出國報告（出國類別：國際會議）

2017 年美國神經醫學會年會

(2017 American Academy of Neurology Annual Meeting)

出國報告

服務機關：臺北榮總神經醫學中心
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摘要

美國神經醫學會年會為神經學領域專家每年最盛大的會議，世界各國及美國各地神經科醫師及相關研究學者約有數千人與會，2017 年美國神經醫學會年會在美國波士頓舉行，此次會議中，我以壁報形式發表北榮神經基因團隊在遺傳性腦部小血管疾病的最新發現，壁報論文主題為: Heterozygous *HTRA1* mutations in Taiwanese patients with cerebral small vessel disease，我們針對 318 位無親屬關係的腦部小血管疾病患者進行 *HTRA1* (high temperature requirement serine peptidase A1)基因定序，共找到八個突變點，生物資訊軟體預測發現這八個突變位置均具高度致病性，我們再利用攜帶有突變點的質體轉殖使細胞表現突變蛋白，並測試突變蛋白對 HTRA1 蛋白質酵素活性的影響，證實其中有兩種突變會造成酵素活性顯著下降。此類病患過去僅在法國及日本各有一篇文章報導，主題具新穎性，因此獲得大會遴選進行簡短口頭報告，與各國學者分享交流。

此次大會有幾個主題讓我受益良多，包括連續多堂的神經傳導與肌電圖判讀教學，由淺而深，指出多項臨床容易誤判的情境，深具實用價值。多發性硬化症免疫藥物的新觀念是針對免疫反應下游的特定分子/受體所研發之單株抗體藥物，治療效果佳且較少產生全身性免疫抑制等副作用，是未來免疫藥物主流。運動神經元疾病有多種新藥試驗結果在今年解盲，基因編輯治療(genetic editing)藥物對於遺傳疾病如裘馨氏肌肉失養症(Duchene muscular dystrophy)或家族性類澱粉沉積神經病變(Familial amyloid neuropathy)有多種藥物正在進行二、三期臨床試驗，這些過去無藥可治的罕見疾病在未來都會有多線藥物進入臨床用途。

關鍵字: ANN, genetic editing therapy, monoclonal antibody, exomic sequencing

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一、 目的

美國神經醫學會年會為神經學領域專家每年最盛大的會議，世界各國及美國各地神經科醫師及相關研究學者約有數千人與會，會議中除了對臨床診斷與治療提出準則(guideline)或最新治療觀念，也有許多議程是報告神經科學研究最新發現，對於我們接軌世界第一手的研究資訊有很大助益。2017 年美國神經醫學會年會在美國波士頓舉行，此次會議中，我以壁報形式發表北榮神經基因團隊在遺傳性腦部小血管疾病的最新發現，壁報論文主題為: Heterozygous *HTRA1* mutations in Taiwanese patients with cerebral small vessel disease，此壁報論文也獲得大會遴選進行簡短口頭報告，與各國學者分享交流。很感謝科技部的補助及台北榮總的支持，讓我有機會參加 2017 年美國神經醫學學會年會，在臨床治療診斷及未來研究方向都受益良多。

二、 過程

此行參加美國神經醫學會本科共有五位醫師同行，於今年4月22日至4月28日在美國波士頓舉行。我們因飛機抵達較晚，錯過第一天的議程，由4月23日開始參加課程，第一天上午我參加的課程為:Clinical EMG: principles and practice NCS and needle EMG，過去美國神經醫學會此類教學課程均要另外付費才能上課，今年開放給所有與會者聆聽不額外收費，因此我參加了很多堂與肌電圖(EMG)診斷相關的臨床課程，希望將我們北榮周邊神經科肌電圖報告的精確度能與美國教學醫院的標準看齊，達到世界一流水準。下午的課程包括: ALS disease mechanism and therapeutics 以及Multiple sclerosis therapy: disease modifying treatment，前者介紹了利用CRISPR 技術進行genome editing 來治療小鼠的運動

神經元疾病模型；後者介紹了多發性硬化症免疫藥物，未來趨勢是使用針對免疫反應下游的特定分子/受體所研發之單株抗體藥物，此類藥物如雨後春筍般有多種藥物都將在一兩年內進入市場，單株抗體藥物治療效果佳且較少產生全身性免疫抑制等副作用，是未來免疫藥物主流。

