很大。然而當同時有粒線體複合體I (即NADH去氫酶)抑制物存在時，就會因嚴重的能量(ATP)缺乏造成大量的肝細胞壞死。粒線體複合體I的突變或基因多型性，已有報告與女性乳癌之風險及女童氣喘病之風險有關(亦即與女性疾病有關)。

因此主持人假設，女性發生INH或其他抗結核藥物性肝炎之風險較高，有一部分是因為粒線體複合體I (即NADH去氫酶)基因之SNP造成。亦即，INH之有毒代謝物hydrazine (本身是粒線體複合體II抑制劑)或其他的抗結核藥物，即使蓄積在體內，但若沒有特定的粒線體複合體I (即NADH去氫酶)基因多型性造成NADH去氫酶受抑制，則並不會造成嚴重的INH或其他的抗結核藥物性肝炎。然而若此病人有某些特定的NADH去氫酶基因多型性，造成NADH去氫酶受抑制，則因粒線體複合體I與II同時受抑制，會引起嚴重的INH或其他的抗結核藥物性肝炎。

本計畫為期一年，研究方法為，分析300位肺結核病人及300位健康受試者白血球粒線體DNA中複合體I (即NADH去氫酶)基因之多型性，包括位於此基因之ND1、ND2、ND3、ND4、ND4L、ND5、ND6等7個subunit中之9個SNP，經PCR增量後作DNA定序。比較肺結核病人與健康成人9個SNP之基因型(genotype)與單套型(haplotype)，以及有發生INH或其它抗結核藥物性肝炎與未發生肝炎者，其9個SNP之genotype與haplotype。分析NADH 去氫酶基因中，此9個SNP之genotype與haplotype，與女性之INH或其他抗結核藥物性肝炎之風險較高有無相關性。

中 文 關 鍵 詞 ： 結核病、抗結核藥物性肝炎、isoniazid (INH)、hydrazine、女性、粒線體、NADH去氫酶基因、複合體I、複合體II、單核苷酸多型性

、基因型、單套型

英 文 摘 要 ： Hepatitis during anti-tuberculosis (TB) treatment (HATT) is the most important adverse event of anti-TB chemotherapy. In our previous study we found HATT developed in 18.9% of TB patients, of whom 16.5% were due to anti-TB drugs. Risk factors for drug-induced hepatitis were: 1) women; 2) NAT2 slow acetylator; 3) High HBV viral load, and 4) end-stage renal disease.

However, the exact molecular mechanisms leading to final steps of hepatic necrosis in drug-induced hepatitis remain unknown. Whether females have higher risk in the final steps of hepatic necrosis is also unknown. Recently it was discovered that INH and its metabolite hydrazine (both being mitochondrial complex II inhibitors) may interfere with mitochondrial function. Yet such changes of usually won't cause overt liver damage, because mitochondria have large reserve. Yet when there is coexisting genetic or pharmacologic inhibitors of mitochondria complex I, the inhibition of both complex I and complex II would lead to severe ATP shortage and massive hepatic necrosis.

Our hypothesis is: Mitochondrial complex I (or NADH dehydrogenase) SNPs are associated with mitochondrial complex I deficiency and could trigger INH-induced, or other drug-induced HATT, because INH metabolite hydrazine itself is a known mitochondrial complex II inhibitor . The genotype/haplotype distribution of NADH dehydrogenase SNPs might be different in females than in males, contributing to the higher risk of INH- or other drug-induced HATT in females.

We'll sample peripheral blood and sequence 9 SNPs in NADH dehydrogenase gene (in subunit ND1, ND2, ND3, ND4, ND4L, ND5, ND6), compare genotype/haplotype distribution between TB patients with drug-induced HATT and those without, and analyze if SNPs in NADH dehydrogenase could contribute to the higher risk of drug-induced HATT in females than males.

英 文 關 鍵 詞 ： tuberculosis, hepatitis during anti-TB treatment (HATT), INH, hydrazine, mitochondria complex I or complex II deficiency, NADH dehydrogenase, single nucleotide polymorphism (SNP), genotype, haplotype, female, male

Isoniazid 藥物性肝炎之性別特異性風險與粒線體 NADH 去氫酶基因多型性之關聯性（結案報告）

目錄

(一) 前言

Introduction

[Hepatitis during anti-tuberculosis treatment: the importance]

Tuberculosis (TB) remains a major infectious cause of death worldwide even in the 21st century, more than 40 years after the birth of the effective anti-TB medicine rifampicin. In Taiwan, one of the main causes of a persistently high TB incidence (53 per 100,000 in 2012) is low treatment success rate (70% in 2011, far below the WHO's goal: 85%).[1] The most important cause of low treatment success rate is the development of hepatitis during anti-TB treatment (HATT).[2] In our previous studies, it resulted in modification or discontinuation of anti-TB treatment in 17-19 % of our TB patients,[3,4] and is difficult to manage.

The best documented risk factor for HATT is slow acetylator phenotype/genotype of the *N-acetyltransferase 2* (*NAT2*) gene, which is associated with isoniazid (INH)-induced hepatotoxicity.[5,6] Other factors such as cytochrome P450 2E1 (*CYP2E1*),[7] glutathione S-transferase,[8] old age,[5,9-12] low body-mass index (BMI),[12] alcoholism,[12,13] concomitant hepatitis B virus (HBV),[12,14,15] hepatitis C virus (HCV),[12,16,17] and HIV infection[17,18] have been inconsistently associated with HATT.

[Hepatitis during anti-TB therapy: our first previous study]

In 2007 to 2008 we conducted a prospective study at National Taiwan University Hospital to evaluate the risk factors of HATT in TB patients, and observe the influence of HBV/HCV viral load in those who had concomitant HBV/HCV infection. In that study we also performed *NAT2* and *CYP2E1* genotyping to observe the simultaneous effects of genetic susceptibility and viral hepatitis.

