出國報告（出國類別：國際會議）

參加第 14 屆國際神經肌病會議

(14th International Congress on Neuromuscular Diseases) 心得報告

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摘要

感謝科技部專題研究計畫的補助及台北榮總的支持，讓我有機會參加 2016 年 7 月 5 日至 7 月 9 日在加拿大多倫多由世界神經聯盟神經肌病研究群(Research Group on Neuromuscular Diseases-World Federation of Neurology; RGNMD-WFN)所舉辦的第十四屆國際神經肌病會議 (14th International Congress on Neuromuscular Diseases)。 此會議是周邊神經及肌肉疾病領域最大的學術會議，第一次在 1969 年舉辦，之後便 4 年舉辦一次，而今年是第一次開始隔兩年辦一次。 本次會議仍能秉持過去高品質的傳統，安排了許多內容豐富精彩的演講及 workshops，Plenary Sessions 包含了 Genetics、Hot Topics、肌肉萎縮症、及運動神經元疾病等四大主題，其中比較吸引我的 plenary lecture 主題包括了 Genomic Approaches to Diagnosis of Rare Muscle Disease, Gene Discovery in Charcot-Marie-Tooth Neuropathies, Antisense Therapy for Myotonic Dystrophy、CRISPR Based Gene Editing for Muscular Dystrophy, Biology of C9ORF72 Disease、以及 Antisense Therapy for spinal Muscular Atrophy。 而我同時也以學術壁報的形式發表了我們新近對於遺傳性運動感覺病變 X1 型的分子病理機制的研究, 主題為 Biophysical Characteristics and Clinical Correlation of GJB1 Mutations in Charcot-Marie-Tooth Disease Type X1，由於這個研究首次運用鈣離子影像技術評估突變的 GJB1 蛋白所形成的細胞間 gap junction channel 的通透障礙，因而引起一些相關領域研究者的興趣。 整體而言，本次會議內容新穎豐富，有許多地方值得國內神經學界參考。

關鍵字: 國際神經肌病會議、肌肉病變、周邊神經病變、基因治療、及運動神經元疾病

目次

一、 目的

周邊神經及肌肉相關疾病是我們周邊神經科醫療服務及學術研究的主要負責主題，身為周邊神經科主治醫師的我當然對這些領域的最新相關發展非常關心，也希望能將自己的微小研究發現能作一個適當的發表；因此選擇參加周邊神經及肌肉疾病領域裏最大的會議，也就是國際神經肌病會議 (International Congress on Neuromuscular Diseases)。 我從 2002 年當總醫師時第一次參加這個會議以來，就規律地每次都參加了這個會議，這是我第六次參加此會議。

我大概從十二年前起開始對遺傳性運動感覺神經病變 (Charcot-Marie-Tooth disease; CMT) 的基因研究開始感到興趣。 CMT 是一群具有相似臨床症狀但不同致病基因的遺傳性周邊神經病變，它最早的完整病例報告描述是在西元 1886 年由法國的 Jean-Martin Charcot 和 Pierre Marie，以及英國的 Howard Henry Tooth 所發表，這三位專家的姓氏就是 CMT 病名的由來。 CMT 的發病年齡從剛出生到老年都有可能，但最常見到的是在兒童、青春期、及年輕成人時期。 這類病患的周邊神經會緩慢逐漸地退化，通常由肢體遠端開始，因而所造成的症狀也是緩慢逐漸地發生。 一開始的症狀往往是病患的腳掌變形，足部的足弓會變得很彎很高，這是因為腳掌內部肌肉萎縮，因而無法保持正常腳掌的型態。 接著小腿的肌肉也會跟著萎縮，下肢肌肉的萎縮常會造成病患行走、跑步及平衡的問題，因而病患也常會跌倒扭傷足踝。 當疾病更為進展時，許多病患需要枴杖或輪椅來幫助行動。 隨著疾病的進行，手掌的肌肉也會萎縮，造成病患寫字、拿筷子，手部精細活動的障礙。 由於感覺神經也同時受損，有些病患也會感覺到肢體末端麻木或感覺異常。 感覺的缺失常發生在肢體的遠端，對疼痛、溫度、振動和本體感覺等不同型式感覺常有不同程度的損害。 台灣目前並沒有 CMT

