附件四

財團法人罕見疾病基金會九十八年度委託研究計畫

期末報告

計畫名稱：實驗室診斷台灣地區急性間斷性紫質症發病者盛行率

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一、**中文摘要**：請於一千五百字內就本計畫內容作一概述，並依本計畫性質自訂 3~5 個關鍵詞。摘要之內容應包括研究目的、研究方法、主要發現、結論及建議事項。

關鍵詞：急性間斷性紫質症、尿液 PBG 及 δ-ALA 濃度檢測、PBGD 酵素活性

急性間斷性紫質症(Acute intermittent porphyria, AIP)是一種染色體顯性遺傳為位於第 11 對染色體(11q24)上的一個與血基質(Heme)合成有關的酵素 porphobilinogen deaminase(PBGD)的基因突變所造成。跟據流行病學的統計，白種人約每萬至十萬人有一人帶有此突變基因，並且帶有此突變基因的人中每十人就有一位可能發病，常好發於年輕女性，而黃種人的盛行率較低。

此疾病的臨床症狀以復發性腹痛常見，常合併神經學症狀。目前此疾病診斷方式，除臨床特徵外，尿液靜放一段時間呈深紅色，實驗學檢測包括尿液定性篩檢(Watson-Schwarts test) ，24 小時尿液中 PBG 及 δ-ALA 濃度檢測，PBGD 酵素活性及分子基因突變檢驗等。這些檢查中，尿液定性篩檢效度低，酵素活性及分子基因突變檢測耗時耗費。

本研究方法，由醫師轉檢臨床疑似病患尿液和血液檢體。經由 24 小時尿液 PBG 及 δ-ALA 濃度快速檢測，而 24 小時尿中 PBG 及 δ-ALA 濃度可在收集完尿液後 1 工作天後獲得結果，配合 PBGD 酵素活性檢測。

經由此轉介平台，篩選 24 位臨床上復發性急性腹痛病患，其中六位 24 小時尿液 PBG 或 δ-ALA 值異常，配合 PBGD 酵素，活性有 4 位診斷為 AIP 病患，另外二位配合臨床表現為其他型急性肝性紫質症，另一位 24 小時尿液 PBG 和 δ-ALA 值正常而 PBGD 酵素異常，經回顧其以往病史和 24 小時尿液 PBG 和 δ-ALA 值，其多次紫質發作時皆明顯升高。

本研究結果顯示經由台灣西岸中部以北醫學中心轉送臨床疑似病患有三分之一(7/24)為急性肝性紫質症病患其中七分之五為急性間斷性紫質症病患。

此結果應可將此檢測方法推廣至台灣其他地區和中小型醫院。以幫助診斷此類病患。

二、**英文摘要**：請於一千五百字內就本計畫內容作一概述，並依本計畫性質自訂 3~5 個關鍵詞。摘要之內容應包括研究目的、研究方法、主要發現、結論及建議事項。

關鍵詞：Acute intermittent porphyria, urine PBG andδ-ALA level, PBGD activity

Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by a partial deficiency of porphobilinogen deaminase (PBGD) in heme biosynthesis. The responsible gene, PBGD, located at chromosome 11q23 encodes the enzyme, PBGD, the third enzyme in the cascade for heme production. The prevalence rate in Asian was lower than that in Caucasian population.

We enrolled 24 patients with recurrent abdominal pain, transferred from several medical centers of mid-western Taiwan. A rapid analysis for 24-hour urine porphobilinogen (PBG) and -aminolevulinic acid (ALA) level was done and correlated with the PBGD activity. Six patients had elevation of δ-aminolevulinic acid (ALA) and/or PBG in 24 hour-urine collection, including 4 patient being confirmed as a diagnosis of AIP incorrelation with PBG deaminase activity in red blood cell and 2 patient being suspected other form of hepatic porphyria according to clinical manifestation. One patient with AIP carrier had a normal limitation in detection the PBG and ALA level of 24-hr urine.

The study suggests that the hepatic porphyria, particular the AIP type, is not uncommon in Taiwan. The analysis of urinary ALA and PBG level is rapid and high sensitive tool in diagnosis of hepatic porphyria.