We found that among the 360 TB patients we studied, HATT developed in 68 patients (18.9%), of which 59 (16.4%) was drug-induced hepatitis, and 9 (2.5%; all male) was acute flare-up of B or C viral hepatitis. Kaplan-Meier analysis and log-rank test revealed that: 1) women were twice more likely to have drug-induced HATT than men. 2) The risk of both drug- and virus-induced HATT were higher in patients with high initial viral load than those with low initial viral load or those without viral hepatitis (Table 1)[4].

Table 1. Risk factors of hepatitis during anti-TB treatment (HATT) in different subgroups of patients by univariate analysis[4]

Factor		Drug-induced HATT			Virus-induced HATT		
		No. at risk	No. (%) with HATT	*p*	No. at risk	No. (%) with HATT	*p*
Age	>65 y	151	25 (16.6)	0.881	31	4 (13)	0.869
	≦65 y	209	34 (16.3)		35	5 (14)	
Sex	Female	128	31 (24.2)	**0.003**	18	0	**0.045**
	Male		28 (12.1)		48	9 (19)	
HBV or HCV infected	High initial HBV VL*	23	9 (39.1)	**< 0.001**	23	5 (22)	**0.064**
	Low initial HBV VL	19	2 (10.5)		19	0	
	High initial HCV VL	12	4 (33.3)		12	3 (25)	
	Low initial HCV VL	12	2 (16.7)		12	1 (8)	
End-stage renal disease	Yes, under dialysis	9	2 (22.2)	0.079	2	0	**0.004**
	Yes, no dialysis	11	3 (27.3)		2	1 (50)	
NAT2 genotype	Slow acetylator	82	22 (26.8)	**0.002**	17	3 (18)	0.521
	Rapid acetylator	278	37 (13.3)		49	6 (12)	
CYP2E1 genotype	c1c1	215	36 (16.7)	0.801	45	6 (13)	0.942
	c1c2 or c2c2	145	23 (15.9)		21	3 (14)	

(*VL: viral load)

[Drug-induced hepatitis during anti-TB therapy: why women are twice risky than men?]

After completing our previous study, we want to answer why women are twice risky to develop drug-induced HATT than men (24% vs. 12%, Table 1). Actually for a long time women have been reported to have a higher risk of HATT than men, the hazarrd ratio was 1.5 to 3.3 [4,10,19]. One possible reason is the activity of cytochrome P450 3A4 (CYP3A4), the most abundant enzyme in the hepatic cytochrome P450 family that catalyzes phase I reaction of many drugs and produces toxic intermediates, is higher in women [20].

Yet the exact mechanism that leads to higher CYP3A4 activity in women than

men is also 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 [21]. The pregnane X receptor (PXR), a member of the nuclear receptor superfamily, is a known regulator of the *CYP3A4* gene. Because SNPs in the transcription factor binding sites of the *PXR* regulatory region (the promoter and intron1) have been associated with altered *PXR* and *CYP3A4* expressions [22], it is possible that gene variants in the *PXR* regulatory region may contribute to differences in risk of drug-induced HATT between males and females.

[The association between PXR SNPs and higher risk of drug-induced HATT in women: our previous study (NSC 102-2629-B-002 -001 -)]

in this NSC study 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, contributing to the increased risk of HATT in females.

We selected 6 SNPs in the regulatory region of *PXR* gene based on known association with diseases [22,23]: rs3814055 (located in the 5' untranslated region), rs1248820, rs2461823, rs7643645 (all located in intron 1), rs6785049 (located in intron 5), and rs3814057 (located in the 3' untranslated region).

We enrolled TB patients, extracted genomic DNA from peripheral blood, sequenced these SNP sites and correlated with drug-induced hepatitis during anti-TB treatment. We found that among 355 TB patients (male, 65.6%), 70 (19.7%) developed HATT. Genotypes at **rs2461823** and **rs7643645** were significantly associated with risk of HATT **only in females**. AA at rs2461823 was a risk genotype, while AA at rs7643645 was a protective genotype (Table 2) [24].

Table 2. Frequency of drug-induced hepatitis during anti-tuberculous treatment (HATT) in males and females with different genotypes at six single nucleotide polymorphism (SNP) sites of the *PXR* gene

SNP	Loca-tion	Geno-type	Study population			Male			Female		
			No.	% with HATT	*p*	No.	% with HATT	*p*	No.	% with HATT	*p*
rs3814055	5'UTR	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	CC	337	20	0.747	216	17	>0.999	121	26	>0.999
		TT	16	13		15	13		1	0	
rs2461823	Intron 1b	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	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	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	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	

The association with female hepatitis remained significant even by multivariate logistic regression analysis: genotype AA at rs2461823 (OR: 6.87 [2.55–18.52]) was associated with a > 6 times risk, and genotype AA at rs7643645 (OR: 0.14 [0.02–1.02]) was associated with a one-seventh risk of HATT **only in females**. Haplotype analysis showed that h001101 (OR: 2.30 [1.22–4.32]) and h000110 (OR: 2.25 [1.08–4.69]) haplotype were associated with increased risk of HATT **only in females**.

[The association between PXR SNPs and risk of INH-, RIF- or PZA-induced HATT in females] [24]:

We further separated HATT into those due to isoniazid (INH), rifampicin (RIF) or pyrazinamide (PZA). We found that for INH-induced hepatitis, *NAT2* slow acetylator genotype and malnutrition were independent risk factors for INH-induced hepatitis in both males and females, but genotype AA at rs2461823 (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** **(Table 3)**.

Table 3. Factors associated with isoniazid-induced hepatitis, by multivariate logistic regression analysis

Variables	***p***	**OR (95% CI)**
NAT2 slow acetylator	0.012	9.53 (1.65 – 55.0)
Malnutrition	0.034	5.87 (1.14 – 30.1)

AA in rs2461823 of *PXR* gene in women	<0.001	10.5 (1.91 – 58.1)
No. of A allele at rs6785049 in women	0.045	11.7 (1.06 – 129)

For RMP-induced hepatitis, we found 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** (**Table 4**).

