罕見疾病基金會第三屆博碩士獎助論文摘要

國立陽明大學遺傳學研究所碩士論文

華人丙酸血症之分子遺傳學研究

劉又寧

論文摘要

丙酸血症為體染色體隱性遺傳疾病 (MIM No.: 232000, 232050)，為先天性代謝異常疾病中常見之有機酸血症之一。患者臨床症狀之異質性 (clinical heterogeneity) 很高，常見的臨床症狀為 vomitting、hypotonia、neutropenia、ketosis 和 hyperammonemia 等，為丙醯輔酶 A 羧化酶缺失造成。丙醯輔酶 A 羧化酶是一粒線體酵素，將體內 propionyl-CoA 轉換成 D-methylmalonyl-CoA，若此酶發生機障則會導致丙酸在血液中大量堆積。丙醯輔酶 A 羧化酶由兩種次單體 α 及 β 蛋白鏈，構成 $\alpha_6\beta_6$ 的四級結構，α 及 β 蛋白鏈分別由 *PCCA* 及 *PCCB* 基因譯解出來，在 α 鏈上含有 biotin 與 ATP 的 binding site 及 biotin carboxylate domain，在 β 鏈上則有 carboxybiotin 與 propionyl-CoA 的 binding site。

本研究中分析 *PCCA* 基因的 24 exons 及 PCCB 基因上之 15 個 exons 之核酸序列，於 PCCA 基因發現 c.1601G/T 與 IVS18-31A/G 兩個 SNP，在正常華人族群中 c.1601G 與 c.1601T 的發生率分別為 0.98 與 0.02，heterozygosity 觀察值為 0.04，在族群中已趨於平衡 ($\chi^2 = 0.003$, $p > 0.05$)。IVS18-31A 與 IVS18-31G 的發生率分別為 0.53 與 0.47，heterozygosity 觀察值為 0.50 在族群中已趨於平衡 ($\chi^2 = 0.0003$, $p > 0.05$)。此外在 *PCCB* 基因中發現 IVS3+31T/C

一個 SNP，在正常華人族群中 IVS3+31T 與 IVS3+31C 的發生率分別為 0.85 與 0.15，heterozygosity 觀察值為 0.27 在族群中已趨於平衡 ($\chi^2 = 0.04$, $p > 0.05$)。

本研究中分析六個血緣關係丙酸血症家庭共七名華人丙酸血症患者之 *PCCA* 及 *PCCB* 基因，其中 PA001 家庭在 *PCCA* 與 *PCCB* 基因的轉碼區 (coding region) 中除上述 SNP 外均未發現核酸序列改變。在 PA009 家庭中除上述 SNP 外，於 *PCCB* 基因轉碼區中並未發現其他任何核酸序列改變，而於 *PCCA* 基因的一個 allele 中發現 c.1193C>T 的核酸序列改變，造成胺基酸序列 P398L 的變化，於 100 個正常華人 allele 未發現此 c.1193C>T 改變，而在日本的丙酸血症患者曾發現此變異。PA009 患者於 *PCCA* 基因上尚未確認之另一個突變，經 mRNA RT-PCR 及 Pre-mRNA 的研究顯示突變不是發生在 promoter 等基因調控區，可能是 mRNA 穩定性或 splicing error 的問題。其餘四名患者 PA002、PA005、PA008 與 PA010 家庭除上述 SNP 外，未於 *PCCA* 基因轉碼區中發現其他核酸序列改變，而於 *PCCB* 基因上發現 c.491C>T (A164V)、c.560_561delinsA (S187X)、c.580T>C (S194P)、c.601G>A (A221T)與 c.1301C>T (A434V)五種序列改變，皆為 PCCB 基因新發現的突變，此在 100 個正常華人 allele8 均未發現這些變異。綜合本研究結果顯示在華人丙酸血症患者所發現的 *PCCA* 基因的 c.1193C>T 及 *PCCB* 基因的 c.491C>T、c.560_561delinsA、c.580T>C、c.601G>A 與 c.1301C>T 可能為造成丙酸血症的致病突變。

