行政院國家科學委員會專題研究計畫成果報告

肢帶型肌肉失養症 Dystrophin 相關醣蛋白的免疫組織學研究

Immunohistochemical Study of Dystrophin Associated Glycoproteins in Limb-girdle Muscular Dystrophies

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一、中文摘要

本計劃針對二十四名診斷為肢帶型肌肉失養症及四名先天型肌肉失養症的患者，以免疫組織化學的分析(使用 Peroxidase -AntiPeroxidase 或 Immnofluorescence)，做進一步的分類。肢帶型的診斷包括了臨床、病理及肌縮蛋白(dystrophin)免疫染色，肌縮蛋白缺損者及基因找到突變者則排除之。 我們先使用 α-,β-,γ-δ sarcoglycan 抗體做免疫組織化學分析，所有的個案中並無發現有缺損者。懷疑先天性肌肉失養症我們再以 merosin 抗體染色亦無發現有缺損者。我們再以 dysferlin 抗體染色則發現有五名患者有顯著減少現象，故可診斷為 LBMD2B。這些患者的 CK 值均很高(>4000U/L)，其中一患者的姊姊以三好氏遠端肌肉病變(Miyoshi myopathy)為表現。免疫組織化學染色對於隱性肢帶型肌肉失養症的患者提供了進一步的分類，而台灣之患者可能大部分非因 sarcoglycan 的缺乏所引起。持續做更大規模的檢驗及運用基因診斷法將可對這些患者做更進一步的診斷。

關鍵詞：肢帶型肌肉失養症，先天型肌肉失養症，肌縮蛋白相關醣蛋白，免疫組織化學

Abstract

To differentiate autosomal recessive limb-girdle muscular dystrophies (LGMD) and congenital muscular dystrophies (CMD) we applied schematic immunostaining on muscle biopsies of 24 LGMD patients and 4 CMD patients with antibodies against **α** -, **β** - **γ** and **δ** - sarcoglycan. Immunostaining with merosin was also applied to patients with CMD. There was no reduced immunoreactivity in all the patients. Dysferlin immunostaining showed markedly reduced staining in 5 patients, thus leading to the diagnosis of LGMD2B. All these 5 patients had very high

CK level (>4000U/L) and various degree of distal weakness (besides proximal weakness). One patient's older sister had the clinical presentation of Miyoshi myopathy. In conclusion, LGMD due to sarcoglycan deficiency was not detected in our cases but 5 patients with LGMD2B were identified.

Keywords: Limb-girdle muscular dystrophy, congenital muscular dystrophy, dystrophin associated glycoprotein, merosin, sarcoglycan, dysferlin, immunohistochemistry

二、緣由與目的

肢帶型肌肉失養症(Limb-girdle muscular dystrophy, LGMD)**是一群包含有許多不同基因異常的肌肉退化疾病。其遺傳方式包括自體顯性**(LGMD1)**及自體隱性**(LGMD2)。LGMD2**目前可再分為**LGMD2A-H。近年來對於肌縮蛋白**(Dystrophin)**及其相關蛋白的研究，讓我們對於肢帶型肌肉失養症有了新的分類。其中有四型**Type 2C、 2D、2E、2F**就是因為**γ-, α-, β 及δ- sarcoglycan**的缺乏所引起的。**LGMD2A**為**calpain3 缺乏, LGMD2B**則為**dysferlin**缺乏。另外在先天型肌肉失養症中，近年來也發現有一群患者是細胞外基質中**Laminin2**的**alpha-2 chain (merosin)**缺乏所引起的。這些發現讓我們對這些疾病有了更進一步的分類。

本計劃將診斷為隱性肢帶型肌肉失養症及先天型肌肉失養症的患者進一步將肌肉切片做免疫組織化學的分析，利用這些蛋白質的抗體希望能對這些患者做更進一步的診斷及分類。

三、結果與討論

Results:

Totally 28 patients with autosomal recessive or sporadic LGMD were identified. Four biopsies with end- stage muscle were excluded for further immunostaining. Four patients with CMD were identified. All these biopsies have normal immunostaining of dystrophin.

Immunostaing of α-, β- γ and δ- sarcoglycan monoclonal antibodies (Novocastra) were all positive. Merosin (Chemicon) immunostaining were positive in patients with CMD.

Five biopsies showed markedly decreased immunostaining of dysferlin (gift from Kevin P. Campbell).

Discussion:

1.In Taiwan, diagnoses of limb-girdle dystrophy were often not specified. Differential diagnosis can be achieved by schematic immunostaining.

2.Recently γ-sarcoglycan deficiency were reported in two Taiwanese adults. In our series we did not find any adult onset LGMD with sarcoglycan deficiency. Sarcoglycan deficiency probably can only explain a small portion of adult LGMD patients but the majority are due to other types.

3.LGMD2B were identified in 5 of our patients, who all have very high CK levels (4910 to 30000U/L). In patients with very high CK level but with normal dystrophin, LGMD2B should be considered.

4. In patients with dysferlin deficiency, various degrees of distal weakness were observed. One patient showed marked posterior compartment weakness in legs. This patient could have been diagnosed as Miyoshi myopathy if examined in an earlier stage. Progression of Miyoshi myopathy can lead to significant proximal weakness and hence be diagnosed as limb-girdle muscular dystrophy. Careful examination of the distal parts may give us some hint.

5.Miyoshi myopathy and limb girdle muscular dystrophy can occur in the same family. Recent data indicate that there is phenotypic heterogeneity among different individuals with the same dysferlin gene mutation. Genetic study of Taiwanese family with MM may further delineate genotype-phenotype relationship.

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四、計畫成果自評

我們將近三年來神經科及小兒科診斷為隱性肢帶型失養症及先天性肌肉失養症的患者做了一初步的分類。我們希望能將此計劃繼續做下去，以便搜集更多台灣本土患者的資料。以目前的結果看來在台灣肢帶型肌肉失養症的患者可能大部份不是因為 sarcoglycan **的缺乏。雖然免疫染色是一種簡單而初步的分類法，對於其它型的診斷則必須使用其它的工具及基因分析。**

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