Table 4. Factors associated with rifampin-induced hepatitis, by multivariate logistic regression analysis

Variables	*p*	OR (95% CI)
End-stage renal disease	0.012	4.83 (1.42 – 16.4)
AG at rs6785049 in females	0.035	3.09 (1.09 – 8.81)
No. of A allele at rs6785049	0.038	3.09 (1.06 – 8.95)
h000010*	0.001	8.01 (2.30 – 27.9)
h001101* in females	0.005	5.51 (1.68 – 18.1)

* 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; rs3824057: A→C.

For PZA-induced hepatitis, 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** (**Table 5**).

Table 5. Factors associated with pyrazinamide-induced hepatitis, by multivariate logistic regression analysis

Variables	*p*	OR (95% CI)
AA at rs2461823 in females	<0.001	7.29 (2.54 – 20.9)

AG at rs7643645	0.007	2.85 (1.33 – 6.11)
No. of G allele at rs7643645 in females	0.006	1.84 (1.19 – 2.85)
h000110* in females	0.001	5.10 (1.92 – 13.5)

* 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; rs3824057: A→C.

Thus our hypothesis is proved that SNPs in *PXR* regulatory region are associated with increased risk of drug-induced HATT among women. However, this gender-dimorphic association between *PXR* and drug-induced HATT may not be the sole factors that lead to increased risk of drug-induced HATT in women. Other factors or genes may also contribute to woman's higher risks of drug-induced HATT. Besides, despite of our observations, we still do not know what substances are directly responsible for the final damage to liver cells. **We now plan to investigate if females are more vulnerable during the final stage of hepatic damage (i.e. the stage of mitochondrial energy supply) of HATT than males.**

[INH-induced cell death is precipitated by underlying mitochondrial complex I dysfunction in mouse hepatocytes]

Isoniazid (INH) is a widely-used first-line anti-TB drug that has been associated with idiosyncratic (host-dependent) liver injury in susceptible patients. The incidence is relatively high. **In our own previous study, INH-induced hepatitis occurred in 7% of our patients who received anti-TB treatment (more than one-third of HATT) [4]**. In studies performed in western countries, up to 20% of INH-treated patients developed increased ALT activity, and **1% of recipients developed more severe hepatotoxicity including liver failure and mortality** [25]. In our own study, risk factors for INH-induced hepatitis included (see Table 3): ***N-acetyltransferase* gene (*NAT2*) slow acetylator and malnutrition (for men and women alike), AA genotype in rs2461823 and number of A allele at rs6785049 of *PXR* gene (only in women). However, we know very little about the molecular mechanisms that cause INH-induced liver injury, and whether or not the molecular mechanisms exert sex-dimorphic influences in men and women.**

Previous experiments showed that INH [26] or its major metabolite hydrazine [27] may interfere with mitochondrial function. However, these mitochondrial changes (mitochondrial oxidant stress) were not sufficient to cause overt hepatocyte

injury, because hepatic mitochondria have a large reserve capacity. Lee KK and colleagues showed that INH alone (≦3000 uM) did not induce cell injury in cultured mouse hepatocytes. However, coexposure of hepatocytes to INH and nontoxic concentrations of the complex I inhibitors rotenone (3 uM) or piericidine (30 nM) resulted in massive ATP depletion and cell death [26]. They found that **the toxic metabolite hydrazine is a solubilized mitochondria complex II inhibitor,** and concluded that underlying **pharmacological or genetic** inhibition of mitochondria complex I (which alone is not acutely toxic), can trigger INH-induced hepatocellular injury.

[Genetic mutations associated with mitochondrial complex I deficiency]

Mitochondria have an inner and an outer membrane. The respiratory chain, located in the inner membrane, is composed of 5 enzyme complexes [28]. The respiratory chain is controlled by 2 separate genetic systems in the genomic and mitochondrial DNA (mtDNA). mtDNA is a small, closed circular and double-stranded molecule containing 37 genes, of which 24 are needed for translation and 13 encode subunits of the respiratory chain [28]. Complex I, also named nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase or NADH dehydrogenase, is composed of at least 46 subunits and its defect are the most frequent deficiencies of the respiratory chain [29].

Polymorphisms or mutations in this part of mtDNA (DNA coding NADH dehydrogenase) have been associated with neurological diseases [29], breast cancer [30], and asthma in girls but not in boys [31]. No such study on the field of pharmacology or toxicology has ever been reported. Because the reported association with breast cancer and asthma in girls shows **gender dimorphism**, we decide to investigate if single nucleotide polymorphisms (SNPs) in mitochondrial NADH dehydrogenase gene are associated with INH-induced hepatitis and the increased risk of INH-induced HATT in females. **Our hypothesis is that mitochondrial *NADH dehydrogenase* gene SNPs, which can be potential causes of mitochondrial complex I deficiency, are associated with INH-induced hepatocellular damage (INH metabolite hydrazine being a known mitochondrial complex II inhibitor) (Figure 1), and that genotypes/haplotypes at SNPs of NADH dehydrogenase gene can be different between females and males, contributing to the increased risk of INH hepatitis in females.**

[*Goals of our current proposal*]

In the current 1-year proposal, we will test our hypothesis by:

1) Investigate SNPs in mitochondrial *NADH dehydrogenase* gene in TB patients who develope INH- or other drug-induced HATT and in TB patients without drug-induced HATT.
2) Compare the distribution of SNP genotypes/haplotypes between the groups in 1) and check if certain SNP genotypes/haplotypes are associated with INH- or other drug-induced HATT.
3) Check if certain SNP genotypes/haplotypes are associated with INH- or other drug-induced HATT **only in female patients**.