三、本文：

1. 研究背景及目的

急性間斷性紫質症(Acute intermittent porphyria, AIP) 是一種顯性遺傳，因為 porphobilinogen deaminase 基因突變造成血基質(heme)合成過程無法完成，導致血基質的前趨產物包括 δ-aminolevulinic acid (δ-ALA)及 prophobilinogen 堆積在人體組織。當急性發作時這些對身體有害的前趨物質快速昇高，並造成神經系統毒性反應，而這成嚴重臨床症狀，其中又以復發性急性嚴重腹痛最常見，病患常因此急診就醫，並轉介腸胃、婦產、一般外科、血液及腎臟等專科。此疾病又常因未能提早診斷而被醫師開立吡咯紫質類藥物（porphyrogenic agent）以緩解臨床症狀，而誘發更嚴重神經學症狀，甚至造成重覆性腹腔開刀等結果。

依據外國流行病學的統計，預估台灣地區約有 2000 位帶原者，其中約 200 位以上已發病。目前台灣地區經由實驗檢驗方法確定診斷為 AIP 病患不超過 50 位。根據林口長庚醫院及台大醫院研究顯示發病者的家屬常為帶因者。而 24 小時尿液的 δ-ALA 及 PBG 皆可快速檢驗出此病患及帶因者。此外對其他遺傳性肝性紫質症(hepatic porphyria)如遺傳性糞紫質症（Hereditary coproporphyria, HCP）、異位型紫質症(Variegate porphyria, VP) ALAD 缺乏紫質症(ALA dehydratase deficiency porphyria)等會引起急性神經學紫質症發作的病患，24 小時尿液 prophobilinogen 及 δ-ALA 濃度也可提供診斷的參考。

本研究的目的，希望提供國內各醫院對急性腹痛病患，疑似紫質病發作病患快速實驗室診斷工具，早期診斷，適當醫療介入，減少病患嚴重神經學症狀的產生。並進一步提供未發病家屬快速篩檢，經由適當衛教以避免發作，以有效降性醫療及社會成本。

2. 研究方法

Determination of PBG and ALA concentration

During porphyric attack, 24-hour urine specimens were collected by standard procedure, and adjusted 24-hour urine specimens to pH 6 with concentrated HCl. The container of urine specimen was protected from light. The concentrations of PBG and ALA in urine sample were measured by ion-exchange chromatography with the BioSystems ALA/ PBG Test (BioSysems S.A. Barcelona Spain). The 24-hour urine specimens were passed consecutively through two chromatographic columns that contain ionic exchange resins: the first one retains PBG, while the second retain ALA. Once the interfering substances were removed by washing, ALA and PBG were eluted and spectrophotometrically quantified by the absorbance at 555 nm of the Ehrlichs reaction product using a Beckman DU 500 spectrophotometer.

PBGD assay

Hemolysates were prepared by the addition of 4.95ml of Triton x-100 buffer to 50ul of RBC before the assay. To 0.5ml of hemolysate, 0.94 ml of Tris buffer was added. The reaction was started by adding 60ul of 3 mmol/L PBG substrate in Tris buffer. The blank tubes were prepared by adding 1 ml of Tris buffer without enzyme substrate (PBG) to 0.5ml of hemolysate for each sample. The reaction mixture was incubated in the dark at 37℃ for 1 hour and stopped by adding 1.5 ml of 25 g/dl trichloroacetic acid. After standing at a room temperature in the dark for 30 min and centrifugation for 10 min at 3000 rpm, the porphyrin formed in the reaction mixture was determined by measuring the fluorescence of the supernatant solution with an aminoco-Bowman spectrophoto-fluorometer (excitation 405nm, emission 596 nm), and using coproporphyrin as the standard. The PBGD activity was expressed as nmol porphyrin formed per hour per ml of RBC.

Molecular analysis

DNA was extracted from peripheral blood leukocytes following the standard methods. The 15 exons and their flanking intron sequences of HMBS gene were amplified using the polymerase chain reaction (PCR).