的盛行率資料，在西方國家，CMT 的盛行率是十萬分之 20.1- 40，而 CMT1 則在每十萬人中有 16.2- 30 人。 如果將此數據套用在台灣的人口數上，預計在台灣應有 4600 至 9200 CMT 患者；這個人數對於神經遺傳疾病來說算是非常多的。

目前有超過 80 種不同的 CMT 致病基因已經被發現，反映出周邊神經的結構與功能的分子複雜性。 其中第二常見發生突變的致病基因為 GJB1 基因，約十分之一的 CMT 病人是因為此基因突變而生病。 此基因的蛋白質產物 GJB1 蛋白可在 Schwann cells 所形成的髓鞘兩端細胞膜上以 hexamers 的型式形成 hemichannel，而與鄰近的 hemichannel 形成 gap junction channel，幫助髓鞘區域的細胞質與細胞核附近的細胞質能快速連通。 我們先前在 24 位 CMT 病人中發現了 19 種不同的 GJB1 突變，在這些病人中，雖然相同是 GJB1 發生突變，不同突變的病人似乎會有不同時期的發病年齡及不同的臨床嚴重程度。 我們對於這個現象感到非常有興趣，因而嘗試分析研究這些突變所造成的 GJB1 分子功能缺損與臨床表徵的相關性。 我們發現不同的突變可能會影響三個不同相面的分子特性，包括 GJB1 蛋白質的表現量是否下降、GJB1 是否能良好地到達細胞膜以發揮功能、到達細胞膜的 GJB1 蛋白能否良好地形成 gap junction channel 並發揮離子或 second messengers 通透的功能。 我們的研究結果發現這些 GJB1 突變的分子特性的確與臨床嚴重程度有關連。 我非常高興參加了此次會議，不僅發表了我們 *GJB1* 的相關研究，同時也聽到許多令人非常欽佩的研究論文的細節內容。整個過程真是令人感到非常享受。

二、 過程

感謝科技部專題計畫的補助及台北榮總的支持，讓我有機會參加2016年7月5日至7月9日在加拿大多倫多由世界神經聯盟神經肌病研究群 (Research Group on Neuromuscular Diseases-World Federation of Neurology; RGNMD-WFN) 所舉辦的第十四屆國際神經肌病會議 (14th International Congress on Neuromuscular Diseases)。 此會議是周邊神經及肌肉疾病領域裏最大的學術會議，第一次在1969年舉辦後，之後便4年舉辦一次，但由於近年來神經肌病領域的知識及研究興盛，因而今年開始第一次隔兩年辦一次。 本次會議仍能秉持過去高品質的傳統，安排了許多內容豐富精彩的演講及workshops，包含了Genetics、Hot Topics、肌肉萎縮症、及運動神經元疾病等四大主題領域中近兩年的新發現及突破，其中，我個人認為特別精彩的演講如下：

1. Genomic Approaches to Diagnosis of Rare Muscle Diseases 由美國 Massachusetts General Hospital 的 Dr. Daniel MacArthur 主講。
2. Gene Discovery in Charcot-Marie-Tooth Neuropathies 由美國 University of Miami 的 Dr. Stephan Züchner 主講。
3. Antisense Therapy for Myotonic Dystrophy 由美國 University of Rochester 的 Dr. Charles Thornton 主講。
4. CRISPR Based Gene Editing for Muscular Dystrophy 由加拿大 The Hospital for Sick Children (SickKids)的 Dr. Ronald Cohn 主講
5. Biology of C9ORF72 Disease 由在美國 Jacksonville, Florida 的 Mayo Clinic 的 Dr. Leonard Petrucelli 主講。
6. Antisense Therapy for spinal Muscular Atrophy 由美國 The Ohio State