(二) 方法

Methods

[*Ethics approval*]

Request for ethics approval for the study has been submitted to the Ethics Committee of National Taiwan University Hospital.

[*Study population*]

The inclusion criteria are (All participants must be ≧ 20 years old):

1. Patients with culture- or histology proved tuberculosis (TB)
2. Healthy controls (people who have no previous history of TB and no symptoms)

The exclusion criteria are:

1. Patients who are treated with corticosteroids or other potentially hepatotoxic drugs.

[*Study protocol*]

1. For TB patients who agree to participate in the study, liver function test (AST, ALT, bilirubin, gamma-GT, alkaline phosphatase, albumin), renal function test (creatinine), fasting blood sugar, uric acid, hemogram, HBsAg and anti-HCV will be checked. (These are the routine blood tests before treatment initiation, not research blood tests.)
2. For TB patients, blood will be drawn at time zero (before initiation of anti-TB therapy) for DNA extraction (for mitochondria *NADH dehydrogenase* gene polymorphisms). (These are for the purpose of research.)
3. For TB patients, blood will also be drawn 2, 4, 6, 8, 10 and 12 weeks after the initiation of anti-TB therapy to check for hepatitis during anti-TB therapy, and HBV/HCV viral load if needed. (These are routine tests, not for research purpose.)
4. For healthy controls blood will be drawn when they join the study for mitochondrial *NADH dehydrogenase* gene polymorphism.

[*Definition of hepatitis during anti-TB treatment (HATT)*]

Hepatitis during anti-TB treatment (HATT) was defined as an increase in serum AST and/or ALT to > 3 times the upper limit of normal (ULN) if symptomatic, or > 5 times the ULN if asymptomatic.

[*Anti-TB therapy*]

All TB patients receive standard anti-TB treatment including daily INH (H), RIF (R), ethambutol (E), plus pyrazinamide (PZA, Z) in the first 2 months, and daily HR for the following 4 months. The regimen would be modified if necessary by the primary care

physician in case of concomitant hepatic/renal diseases, adverse events or susceptibility test.

[*Diagnosis of drug-induced HATT*]

The diagnosis of INH- or RIF-induced HATT requires a positive rechallenge test (at least doubling of serum AST or ALT level after rechallenge), whereas PZA-induced HATT is diagnosed either by rechallenge or by exclusion.

[*SNP study of mitochondrial NADH dehydrogenase gene*]

After participants give informed written consent, peripheral blood is drawn into sterile tubes. Leukocyte genomic DNA is extracted, and mtDNA is amplified using primers for 9 SNP sites located in 7 subunits as listed in Table 6 [30,32].

Table 6 Mitochondrial *NADH dehydrogenase* gene primers for PCR-sequencing

NADH dehydrogenase subunit (ND)	Primer name	Primer sequence
ND1	ONP82 [F]	5’ CTC AAC TTA GTA TTA TAC CC 3’
	ONP84 [R]	5’ GAG CTT AGC GCT GTG ATG AG 3’
ND2	ONP64 [F]	5’ GTC ATC TAC TCT ACC TAC TT 3’
	ONP89 [R]	5’ GGC GGG AGA AGT AGA TTG AA 3’
ND3	ONP91 [F]	5’ CAC TAT CTG CTT CAT CCG cc 3’
	ONP94 [R]	5’ GAG CGA TAT ACT AGT ATT CC 3’
ND4	ONP9 [F]	5’ TCT CCA ACA CAT ATG GCC TA 3’
	ONP203 [R]	5’ ACT GTG AGT GCG TTC GTT CGT AGT TTG AG 3’
	ONP14 [F]	5’ GCG CAG TCA TTC TCA TAA TC 3’
	ONP46 [R]	5’ TTT GTT AGG GTT AAC GAG GG 3’
ND4L	ONP93 [F]	5’ TCT GGC CTA TGA GTG ACT AC 3’
	ONP203 [R]	5’ ACT GTG AGT GCG TTC GTT CGT AGT TTG AG 3’
ND5	ONP11 [F]	5’ TTT TGG TGC AAC TCC AAA 3’
	ONP74 [R]	5’ GGT TGA CCT GTT AGG GTG AG 3’
	ONP21 [F]	5’ GCA GTC TGC GCC CTA CA 3’
	ONP12 [R]	5’ TCA GGG TTC ATT CGG GAG GA 3’
ND6	ONP204 [F]	5’ CTC CAA AGA CCA CAT CAT CGA AAC 3’
	ONP318 [R]	5’ TTC ATC ATG CGG AGA TGT TGG ATG GGG TGG 3’

[*Sequencing*]:

Sequencing was performed using two methods: 1) Next generation sequencing (NGS) performed using Illumina HiSeq 2000 (Illumina, San Diego, CA); the technique sequenced the entire mitochondria DNA; 2) sanger sequencing: PCR products are purified using a QIAquick PCR purification kit (QIAGEN) and directly sequenced.

Sequencing reactions are determined using PE Biosystems' capillary 3700 DNA Analyzers (Foster City, CA). All genotypes resulting in amino acid exchanges are confirmed by independent PCR followed by sequencing.

[*Statistical analysis*]

The distribution of genotypes and haplotypes at 9 *NADH dehydrogenase* SNP sites are compared between:

- ◆TB patients and healthy subjects
- ◆TB patients with drug-induced HATT and those without
- ◆TB patients with **INH-induced** hepatitis and those without
- ◆Female TB patients with drug-induced HATT and female TB patients without
- ◆Male TB patients with drug-induced HATT and male TB patients without
- ◆Female TB patients with INH-induced HATT and female TB patients without
- ◆Male TB patients with INH-induced HATT and male TB patients without
- ◆Our cohort and cohorts of other countries

Using *chi-*x^2 or Fisher's exact test.