第二天的議程為Therapy of neuromuscular disease: ALS, inflammatory neuropathies, myopathies and myasthenia gravis，這些免疫相關的周邊神經病變或、發炎性肌炎、重症肌無力症過去很少大型臨床試驗，以致常沒有公認的藥物治療準則，如何用藥、選藥大部分是根據專家意見或臨床經驗做調整，此次會議中很詳盡且實用的列舉每種疾病第一線第二線第三線藥物的建議為何。第三天議程重點包括介紹了運動神經元疾病(ALS, amyotrophic lateral sclerosis) 最近剛完成第三期臨床試驗的藥物，及一些剛開始進行的第三期藥物試驗，這些藥物雖然無法治癒運動神經元疾病，但統計結果看來至少與安慰劑相較之下新藥物可以顯著延緩病程惡化或減少bulbar function 退步，為此不治之症帶來一線新曙光。

第四天議程中多位講者都提到基因治療，包括Epigenome editing by CRISPR/Cas9及adeno-associated virus (AAV)進行exon-skipping治療裘馨氏肌肉失養症 (Duchene muscular dystrophy)，或利用engineered nuclease 進行genome editing 將exon 44-50這一段常會出現frame-shift突變的片段剪切掉以治療裘馨氏肌肉失養症，利用RNAi 治療家族性類澱粉沉積神經病變(Familial amyloid neuropathy)等等，可以預見基因治療對於神經遺傳疾病來說是指日可待。第五天議程亮點是next-generation sequencing (NGS)技術及whole exome sequencing (WES)技術是未來診斷神經遺傳疾病的顯學，此二技術因有許多商業的實驗室支應，取得定序結果的難度與價格都在快速遞減中，在不遠的將來即變成臨床診斷

工具，而非僅限研究用途，但如何正確判讀定序結果，區辨真正的突變位點跟 variant of unknown significance (致病力未明的基因變異點)給予適切的遺傳諮詢，將是臨床醫師的一大挑戰。

此次會議中，我以壁報形式發表北榮神經基因團隊在遺傳性腦部小血管疾病的最新發現，壁報論文主題為: Heterozygous HTRA1 mutations in Taiwanese patients with cerebral small vessel disease，我們針對318 位無親屬關係的腦部小血管疾病患者進行HTRA1 (high temperature requirement serine peptidase A1)基因定序，共找到八個突變點，生物資訊軟體預測發現這八個突變位置均具高度致病性，我們再利用攜帶有突變點的質體轉殖使細胞表現突變蛋白，並測試突變蛋白對HTRA1 蛋白質酵素活性的影響，證實其中有兩種突變會造成酵素活性顯著下降。此類病患過去僅在法國及日本各有一篇文章報導，主題具新穎性，因此獲得大會遴選進行簡短口頭報告，與各國學者分享交流。

三、 心得

基因診斷及治療是未來神經學發展重要的顯學與趨勢，次世代定序技術即將變成臨床診斷利器，也是必備的武器。基因領域的知識演進日新月異，如果我們不跟國際接軌，很容易被潮流所淘汰。基因治療號稱可以將突變的基因重新編輯修正為正確序列，對於過去我們束手無策的神經遺傳疾病提供全新的治療契機，但此類罕見疾病很難進行臨床雙盲試驗，基因治療此領域對於台灣醫界又是陌生的領域，需要很多觀念與制度的革新才可能改變現況，能夠有機會將基因治療應用在我們的病人身上。

四、 建議事項（包括改進作法）

次世代定序技術包括 whole genome sequencing 及 whole exome sequencing 很快就將變成臨床診斷的利器且也是必備的武器，如何掌握這項工具將是我們最重要的課題，需要有更多人力與資源的挹注才能達成。

附錄（所發表的學術海報）

Heterozygous *HTRA1* mutations in Taiwanese patients with cerebral small vessel disease

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Introduction

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is an autosomal recessive inherited cerebral small vessel disease (CSVD) caused by mutations in the high-temperature requirement serine peptidase A1 gene (*HTRA1*). Recently, heterozygous *HTRA1* mutations were found in patients with autosomal dominant CSVD, the presentation of which differs from CARASIL by a later age of onset and absence of the typical extra-neurological features like alopecia. Fifteen heterozygous *HTRA1* mutations have been identified, yet the molecular mechanism of these heterozygous variants remains unclear.