University 的 Dr. John Kissel 主講。

另外，在開會期間有兩百二十三篇嚴格挑選的學術海報發表，每篇都是高品質研究的展現。 而我在與會期間同時也以學術海報的方式發表了我們在 CMT 領域的相關研究結果，主題為 Biophysical Characteristics and Clinical Correlation of GJB1 Mutations in Charcot-Marie-Tooth Disease Type X1，由於這個研究首次運用鈣離子影像技術評估突變的 GJB1 蛋白所形成的細胞間 gap junction channel 的通透障礙，因而引起一些相關領域研究者的興趣。 這篇論文目前正投稿於 Annals of Clinical and Translational Neurology 雜誌，已 revise 過一次，希望近期能順利被接受。 整體而言，本次會議內容新穎豐富，我因而受益良多。

三、 心得

這次參加學術會議的期間我聆聽了許多演講報告，也仔細閱讀了許多學術海報，獲益良多。 除了欣賞世界各地許多優秀研究學者的優雅研究外，也增進自身對現今周邊神經疾病及肌肉疾病相關研究的進一步瞭解。 今年在會議中所聽到的訊息中，我覺得有兩個項目具有的影響力最大： 一是利用RNA sequencing來診斷困難診斷的神經肌肉遺傳疾病； 第二是遺傳性神經肌肉疾病的基因治療已日趨成熟。 自次世代核苷酸定序技術 (Next-Generation sequencing; NGS)發展至今，全基因體定序 (whole genome sequencing; WGS)或全外顯子定序 (whole exome sequencing; WES) 在許多國外提供基因診斷的實驗室中已非常成熟。 這些技術定序的標的是所有基因的DNA序列，而所得到的數據結果是數百萬甚至千萬段長度約75-150bp的定序結果再運用資訊運算所拚湊判斷出來。 由於技術上的先天限制，這兩個技術對於超過75-150bp長度的基因缺損或重複是無法診斷出來的。 另外，如果在基因的intron區域內發生基因變異，因為intron的訊息在基因表達的過程中不會傳給mRNA，所以一般不會影響蛋白質產物的胺基酸序列。 但是intron區域內的基因變異在少數的情形下可能會影響mRNA splicing，進而造成錯誤的mRNA序列及蛋白質產物。 RNA sequencing就是利用NGS直接來定序組織中所有的mRNA序列，以此可以更進一步找出運用WGS或WES無法找出的突變。 但較大的限制是需要標的組織，這在肌肉病變或周邊神經病變中是較易達成的。 而關於基因治療方面，在肌肉萎縮症或遺傳性運動神經元疾病的動物模式中，針對不同突變所量身訂做的基因治療，已達成非常顯著的治療效果。 這些方式包括對於突變造成蛋白質產物不足而致病的清況下，運用AAV病毒載體將正常基因表現在標的組織中或運用exome skipping的方法跳過突變區域

而產生仍有部分功能的新穎變異蛋白；而對於顯性遺傳下常見的gain of toxic function機制，也就是突變會造有毒的突變蛋白的情形下，運用antisense oligonucleotide來抑制突變基因的表現。 在聆聽許多基因治療的相關研究後，我深深地覺得基因治療再也不是一個遙遠的概念與理想，而是極可能在5-10年內就會在歐美國家內普遍的醫療技術，而且將會在許多臨床科別內都扮演一個重要的角色。

