

# 行政院國家科學委員會專題研究計畫成果報告

## 粒線體疾病表皮神經分佈的變化

### Epidermal Innervation in Mitochondrial Diseases

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#### 一、中文摘要

粒線體疾病的臨床表現十分多樣化，在神經系統方面可以出現中樞神經，周邊神經及肌肉的症狀。粒線體疾病患者的周邊神經病變在臨床及病理上都有很大的差異性，傳統上評估周邊神經的方法為使用神經傳導速度及腓腸神經的生檢，前者主要評估大口徑神經，後者侵襲性大。對於小口徑神經的病變常須需使用電子顯微鏡。皮膚具有許多感覺及自主神經的支配，特別可以成為評估小口徑神經的「窗口」。本計劃利用侵襲性很小的皮膚切片的方法來評估這群病患表皮中感覺神經的變化。患者有三人為 MELAS 徵候群，一人為 MERRF 徵候群，二人為 NARP 徵候群，一人為粒線體肌病變。我們在小腿外側踝上十公分作皮膚切片，而以免疫組織化學的方法，利用 protein gene product 9.5 這種抗體來標示表皮中的神經末梢，而以每 mm 表皮中的表皮神經分布來作定量分析。在疾病組其表皮神經密度為  $5.33 \pm 3.49/\text{mm}$ ，控制組為  $13.73 \pm 2.54/\text{mm}$ ，疾病組有顯著減少 ( $p < 0.01$ , Mann-Whitney test)。有三位患者臨床檢查、神經傳導、感覺閾值均正常，但表皮神經密度減低。有臨床神經病變的患者有較明顯的減低，其中最明顯的是一名合併有糖尿病的病人。表皮神經分佈的變化對於評估粒線體疾病的周邊神經病變可提供另一種評估的方式。

**關鍵詞：**粒線體疾病，周邊神經病變，皮膚切片，表皮內神經分布，免疫組織化學，

#### Abstract

The phenotypes of mitochondrial disease is extremely broad. The clinical manifestation of neuropathy varies from asymptomatic to mild to moderate sensorimotor symptoms. Both axonal and demyelinating polyneuropathy has been described. Traditionally, peripheral neuropathy is studied with electrodiagnostic test and sural nerve biopsy. Few objective measures are available for the assessment of small caliber sensory fibers. Skin has extensive sensory and autonomic innervation (terminal of C and A fibers), and represents an attractive site to assess small nerve fibers. In this study we apply skin biopsies to examine intracutaneous innervation in patients with mitochondrial diseases. There are 3 patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), 1 patient with myoclonus epilepsy with ragged red fibers (MERRF), and 2 patients with neurogenic weakness, ataxia, and retinitis pigmentosa (NARP), and one patient with mitochondrial myopathy without known mitochondrial DNA mutation. Skin punches are taken from the lateral aspect of the leg 10 cm above the lateral malleolus, and stained with a monoclonal antibody to neuron-specific ubiquitin hydrolase, PGP9.5. Epidermal innervation was quantified in 5 sections from each specimen and expressed as number of epidermal fibers per mm length of epidermis. There was significant reduction in epidermal innervation of

patients with mitochondrial diseases:  $5.33 \pm 3.49$  epidermal nerves/mm in patients compared to  $13.73 \pm 2.54$  epidermal nerves/mm in 20 age- and gender-matched controls ( $p < 0.01$ , Mann-Whitney test). The patients with clinical neuropathy have more significant reduction. Three patient without clinical and electrophysiological abnormality also demonstrate reduction. Skin biopsy offers a sensitive and minimally-invasive way to study neuropathy in mitochondrial diseases.

**Keywords:** mitochondrial diseases, neuropathy, skin biopsy, epidermal innervation, immunocytochemistry

## 二、緣由與目的

The phenotypes of mitochondrial disease are extremely broad. The clinical manifestations of neuropathy vary from asymptomatic to mild to moderate sensorimotor symptoms. Both axonal and demyelinating polyneuropathies have been described. Traditionally, peripheral neuropathy is studied with electrodiagnostic test and sural nerve biopsy. Few objective measures are available for the assessment of small-caliber sensory fibers. Skin has extensive sensory and autonomic innervation (terminal of C and A fibers), and represents an attractive site to assess small nerve fibers. In this study we applied skin biopsies to examine intracutaneous innervation in patients with mitochondrial diseases, with or without clinical symptoms of peripheral neuropathy.

## 三、結果與討論

### 結果:

There was significant reduction in epidermal innervation of patients with mitochondrial diseases:  $5.33 \pm 3.49$  epidermal nerves/mm in patients

compared to  $13.73 \pm 2.54$  epidermal nerves/mm in 20 age- and gender-matched controls ( $p < 0.01$ , Mann-Whitney test)

P't	MtDNA mutation	Clinical neuropathy	NCV	QST	END mean	SD
M/34	T8993G	N	SAP	normal	5.77	2.16
F/26	T8993G	N	SAP	normal	8.22	1.34
M/38	A8344G	Y	SAP	abnormal	1.70	0.96
F/53	A3243G	Y	SAP	abnormal	0.11	0.19
M/39	A3243G	N	normal	normal	4.22	1.07
F/59	A3243G	N	normal	normal	9.66	1.15
M/28	unknown	N	normal	normal	7.55	3.86

NCV: nerve conduction velocity

QST: quantitative sensory threshold

END: epidermal nerve density

### 討論:

1. Skin biopsies demonstrated significant reduction in epidermal nerve fiber densities in patients with mitochondrial diseases. This simple technique is a reliable methods for quantitation of small cutaneous sensory fibers.
2. Epidermal nerve density reduction was observed in patients without clinical signs of peripheral neuropathy. In three patients, neither routine NCV nor QST study showed abnormality.
3. The patients with clinical neuropathy have more significant reduction. Patient 4 has concomitant diabetes mellitus, which may play a part besides mitochondrial dysfunction.

## 四、計畫成果自評

The study is finished as scheduled. We will use this method to study more patients with mitochondrial diseases and other patients with neuropathy.

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