Methods

Mutational analyses of *HTRA1* were carried out by Sanger sequencing in a cohort of 318 unrelated Taiwanese patients with clinically diagnosed CSVD, in whom pathogenic *NOTCH3* mutations had been excluded. Functional effects of the identified heterozygous *HTRA1* mutations were evaluated by comparing the HTRA1 protease activities using Pierce® Fluorescent Protease assay kit in HEK293 cells expressing wide-type (WT) or mutant *HTRA1* construct .

Table 1. Clinical features and *in silico* predictions of *HTRA1* mutations

HTRA1 mutations	Ex	Location: Protein domain	Bioinformatics prediction: Mutation Taster	SIFT	PolyPhen2	Population genetics: GnomAD	500 Taiwan controls	Clinical features of patients: AO/Sex	Stroke	Cognitive/ Psychiatric	Spinal lesions	Lacunar infarcts	EMBs	WMH
c.559G>A, p.Gly120Asp	1	Kazal type	Disease causing	Deleterious	Probably damaging	not found	not found	52 F	ICH, CI	-	-	BG, pons	NA	WM 1/1, EC 0/0, AT 0/0
c.536T>A, p.Ile179Asn	2	None	Disease causing	Deleterious	Probably damaging	not found	not found	48 M	-	-	Cv, Ls spine	BG	-	WM 2/1,EC 0/0, AT 0/0
c.543delT, p.Ala182Profs*33	2	None	Disease causing	NA	NA	not found	not found	62 M	CI, ICH	memory ↓, obsessive compulsive	-	Thalamus, pons	+	WM 4/4, EC 4/4, AT 1/0
c.754G>A, p.Ala252Thr (rs113893568)	3	Serine protease	Disease causing	Deleterious	Probably damaging	1/246240 (European)	not found	35 F	TIA	-	Cv spine	BG,CR, pons,	NA	NA
c.767T>C, p.Ile256Thr (rs201305795)	3	Serine protease	Disease causing	Deleterious	Probably damaging	2/246206 (African)	not found	54 M	-	dementia, depression, delusion	Cv spine	thalamus, pons	NA	WM 8/6, EC 6/6, AT 0/0
c.827G>C, p.Gly276Ala	4	Serine protease	Disease causing	Deleterious	Probably damaging	not found	not found	49 F	CI, ICH	-	Cv spine	thalamus, CR	+	WM 3/4, EC 2/2, AT 0/0
c.865C>T p.Gln288X	4	Serine protease	Disease causing	NA	NA	not found	not found	62 F	TIA	-	Cv, Ls spine	pons	-	WM 4/4, EC 3/3, AT 6/5
c.971A>C, p.Asn324Thr	4	Serine protease	Disease causing	Tolerated	Probably damaging	not found	not found	60 M	CI	-	Cv, Ls spine	CR	-	WM 6/6, EC 2/3, AT 0/1

Results

Eight heterozygous *HTRA1* variants were identified (Fig 1). All have an allele frequency less than 0.00001 in GnomAD database and are absent in 1000 ethnic control chromosomes (Table 1). Cerebral infarcts or intracranial hemorrhage at middle age, mentality decline and diffuse spondylosis were common presentations. Multiple lacunar infarcts at pons, thalamus, and basal ganglia and diffuse white matter hyperintensities at corona radiate were frequently found in brain MRI.

Fig.1 *HTRA1* mutations in Taiwanese CSVD families

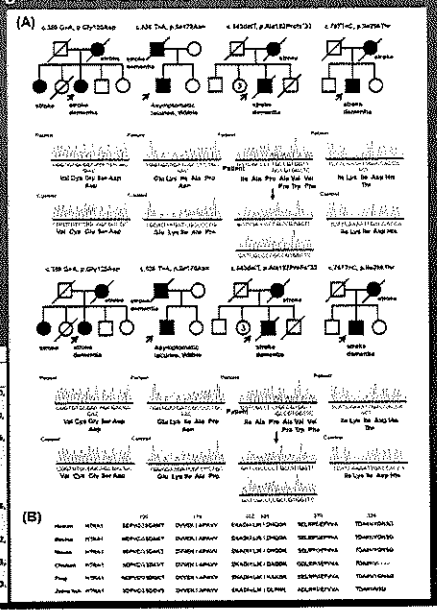
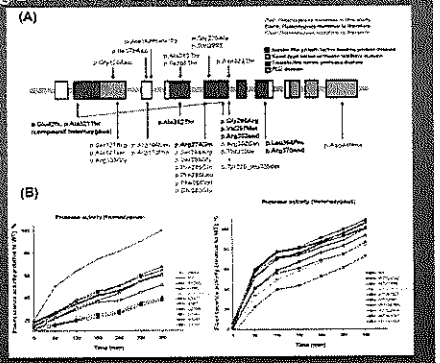