四、 建議事項（包括改進作法）

以我們目前的環境，要創新發展在臨床應用上切實可行的基因治療，可能相當困難。 但是我們還是應該要為基因治療時代的來臨預作準備。 基因治療的基礎除了先進的分子生醫技術外，也必須對標的疾病的各樣式分子病理機制清楚掌握，才能夠正確適當的矯正或補償造成疾病的不正常分子徑路。 最基本的事項之一是我們需要增進我們對所有與基因相關疾病的分子診斷技術，同時也必須深究所有致病突變背後的分子機制。 運用次世代核苷酸定序技術來進行臨床診斷的能力是重要的基礎之一。

附錄（所發表的學術海報）

Biophysical Characteristics and Clinical Correlation of GJB1 Mutations in Charcot-Marie-Tooth Disease Type X1

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Introduction

Charcot-Marie-Tooth disease type X1 (CMTX1), which is caused by mutations in the gap junction (GJ) protein beta-1 gene (*GJB1*), is the second most common form of Charcot-Marie-Tooth disease (CMT). *GJB1* encodes the GJ beta-1 protein (GJB1), which forms GJs within the myelin sheaths of peripheral nerves. The process by which GJB1 mutants cause neuropathy has not been fully elucidated. This study evaluated the biophysical characteristics of GJB1 mutants and their correlations with the clinical features of CMTX1 patients.

Methods

Patients

Patients were diagnosed with CMTX1 based on identification of a *GJB1* mutation, the characteristic clinical features and electrophysiological analyses. The CMTX1 patients all received evaluation using the Charcot–Marie–Tooth disease neuropathy score version 2 (CMTNS).[1]

Immunofluorescence studies and Western blotting

The coding region of human *GJB1* was cloned into pcDNA3.1/myc-His (Invitrogen) to generate wild-type (WT) GJB1 expression plasmids. The *GJB1* mutations identified in this study were separately introduced into the WT expression plasmids using a Site-Directed Mutagenesis method. HeLa cells were transfected to express WT or mutant GJB1 alone or together with pDsRed-ER or pDsRed-Monomer-Golgi using Lipofectamine 2000 (Invitrogen). At 48 hours after transfection, the cells were fixed and incubated with anti-GJB1 antibody overnight at 4°C. Images were captured using an Olympus FluoView FV10i confocal laser scanning fluorescence microscopy system (Olympus). HeLa cells transfected with WT or mutant *GJB1* constructs were lysed with RIPA buffer at 48 hours after transfection. 50 μg of proteins from each cell lysate were used for Western blotting.

Ionic permeability of GJB1 GJ channels

The abilities of the mutated GJB1 GJ channels to mediate the intercellular propagation of Ca^{2+} signalling were assessed by Ca^{2+} imaging analyses as previously described.[2-3] HEK293T cells expressing WT or mutant GJB1 were washed with Hanks' Buffered Salt Solution (HBSS) at 48 hours after transfection and loaded with the Ca^{2+} indicator fura-2/AM and Pluronic F-227 detergent (0.02%) at 37°C for 40 minutes. Next, the cells were washed and saturated with 20 μM ATP in HBSS for 20 minutes to block the intercellular Ca^{2+} signaling mediated by purinergic receptors. One transfected cell was stimulated mechanically by temporarily distorting the plasma membrane with a glass micropipette (tip diameter, <1 μm) mounted on a vertical microinjection system (Narishige MHW-3, Tokyo, Japan). An Olympus IX71 microscope equipped with an Olympus UApo/340 40×/1.35 NA objective, a Polychrome V monochromator (Till Photonics, FEI, Hillsboro, Oregon), and a charge-coupled device camera (Hamamatsu ORCA-AG, Shizuoka Pref, Japan) controlled by SimplePCI software (Hamamatsu) was used to monitor changes in the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in the stimulated and surrounding cells with transfection markers. Fura-2 ratio images were acquired at excitation wavelengths of 340 and 380 nm, and the 340/380 nm ratio was calculated to reflect the $[Ca^{2+}]_i$ changes.