The promoter was also screened for a promoter mutation. The primer sequences were redesigned and will be available on request. PCR amplification was performed in a 50μl

reaction volume containing 50~100ng genomic DNA, 15pmol of each primer, 0.2mM dNTPs, 1u Taq DNA polymerase (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA) and 1x PCR buffer (Applied Biosystems). Amplification was achieved using the following protocol: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing with a 3°C touch-down to the appropriate temperature for each primer pair (i.e., a reduction of 0.1°C per cycle) for 30 sec (information available upon request), extension at 72°C for 45 sec, then another 10 cycles of further amplification with a final extension step at 72°C for 7 min.

Using the ABI Prism BigDye Terminator Cycle Sequencing Reaction Kit, the amplicons were then subjected to the automatic DNA sequencer ABI3100 (Applied Biosystems). Sequences were compiled and analyzed with the software VECTOR NTI (InforMax, North Bethesda, MD).

3. 研究結果

Table 1

No	Age/Sex	PBG deaminase 活性 (nmol/hr/ml RBC) Normal range:30.3-73.7	送檢單位	24 hrs. urine		Diagnosis
				δ-ALA mg/day (1.3-7.0)	porphobilinogen mg/day (0-2)	
1	51/ F	32.3	林口長庚	2.14	0.57	Non-hepatic porphyria
2	49/ F	15.2	林口長庚	6.72	1.42	AIP
3	50/ M	21.8	林口長庚	1.84	0.86	AIP
4	20/ F	32.7	林口長庚	3.04	0.24	Non-hepatic porphyria
5	16/ M	33.2	林口長庚	2.84	0.12	Non-hepatic porphyria
6	14/ F	23	林口長庚	2.47	0.14	AIP
7	49/ F	50.7	林口長庚	2.29	0.25	Non-hepatic porphyria
8	38/ F	47.7	林口長庚	1.53	0.21	Non-hepatic porphyria
9	30/ M	47.2	其他醫院-台中	15.66	22.23	hepatic porphyria
10	41/ F	17.1	林口長庚	6.52	7.02	AIP
11	29/ F	20.2	林口長庚	44.27	80.02	AIP
12	33/ F	x	其他醫院-新北市	3.73	0.56	Non-hepatic porphyria
13	59/ F	x	其他醫院-新北市	3.53	0.97	Non-hepatic porphyria
14	38/ F	35.1	其他醫院-新北市	2.46	0.36	Non-hepatic porphyria
15	16/ F	31.4	其他醫院-台中	2.21	0.95	Non-hepatic porphyria
16	31/ F	32.4	林口長庚	1.74	0.14	Non-hepatic porphyria
17	45/ F	44	其他醫院-新北市	0.41	2.21	Non-hepatic porphyria
18	16/ F	33.3	林口長庚	3.23	1.05	Non-hepatic porphyria
19	32/ F	30.5	其他縣市-台中	1.68	0.74	Non-hepatic porphyria
20	14/ F	39.1	林口長庚	1.98	0.48	Non-hepatic porphyria
21	22/ F	32.3	林口長庚	2.58	2.71	Non-hepatic porphyria
22	54/ F	48.4	其他醫院-雲林	0.22	0.52	Non-hepatic porphyria
23	47/ F	37.7	其他醫院-台中	58.74	157.47	hepatic porphyria
24	50/ F	x	其他醫院-台中	2.84	0.31	Non-hepatic porphyria

Table 2 abnormal 24 hours urine PBG and/or δ-ALA in hepatic porphyria

	Yes	No
Positive	6	0
Negative	0	0

4. 結論與建議：

結論：

1. 雖然樣本數小，但台灣地區急性復發性腹痛病患，特別是年輕女性，約有 1/3 病患可能是急性肝性紫質症。

2. 24 小時 urine PBG 和 δ-ALA 值，可在收集完檢體 1-3 天內，提早診斷急性肝性紫質症。

3. 24 小時 urine PBG 和 δ-ALA 值，對診斷 hepatic porphyria 有高的 sensitivity。

4. 台灣地區的 hepatic porphyria 中，有大部分為 AIP。

建議：

對於不明原因的急性復發性腹痛，可做 24 小時 PBG 和 δ-ALA 檢測，並考慮 hepatic porphyria 為其中一個鑑別診斷。

5. 參考文獻：

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