Fig.2 Functional consequence of *HTRA1* mutations



The HTRA1 protease activities were significantly reduced in cells expressing any of the 8 mutant *HTRA1* constructs than those with WT construct (Fig 2). The enzyme activities of the two nonsense variants (p.A182Pfs*33 and p.Q289X) and S328A (negative control with abolished enzyme activity) were comparable to empty vector. Cells transfected with half-dose of Q289X and half-dose of WT constructs had an even lower protease activities than those expressing half dose of S328A and half dose of WT *HTRA1* constructs, supporting an dominant-negative effect of Q289X mutation.

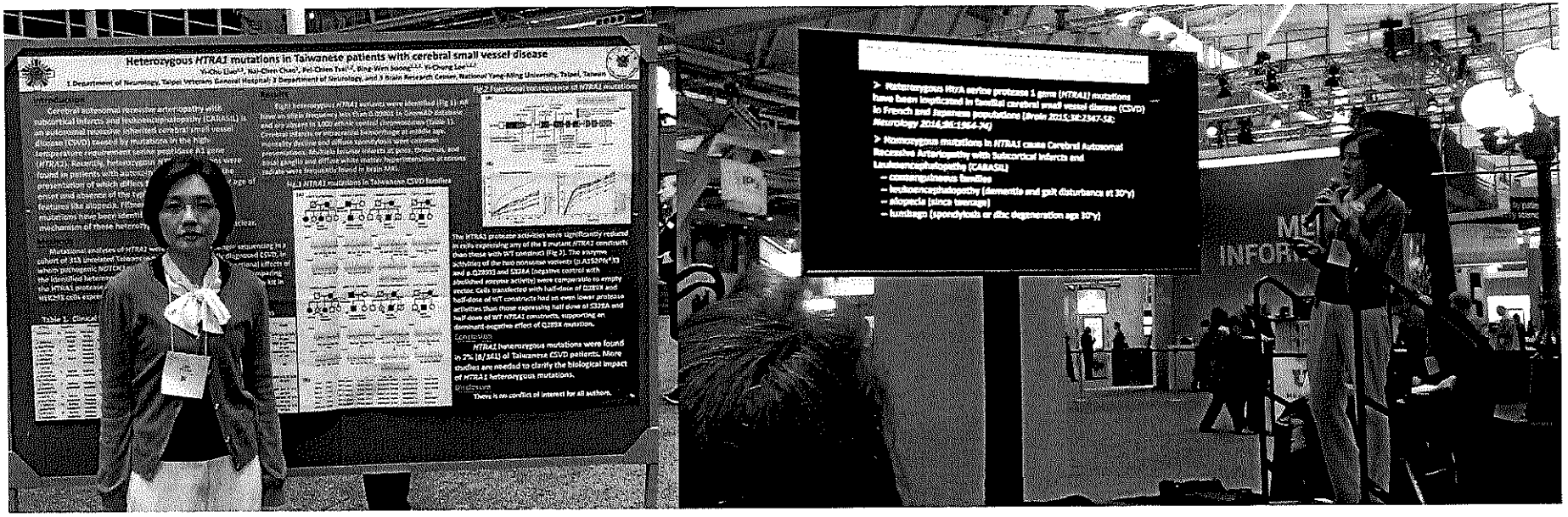
Conclusion

HTRA1 heterozygous mutations were found in 2% (8/381) of Taiwanese CSVD patients. More studies are needed to clarify the biological impact of *HTRA1* heterozygous mutations.

Disclosure

There is no conflict of interest for all authors.

壁報發表及口頭報告


Heterozygous MTRA1 mutations in Taiwanese patients with cerebral small vessel disease
Introduction
Results
Conclusion
Disclosure
There is no conflict of interest for all authors.
> Heterozygous HtrA serine protease 1 gene (HTRA1) mutations have been implicated in familial cerebral small vessel disease (CSVD) in French and Japanese populations (Brain 2013;136:3947-58; Neurology 2014;83:1964-74)
> Heterozygous mutations in HTRA1 cause Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL)
– consanguineous families
– leukoencephalopathy (dementia and gait disturbance at 30's)
– alopecia (since teenage)
– lumbago (spondylosis or disc degeneration age 30's)