Result

Nineteen *GJB1* mutations were identified in 24 patients with a clinical diagnosis of CMT. Six are novel mutations: p.L6S, p.I20F, p.I101Rfs*8, p.F153L, p.R215P and p.D278V (Figure 1; Table 1). Diverse pathological effects of the mutations were demonstrated, including intracellular mislocalization (Figure 2), reduced GJB1 expression (Figure 3) and altered GJ function (Figure 4). *GJB1* mutations that caused a complete loss of GJ Ca^{2+} permeability were associated with earlier disease onset and faster progression of symptoms, whereas those resulting in preservation of GJ permeability and with predominant cell membrane expression were associated with a later onset and more insidious disease course (Table 2).

Conclusion

This study demonstrated that the degree of loss of GJ function caused by the *GJB1* mutations was associated with the onset and progression of neuropathic symptoms in CMTX1.

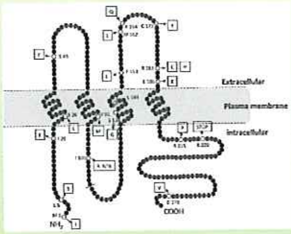

Figure 1. Schematic representations of the membrane topologies of GJB1 and the mutations identified in this study. The p.M1I, p.L6S and p.I20F mutations are in the N-terminal domain. The p.S26L, p.V91M, p.S138G, and p.L144del mutations are in the transmembrane domains. The p.I101Rfs*8 mutation leads to a premature truncation in the cytoplasmic loop. The p.S49Y mutation is located in the first extracellular loop. Further, the p.F153L, p.M162L, p.R164Q, p.C173Y, p.R183C, p.R183H, and p.E186K mutations are located in the second extracellular loop, and the p.R215P, p.R220X, and p.D278V mutations are located in the C-terminal domain.42

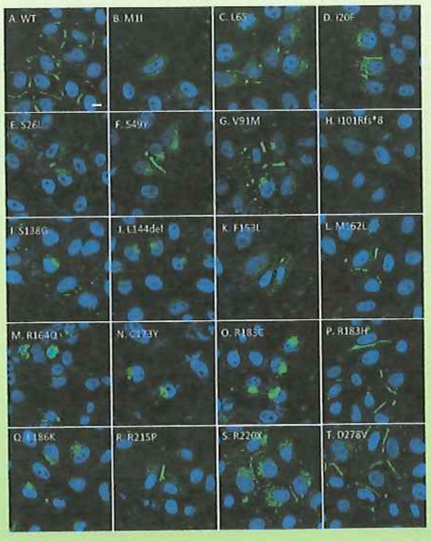

Figure 2. Immunolocalization of wild-type and mutant GJB1 in transfected HeLa cells. Numerous large gap junction plaques (GJPs) were observed in cells expressing wild-type GJB1 (A) and in those expressing the mutant M162L (L), R183H (P), or D278V (T). In cells expressing the I20F (D), S49Y (F), V91M (G), or R215P (R) GJB1 mutant, large-sized but lower amounts of GJPs were observed at intercellular boundaries with increased cytoplasmic GJB1-immunoreactive staining, and smaller GJPs were observed in cells expressing the L6S (C), S26L (E), S138G (I), F153L (K), R164Q (M), or R220X (S) mutant. In contrast, the mutants C173Y (N), R183C (O), E186K (Q), and L144del (J) were retained intracellularly and were incapable of forming detectable GJPs. M1I (B) and I101Rfs*8 (H) appeared to be localized to the cytoplasm, with very weak and diffuse staining patterns.