Results 結果

We analyzed the entire mitochondrial genome of leukocytes using NGS technique and compared results between 19 tuberculosis (TB) patients (male: 9) with, and 19 TB patients (male: 9) without hepatitis due to anti-TB agents. The comprehensive NGS approach identified all mitochondrial DNA point mutations. All the point mutations detected by NGS were validated by sanger sequencing.

Among 19 patients with hepatitis due to anti-TB drugs, 5 were due to RIF, 8 PZA, and 6 INH. The number of mitochondrial DNA variants detected in the hepatitis group and control group were: 63, 51 in D-loop, 15, 15 in two ribosomal RNA segments, 19, 10 in ND1 gene, 16, 12 in ND2, 13, 13 in COX1, 7, 8 in COX2, 3, 4 in ATP8, 10, 12 in ATP6, 8, 6 in COX3, 6, 5 in ND3, 5, 7 in ND4L, 16, 13 in ND4, 28, 24 in ND5, 4, 5 in ND6, 19, 15 in CYTB, and 8, 0 in the entire 19 tRNA segments, respectively (Table 7).

Table 7 Number of mitochondrial DNA SNP detected in hepatitis and control group

mtDNA	Nucleotide No.	Product	No. of SNP	
			Hepatitis group	Control group
D-loop	16024..16569, 1..577	(-) (DNA control region)	63	51
tRNA	578..648	“tRNA-Phe”	0	0
rRNA	649..1602	“12S ribosomal RNA”	5	4
tRNA	1603..1671	“tRNA-Val”	1	0
rRNA	1672..3229	“16S ribosomal RNA”	10	11
tRNA	3230..3304	“tRNA-Leu”	1	0
Gene ND1	3307..4262	“NADH dehydrogenase subnunit 1”	19	10
tRNA	4263..4331	“tRNA-Ile”	0	0
tRNA	4329..4400	“tRNA-Gln”	0	0
tRNA	4402..4469	“tRNA-Met”	1	0
Gene ND2	4470..5511	“NADH dehydrogenase subnunit 2”	16	12
tRNA	5512..5576	“tRNA-Trp”	0	0

tRNA	5587..5655	“tRNA-Ala”	0	0
tRNA	5657..5729	“tRNA-Asn”	0	0
tRNA	5761..5826	“tRNA-Cys”	1	0
tRNA	5826..5891	“tRNA-Tyr”	0	0
Gene COX1	5904..7445	“cytochrome c oxidase subunit I”	13	13
tRNA	7445..7516	“tRNA-Ser”	0	0
tRNA	7518..7585	“tRNA-Asp”	0	0
Gene COX2	7586..8269	“cytochrome c oxidase subunit II”	7	8
tRNA	8295..8364	“tRNA-Lys”	0	0
Gene ATP8	8366..8572	”ATP synthase F0 subunit 8”	3	1
Gene ATP6	8527..9207	”ATP synthase F0 subunit 6”	10	12
Gene COX3	9207..9990	“cytochrome c oxidase subunit III”	8	6
tRNA	9991..10058	“tRNA-Gly”	2	0
Gene ND3	10059..10404	product=”NADH dehydrogenase subunit 3”	6	5
tRNA	10405..10469	“tRNA-Arg”	0	0
Gene ND4L	10470..10766	”NADH dehydrogenase subunit 4L”	5	7
Gene ND4	10760..12137	”NADH dehydrogenase subunit 4”	16	13
tRNA	12138..12206	“tRNA-His”	1	0
tRNA	12207..12265	“tRNA-Ser”	0	0
tRNA	12266..12336	“tRNA-Leu”	0	0
Gene ND5	12337..14148	”NADH dehydrogenase subunit 5”	28	24
Gene	14149..14673	”NADH	4	5

ND6		dehydrogenase subunit 6”		
tRNA	14674..14742	/product= “tRNA-Glu”	0	0
Gene CYTB	14747..15887	”cytochrome b”	19	15
tRNA	15888..15953	“tRNA-Thr”	2	0
tRNA	15955..16023	“tRNA-Pro”	0	0

Non-synonymous SNPs that occurred in 13 mitochondrial genes and resulted in amino acid changes (Table 8) occurred in similar frequencies in hepatitis and control group, except for: 1) The 4 non-synonymous variants in COX3 gene occurred only in 5 patients in the hepatitis group, but in none of the control group, and 2) Non-synonymous variant at ND5 gene's 13928 nucleotide occurred in 9 patients in hepatitis group, but only 2 in control group.

Table 8 Non-synonymous mitochondrial DNA variations in TB patients with drug-induced hepatitis due to anti-TB agents and those without hepatitis (controls)

Gene	Product	Nucleotide		Amino acid change	No.(%) with nucleotide change		p
		Position	Change		Hepatitis group (drug causing hepatitis)	Control	
ND1	NADH dehydrogenase subunit 1	3316	gcc..acc	Ala..Thr	1 (5): RIF	0	0.959
		3397	ata..gta	Met..Val	1 (5): RIF	0	0.959
		3497	gcc..gtc	Ala..Val	1 (5): RIF	0	0.959
		3511	acc..gcc	Thr..Ala	1 (5): PZA	0	0.959
		3571	ctc..ttc	Leu..Phe	1 (5): RIF	0	0.959
		3865	atc..gtc	Ile..Val	0	1 (5)	0.959
		4048	gac..aac	Asp..Asn	1 (5): PZA	2 (11)	0.934
		Total No. (%) of subjects with ND1 variants			5 (26): 3 RIF, 2 PZA	3 (16)	0.720
ND2	NADH dehydrogenase subunit 2	4491	gtc..atc	Val..Ile	0	1 (5)	0.959
		4824	acc..gcc	Thr..Ala	1 (5): INH	0	0.959
		5178	cta..ata	Leu..Met	3 (16): RIF, 2 PZA	1 (5)	0.680
		5263	gcc..gtc	Ala..Val	1 (5): PZA	1 (5)	0.520
		5302	atc..acc/ atc	Ile..Thr	0	1 (5)	0.959
		5442	ttc..ctc	Phe..Leu	2 (11): RIF, INH	0	0.438