本研究中利用微衛星標誌--D3S3528 與 D3S2453 分析研究中發現的 *PCCB* 基因上的 c.491C>T 與 c.1301C>T 突變在族群中是否有連鎖不平衡的情形。結果發現在北方/南方/香港次族群中 heterozygosity 的觀察值分別為 0.38/0.42/0.34 與 0.64/0.67/0.69。其中 D3S3528 在三個華人次族群中皆未達到平衡，而 D3S2453 在三個華人次族群中皆已趨近平衡。於香港華人丙酸血症

患者 *PCCB* 基因上發現的 c.491C>T 突變，與 D3S3528 的 272 bp allele 連鎖，佔 100% (2/2)，且與 D3S2453 的 316 bp allele 連鎖，佔 100% (2/2)，正常華人香港次族群中 D3S3528 272 bp allele 及 D3S2453 316 bp allele 分別只佔 3% (3/100)及 1% (1/102)。南方華人丙酸血症患者 *PCCB* 基因上共發現三個 c.1301C>T alleles，與 D3S3528 中與 270 bp allele 連鎖，佔 100% (3/3)，而於 D3S2453 中與 332 bp 和 336 bp allele 連鎖者，分別佔 33% (1/3) 和 67% (2/3)。正常華人南方次族群中 D3S3528 之 270 bp allele、D3S2453 的 332 bp 和 336 bp allele 分別只佔 11% (16/146)、9% (9/102) 和 2% (2/102)。研究結果顯示於香港華人丙酸血症患者*PCCB*基因上發現的c.491C>T突變及南方華人丙酸血症患者中發現的 c.1301C>T 突變可能有方舟效應 (founder effect)。

Abstract

Propionic acidemia (PA, MIM 232000, 232050) is a rare autosomal recessive metabolic error of propionic acid, the catabolism product of methionine, isoleucine, threonine and valine, odd-numbered chain length fatty acids and cholesterol. The disease is clinically very heterogeneous and characterized by recurrent metabolic ketoacidosis, vomiting, lethargy and hypotonia. It is caused by the deficiency of propionyl CoA carboxylase (PCC, EC 6.4.1.3), a biotin-dependent mitochondrial enzyme that catalyzes the carboxylation of propionyl-CoA to D-methylmalonyl-CoA. The PCC is composed of two types of subunits, an α subunit (74 kDa), containing the covalently attached biotin cofactor, and a β subunit (55 kDa), likely in an $\alpha_6\beta_6$ structure. The *PCCA* and *PCCB* gene, which encode the α and β subunits, have been mapped to chromosomes 13 and 3, respectively. Defect either in α or β subunit will cause PCC deficiency.

In this study, 24 exons of the *PCCA* gene and 15 exons of the *PCCB* gene, were PCR amplified and sequenced to analyze the mutations in Chinese PA families. One PA patient was identified to have c.1193C>T transition resulting in the replacement of Pro for Leu at codon 398 (P398L) in the *PCCA* gene. This c.1193C>T mutation had been reported in a Japanese PA patient. Five novel mutations, designated c.491C>T (A164V), c.560_561delinsA (S187X), c.580T>C (S194P), c.601G>A (A201T) and c.1301C>T (A434V) alteration, were identified in the *PCCB* gene of four PA patients. Two of these patients were homozygote of c.491C>T mutation and c.1301C>T mutation, respectively. All patients were born in a non-consanguineous family. No other mutation was detected in the coding region and exon/intron boundary of *PCCA* and *PCCB* gene for these 5 patients. All of these 6 variations identified in *PCCA* and *PCCB* gene were not detected in 100 Chinese normal alleles. These data indicated the c.1193C>T in the *PCCA gene* and the c.491C>T, c.560_561delinsA, c.580T>C, c.601G>A and c.1301C>T in the *PCCB* gene might be the disease causing mutations of PA.

Two STR markers, D3S3528 and D3S2453, were analyzed to study whether the transmission of c.491C>T and c.1301C>T transition of the PCCB gene identified in the Chinese PA families were linked disequilibrium. The heterozygosity of D3S3528 and D3S2453 were found to be 38% and 64% in Chinese population. The homozygous c.491C>T mutation found in one PA family was linked to the same 272bp allele of D3S3528. Three c.1301C>T alleles identified in two PA families were linked to the same 270bp allele of D3S3528. The 270bp and 272bp allele of D3S3528 were found to be less frequent in normal Chinese population (11.0% and 6.1%, respectively). These data suggested that the c.491C>T and c.1301C>T mutation in Chinese PA patients

might have founder effects.