Table 2. Correlation of biophysical dysfunctions of the mutant GJB1 proteins and the clinical features of the patients with CMTX1

Biophysical dysfunctions			GJB1 mutant	Number of male patients	AN (yr)	AE (yr)	MNCV (m/s)	CMTNS	CMTNS (AE-AN)
A	Complete loss of GJP	Undetectable expression	M1I	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		Complete cytoplasmic protein retention with reduced expression	L144del, C173Y, R183C, E186K						
B	Preserved GJP with predominant cytoplasmic retention		L6S, I20F, S26L, S49Y, V91M, F153L, R164Q, R215P, R220X	13	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	Preserved GJP with predominant cell membrane expression		M162L, R183H, D278V	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
A vs B					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
A vs C					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B vs C					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

AN = age of onset; AE = age at examination; CMTNS = the CMT neuropathy score version 2 at AE; GJP = gap junction plaque; MNCV = median nerve motor nerve conduction velocity at AE. [illegible]

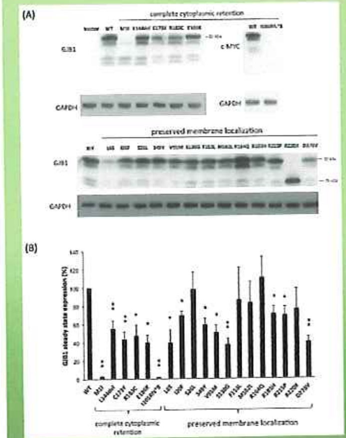

Figure 3. ***In vitro*** **expression of GJB1 in HeLa cells.** (A) Representative images from immunoblotting analyses of GJB1 expression in HeLa cells transfected with plasmids expressing wild-type (WT) or mutant GJB1, as indicated. Cells transfected with WT-GJB1 or the indicated non-truncated mutant showed a major band at 32 kDa, and the truncated mutant R220X produced a band at the expected size of 25 kDa. (B) Densitometric quantification of the GJB1 protein expression levels in cells transfected with plasmids expressing WT or mutant GJB1, as indicated. The GJB1 expression levels were normalized to GAPDH expression and were expressed as a fraction of the WT expression, which was set to 100%. The asterisk indicates a significant difference (*, $p < 0.05$; **, $p < 0.01$).

Acknowledgments

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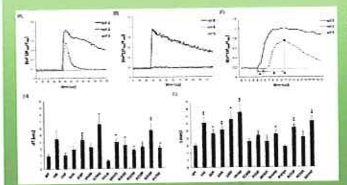

Figure 4. Quantitative measurement of intercellular Ca^{2+} signaling in HEK293T cells expressing wild-type and mutant GJB1 gap junctions. (A) Representative Ca^{2+} traces (fura-2 ratio, excitation at 340 nm and 380 nm) obtained from fura-2-loaded cells expressing wild-type GJB1 in response to mechanical stimulation. The intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in a stimulated cell (labelled cell 1), gap junction-coupled cell (labelled cell 2) and uncoupled cell (labelled cell 3) were plotted. (B) Representative Ca^{2+} traces obtained in response to mechanical stimulation of cells expressing the intracellularly trapped C173Y mutant. The stimulus induced an increase in the $[Ca^{2+}]_i$ in the stimulated cell (labelled cell 1), but this increase failed to transfer to neighbouring cells (labelled cells 2 and 3). (C) Parameters describing intercellular $[Ca^{2+}]_i$ fluxes across GJB1 gap junctions. Mechanical stimulation increased the $[Ca^{2+}]_i$ in the stimulated cell (cell 1, touched) and subsequently induced the amplitude of Ca^{2+} transients that spread to the adjacent gap junction-coupled cell (cell 2, follower cell). dT, the time delay in the onset of the $[Ca^{2+}]_i$ increase in the follower cells; and τ, the onset-to-peak duration in the follower cells. (D), (E) Comparisons of the average dT and τ between the wild-type GJB1 and mutants. The asterisk indicates a significant difference (*, $p < 0.05$; **, $p < 0.01$).