		5460	gcc..acc	Ala..Thr	1 (5): PZA	2 (11)	0.934
		Total No. (%) of subjects with ND2 variants			8 (42): 2 RIF, 4 PZA, 2 INH	6 (32)	0.762
COX 1	Cytochrome C oxidase subunit 1	7158	atc..gtc	Ile..Val	0	2 (11)	0.428
		7353	ata..tta	Met..Leu	0	1 (5)	0.959
		Total No. (%) of subjects with COX1 variants			0	3 (16)	0.223
COX 2	Cytochrome C oxidase subunit 2	7598	gcg..acg	Ala..Thr	1 (5): RIF	0	0.959
		7853	gtc..atc	Val..Ile	2 (11): PZA, INH	3 (16)	0.897
		Total No. (%) of subjects with COX2 variants			3 (16): RIF, PZA, INH	3 (16)	0.658
ATP8	ATP synthase Fo subunit 8	8414	ctc..ttc/ctc	Leu..Phe/Leu	2 (11): RIF, PZA	0	0.438
ATP6	ATP synthase Fo subunit 6	8584	gca..aca	Ala..Thr	1 (5): INH	4 (21)	0.325
		8603	ttt..tct	Phe..Ser	0	1 (5)	0.959
		8654	atc..acc	Ile..Thr	0	1 (5)	0.959
		8684	acc..atc	Thr..Ile	1 (5): INH	0	0.959
		8701	acc..gcc	Thr..Ala	7 (37): 3 RIF, 2 PZA, 2 INH	6 (32)	0.986
		8794	cac..tac	His..Tyr	2 (11): PZA, INH	1 (5)	0.933
		9053	agc..aac	Ser..Asp	3 (16): PZA, 2 INH	0	0.223
		Total No. (%) of subjects with ATP6 variants			12 (63): 3 RIF, 4 PZA, 5 INH	12 (63)	0.737
COX 3	Cytochrome C oxidase subunit 3	9468	acc..gcc	Thr..Ala	2 (11): PZA, INH	0	0.438
		9682	ata..aca	Thr..Met	1 (5): RIF	0	0.959
		9845	ttt..ctt	Phe..Leu	1 (5): PZA	0	0.959
		9861	ttc..ctc	Phe..Leu	1 (5): INH	0	0.959
		Total No. (%) of subjects with COX3 variants			5 (26): RIF, 2 PZA, 2 INH	0	0.057
ND3	NADH dehydrogenase subunit 3	10398	acc..gcc	Thr..Ala	8 (42): 3 RIF, 3 PZA, 2 INH	10 (53)	0.723
ND4L	NADH dehydrogenase subunit 4L	10609	ata..aca	Met..Thr	3 (16): 1 PZA, 2 INH	1 (5)	0.564
ND4	NADH dehydrogenase	11016	agt..aat	Ser..Asn	0	1 (5)	0.959
		11087	ttc..ctc	Phe..Leu	0	1 (5)	0.959

	subunit 4	11318	tca..cca	Ser..Pro	1 (5): INH	0	0.959
		11696	gtc..atc	Val..Ile	0	1 (5)	0.959
		12026	att..gtt	Ile..Val	1 (5): PZA	0	0.959
		12090	att..act	Ile to Thr	0	1 (5)	0.959
		Total No. (%) of subjects with ND4 variants			2 (11): PZA, INH	4 (21)	0.690
ND5	NADH dehydrogenase subunit 5	12338	ata..aca	Met..Thr	2 (11): 2 PZA	2 (11)	0.604
		12358	acc..gcc	Thr..Ala	0	1 (5)	0.959
		12361	acc..gcc	Thr..Ala	0	1 (5)	0.959
		12406	gtt..att	Val..Ile	2 (11): 2 INH	3 (16)	0.981
		12501	atg..atc	Met..Ile	1 (5): PZA	0	0.959
		12952	gct..act	Ala..Thr	1 (5): RIF	0	0.959
		12811	tac..cac	Tyr..His	0	2 (11)	0.438
		13145	agc..aac	Ser.. Asn	0	1 (5)	0.959
		13708	gca..aca	Ala..Thr	1 (5): PZA	1 (5)	0.457
		13759	gca..aca	Ala..Thr	2 (11): PZA, INH	2 (11)	0.604
		13928 (rs2835 9184)	agc..acc	Ser..Thr	9 (47): 5 PZA, 4 INH	2 (11)	0.037
		13966	Tag acg..gcg	Thr..Ala	1 (5): PZA	0	0.959
		14053	acc..gcc	Thr..Ala	0	1 (5)	0.959
		Total No. (%) of subjects with ND5 variants			10 (53): RIF, 6 PZA, 3 INH	11 (58)	0.987
ND6	NADH dehydrogenase subunit 6	14308	tcc..ccc	Ser..Pro	0	1 (5)	0.959
		14392	cac..tac/ cac	His..Tyr/His	1 (5): INH	0	0.959
		14470	tcc..ccc	Ser..Pro	1 (5): INH	0	0.959
		14668	cat..tat	His..Tyr	2 (11): RIF, PZA	1 (5)	0.933
		Total No. (%) of subjects with ND6 variants			4 (21): RIF, PZA, 2 INH	2 (11)	0.690
CYT B	Cytochrome b	14769	aac..agc	Asn..Ser	0	1 (5)	0.959
		14979	atc..acc/ atc	Ile..Thr/Ile	1 (5): PZA	0	0.959
		15024	tgc..tac	Cys..Tyr	0	1 (5)	0.959
		15038	atc..gtc	Ile..Val	1 (5): RIF	0	0.959
		15236	atg..gtg	Met..Val	1 (5): PZA	0	0.959

		15479	ttc..ctc	Phe..Leu	1 (5): PZA	0	0.959
		15662	atc..gtc	Ile..Val	0	1 (5)	0.959
		15884	gcc..acc	Ala..Thr	0	1 (5)	0.959
		Total No. (%) of subjects with CYTB variants			4 (21): RIF, 3 PZA	4 (21)	0.690

Discussion

In the current study we found that mitochondrial DNA variants occurred with different frequencies between TB patients with and those without hepatitis due to anti-TB drugs. The main difference occurred in these 3 regions: 1)mitochondrial gene coding for tRNAs; 2) non-synonymous SNPs that occurred in mitochondrial gene coding for cytochrome oxidase subunit III (COX3, a subunit of complex IV), 3) non-synonymous SNPs in mitochondrial ND5 gene's 13928 nucleotide.

COX3 gene codes for cytochrome oxidase 3, a subunit of complex IV (the terminal enzyme of electron transfer chain, ETC). ND5 gene codes for NADH dehydrogenase subunit 5, a subunit of complex I. Non-synonymous variants in these regions may affect energy supply and increase susceptibility to drug-induced hepatitis by anti-TB drugs.

References:

1. www.cdc.gov.tw/professional/
2. American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of Tuberculosis. MMWR Recomm Rep 2003; 52 (RR-11):1-77.
3. Wang JY, Wang JT, Tsai TH, Hsu CL, Yu CJ, Hsueh PR, Lee LN, Yang PC. Adding moxifloxacin is associated with a shorter time to culture conversion in pulmonary tuberculosis. Int J Tuberc Lung Dis 2010;14(1):65-71.
4. Wang JY, Liu CH, Hu FC, Chang HC, Liu JL, Chen JM, Yu CJ, Lee LN, Kao JH, Yang PC. Risk factors of hepatitis during anti-tuberculous treatment and implications of hepatitis virus load. J Infection 2011;62:448-55.
5. Huang YS, Cherng HD, Su WJ, et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for anti-tuberculosis drug-induced hepatitis. Hepatology 2002;35(4):883-9.
6. Possuelo LG, Castelan JA, de Brito TC, et al. Association of N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from southern Brazil. Eur J Clin Pharmacol 2008;64(7):673-81.
7. Shen C, Meng Q, Zhang G, Hu W. Rifampicin exacerbate isoniazid-induced toxicity in human but not in rat hepatocytes in tissue-like cultures. Br J Pharmacol 2008;153(4):784-91.
8. Huang YS, Su WJ, Huang YH, et al. Genetic polymorphisms of manganese superoxide dismutase NAD(P)H:quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. J Hepatol 2007;47(1):128-34.
9. Pande JN, Singh SP, Khilnani GC, Khilnani S, Tandon RK. Risk factors for hepatotoxicity from antituberculosis drugs: a case-control study. Thorax 1996;51(2):132-6.
10. Teleman MD, Chee CB, Earnest A, Wang YT. Hepatotoxicity of tuberculosis chemotherapy under general programme conditions in Singapore. Int J Tuberc Lung Dis 2002;6(8):699-705.
11. Sharma SK, Balamurugan A, Saha PK, Pandey RM, Mehra NK. Evaluation of clinical and immunogenetic risk factors for the development of hepatotoxicity during antituberculosis treatment. Am J Respir Crit Care Med 2002;166(7):916-9.
12. Fernandez-Villar A, Sopena B, Fernandez-Villar J, et al. The influence of risk factors on the severity of anti-tuberculosis drug-induced hepatotoxicity. Int J

Tuberc Lung Dis 2004;8(12):1499-505.

13. Shakya R, Rao BS, Shrestha B. Incidence of hepatotoxicity due to antitubercular medicine and assessment of risk factors. Ann Pharmacother 2004;38(6):1074-9.
14. Lee BH, Koh WJ, Choi MS, et al. Inactive hepatitis B surface antigen carrier state and hepatotoxicity during antituberculosis chemotherapy. Chest 2005; 127(4):1304-11.
15. Won WM, Wu PC, Yuen MF, et al. Antituberculosis drug-related liver dysfunction in chronic hepatitis B infection. Hepatology 2000;31(1):201-6.
16. Kwon YS, Koh WJ, Su GY, et al. Hepatitis C virus infection and hepatotoxicity during antituberculosis chemotherapy. Chest 2007;131(3): 803-8.
17. Ungo JR, Jones D, Ashikin D, et al. Antituberculosis drug-induced hepatotoxicity. The role of hepatitis C virus and the human immunodeficiency virus. Am J Respir Crit Care Med 1998;157(6 pt 1): 1871-6.
18. Ozick LA, Jacob L, Comer GM, et al. Hepatotoxicity from isoniazid and rifampicin in inner-city AIDS patients. Am J Gastroenterol 1995;90(11): 1978-80.
19. van Hest R, Baars H, Kik S*, et al.* Hepatotoxicity of rifampin-pyrazinamide and isoniazid preventive therapy and tuberculosis treatment. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*. 2004;**39**:488-96.
20. Hunt CM, Westerkam WR, Stave GM. Effect of age and gender on the activity of human hepatic CYP3A. *Biochem Pharmacol*. 1992;**44**:275-83.
21. Lamba JK, Lin YS, Schuetz EG*, et al.* Genetic contribution to variable human CYP3A-mediated metabolism. *Advanced drug delivery reviews*. 2002;**54**:1271-94.
22. 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 metabolism and disposition: the biological fate of chemicals*. 2008;**36**:169-81.
23. Sookoian S, Castano GO, Burgueno AL*, et al.* The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. *Pharmacogenetics and genomics*. 2010;**20**:1-8.
24. Wang JY, Tsai CH, Lee YL, Lee LN, Hsu CL, Chang HC, Chen JM, Hsu CA, Yu CJ, Yang PC. Sex-dimorphic impact of PXR on hepatotoxicity during anti-tuberculosis treatment. (Submitted)
25. Steele MA, Burk RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis.