Table 1. Biophysical characteristics of mutant GJB1 proteins and clinical features of the patients with these *GJB1* mutations

GJB1 mutations			Biophysical characteristics of mutated proteins					Clinical features of patients							
Predicted domain	A.A. change	Nucleotide change	Localization	Expression	GJ Ca^{2+} permeability	dT	τ	Pat. no.	Sex	Initial symptom	AN (yr)	AE (yr)	CMTNS	CMAP (mV)	MNCV (m/s)
N-terminal	M1I*	c.3G>T	U	U	-	-	-	1	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	L6S*	c.17T>C	Mem, Cyt	↓	P	NL	↑	2	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	I20F*	c.58A>T	Mem, Cyt	↓	P	NL	↑	3	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
								4	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T	S26L	c.77C>T	Mem, Cyt	NL	P	NL	↑	5	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
								6	M	UW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
								7	F	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E-loop	S49Y	c.146C>A	Mem, Cyt	↓	P	NL	↑	8	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T	V91M	c.271G>A	Mem, Cyt	↓	P	NL	↑	9	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
								10	M	LN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C-loop	I101Rfs*8*	[illegible]	U	U	-	-	-	11	F	HNPP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T	S138G	c.412A>G	Mem, Cyt	↓	P	NL	NL	12	F	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	L144del	[illegible]	Cyt	↓	L	-	-	13	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E-loop	F153L*	c.457T>C	Mem, Cyt	NL	P	NL	NL	14	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	M162L	c.484A>T	Mem	NL	P	↑	NL	15	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	R164Q	c.491G>A	Mem, Cyt	NL	P	↑	↑	16	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	C173Y	c.518G>A	Cyt	↓	L	-	-	17	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	R183C	c.547C>T	Cyt	↓	L	-	-	18	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	R183H	c.548G>A	Mem	↓	P	NL	NL	19	M	FW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	E186K	c.556G>A	Cyt	↓	L	-	-	20	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C-terminal	R215P*	c.644G>C	Mem, Cyt	↓	P	NL	↑	21	M	LW	[illegible]	[illegible]	[illegible]	A	A
								22	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	R220X	c.658C>T	Mem, Cyt	NL	P	↑	NL	23	M	LW	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	D278V*	c.833A>T	Mem	↓	P	↑	↑	24	M	LN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

GJB1 **mutations:** A.A = amino acid; C-loop = cytoplasmic loop; E-loop = extracellular loop; T = Transmembrane; *First reported in patients with hereditary peripheral neuropathy.
Biophysical characteristics of mutated proteins: Cyt = cytoplasmic protein retention; GJ = gap junction; L = loss completely; Mem = protein expressed in membrane; NL = within normal limits; P = preserved; U = undetectable; dT = delayed onset of Ca++ increase in follower cells; τ = time from onset of Ca++ increase to peak in the follower cells; ↓ = significantly decreased protein expression level; ↑ = significantly prolonged time to peak Ca++; – not measured.
Clinical features of patients: A = absent evoked response; AN = age of onset; AE = age at examination; CMAP = median nerve compound motor action potential at AE; CMTNS = Charcot Marie-Tooth neuropathy score version 2 at AE; F = female; M = male; MNCV = median nerve motor nerve conduction velocity at AE; Pa No. = identify number for each patient; SD = standard deviation.
Initial symptoms: FW = distal four limbs weakness; LW = distal lower limbs weakness; LN = distal lower limbs numbness; UW = distal upper limbs weakness; UN = distal upper limbs numbness; HNPP = Hereditary neuropathy with liability to pressure palsy

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Zhang Y, Hao H. Conserved glycine at position 45 of major cochlear connexins constitutes a vital component of the Ca^{2+} sensor for gating of gap junction hemichannels. Biochem Biophys Res Commun 2013;436:424-9.

參加證明

Certificate of Attendance

This is to certify that

Yi-Chung Lee

from

Taipei Veterans General Hospital

has attended the

14th International Congress on Neuromuscular Diseases (ICNMD 2016)

which took place on July 4-9, 2016

at the Sheraton Centre Toronto Hotel, in Toronto, Ontario, Canada.

Dr. Vera Bril
Congress President, ICNMD 2016