Chest 1991;99(2):465-71.
26. Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha DR, Dhali GK. Mitochondrial oxidative stress and permeability transition in isoniazid and rifampin induced liver injury in mice. J Hepatol 2006;45:117-26.
27. Tafazoli S, Hasgregi M, O'Brien PJ. Role of hydrazine in isoniazid-induced hepatotoxicity in a hepatocyte inflammation model. Toxicol Appl Pharmacol 2008; 229:94-101.
28. DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. Am J Med Genet 2001;106:18-26.
29. Keogh MJ, Daud D, Pyle A, Duff J, Griffin H, He L, Alston CL, Steele H, Taggart S, Basu AP, Taylor RW, Horvath R, Ramesh V, Chinnery PF. A novel de noro *STXBP1* mutation is associated with mitochondrial complex I deficiency and late-onset juvenile-onset parkinsonism. Neurogenetics 2015;16:65-7.
30. Czarnecka AM, Klemba A, Krawczyk T, ZdroznyM, Arnold RS, Bartnik E, Petros JA. Mitochondrial NADH-dehydrogenase polymorphisms as sporadic breast cancer risk factor. Oncol Reports 2010;23:531-5.
31. Flaquer A, Heinzmann A, Rospleszcz S, Mailaparambil B, Dietrich H, Strauch K, Grychtol R. Association study of mitochondrial genetic polymorphisms in asthmatic children. Mitochondrion 2014;14(1):49-53.
32. Rezaee AR, Azadi A, Houshmand M, Mahmoodi F, Purpak Z, Safaei S, Karimi P, Ghabaee M, Sahraian MA. Mitochondrial and nuclear genes as the cause of complex I deficiency. Genet Mol Res 2013;12(3):3551-4.
33. Barrett JC, Fry B, Maller J*, et al.* Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
34. 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. Human heredity 2003;55:179-90.

自評表:

我國結核病患者約有 17%在治療當中會發生抗結核藥物性肝炎，嚴重影響抗結核治療之進行及完成。發生藥物性肝炎之危險因子包括： 1)女性；2) N-acetyl transferase 2 (NAT2)基因之 slow acetylator 等。本計畫旨在研究粒線體複合體 I (mitochondria complex I，即 NADH 去氫酶)之單核甘酸多型性(即 SNP)是否與抗結核藥物性肝炎之發生有關，並探討粒線體複合體 I 之 SNP 是否與女性較易發生抗結核藥物性肝炎有關。主持人使用次世代定序法(Next Generation Sequencing)，檢測 19 位有發生(肝炎組)，及 19 位未發生(對照組)抗結核藥物性肝炎之結核病病人，白血球中的粒線體基因，總共 1.6KB，包括 13 個蛋白質基因(complex I、complex III、complex IV、ATP synthase)，2 個 ribosomal RNA 基因，22 個 transfer RNA (即 tRNA) 基因，以及 DNA control 區。結果發現，肝炎組與對照組粒線體基因之 SNP，在以下三區塊有顯著之不同: 1) tRNA 基因之 SNP，在肝炎組有 8 人發生，但在對照組則沒有任何人發生；2) Complex I 之 ND5 基因之 non-synonymous SNP 發生於 9 位肝炎組病人，2 位對照組病人；3) Complex IV 之 COX III (cytochrome C oxidase subunit III) 基因之 non-synonymous SNP 發生於 5 位肝炎組病人，0 位對照組病人。雖然由於病例數不多，性別之差異目前尚難斷定，但本計畫的確發現，粒線體基因之多型性，與抗結核藥物性肝炎之發生有相關性。過去並無任何類似的研究被發表過。本研究仍在繼續進行中。

科技部補助計畫衍生研發成果推廣資料表

日期:2017/02/14

科技部補助計畫	計畫名稱: Isoniazid 藥物性肝炎之性別特異性風險與粒線體NADH去氫酶基因多型性之關聯性
	計畫主持人: 李麗娜
	計畫編號: 104-2629-B-002-001- 學門領域: 性別主流科技計畫

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：李麗娜	**計畫編號：**104-2629-B-002-001-
計畫名稱：Isoniazid 藥物性肝炎之性別特異性風險與粒線體NADH去氫酶基因多型性之關聯性	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			0	篇	
		研討會論文			1		將於2017年5月21日在美國胸腔學會年會發表。
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		

		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
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。）			聘用兼任研究助理一人		

科技部補助專題研究計畫成果自評表

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（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）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（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   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200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）
   我國結核病患者約有17%會發生抗結核藥物性肝炎，發生藥物性肝炎之危險因子包括： 1)女性；2) NAT2基因之slow acetylator等。本計畫旨在研究粒線體基因之SNP是否與抗結核藥物性肝炎有關，及其是否與女性較易發生抗結核藥物性肝炎有關。主持人使用次世代定序法，檢測19位有發生(肝炎組)，及19位未發生(對照組)抗結核藥物性肝炎之病人，白血球中的粒線體基因，包括13個蛋白質基因，2個ribosomal RNA 基因，22個tRNA 基因。結果發現，肝炎組與對照組粒線體基因之SNP，在以下三區有顯著不同: 1) tRNA基因之SNP，在肝炎組有8人發生，在對照組則沒有；2) Complex I之ND5基因之non-synonymous SNP發生於9位肝炎組，2位對照組病人；3) Complex IV之COX III基因之non-synonymous SNP發生於5位肝炎組，0位對照組病人。雖然由於病例數不多，性別之差異尚難斷定，但本計畫的確發現，粒線體基因之多型性，與抗結核藥物性肝炎之發生有相關。過去並無類似的研究被發表過。本研究仍在進行中。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是

說明：（以150字為限）