行政院國家科學委員會專題研究計畫成果報告

題目(中)：21-羥　缺乏所致之先天性腎上腺增生的分子病變
題目(英)：Molecular pathology of congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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一、中文摘要

典型 21-**羥　缺乏所致之先天性腎上腺增生為孩童常見的內分泌疾病。本研究共分析來自** 57 **家庭的** 62 **病人，結果顯示常見的突變依序為** I172N(22.8%)**, 介入子** 2 **突變**(21.1%)**，脫失或大基因轉換**(14%)**及** R356W(8.8%)**。於一單純雄性化型患者，除了發現** I172N **突變外，於外介子** 9 **尚發現一新的突變。我們的結果亦證實基因型與表型間有很好的相關性。所有具無效的偶對基因者均為失鹽型患者, 具有內介子** 2 **患者中僅有一名為單純雄性化型，而所有具** I172N **患者均為單純雄性化型。**

關鍵詞：

Abstract

Classic congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency is a common endocrine disorder in children. In the present study, we genotyped 62 patients from 57 unrelated families. Our results showed that the most common mutation was I172N (22.8%), followed by intron 2 splicing mutation (21.1%), deletion or large gene conversion (LGC)(14%) and R356W(8.8%). One novel mutation in exon 9 was detected in a simple virilizer who also carried the I172N mutation. There was good correlation between genotype and phenotype. All patients with both null alleles are salt losers. Patients with intron 2 as the determining alleles are all salt losers except one simple virilizer. All patients carrying I172N are simple virilizer.

二、Introduction

Classic CAH due to 21-hydroxylase deficiency is a common disorder in children. Forty-nine mutations of the steroid 21-hydroxylase gene has been reported [1-3]. Although some discrepancies have been reported, the genotype generally correlates with the phenotype [4,5]. The mutation study of CAH patients in Taiwan has been reported [6-8]. However, the phenotype-genotype correlation has not been reported before. In the present study, we analyzed the molecular pathology of 62 classic CAH patients with allele-specific PCR and PCR-single strand conformation polymorphism (SSCP) in order to provide information for the rapid diagnosis in the suspected neonates and prenatal diagnosis in high-risk families.

Keywords: congenital adrenal hyperplasia
21-hydroxylase deficiency

二、Results

One hundred and fourteen chromosomes from 57 unrelated families were studied. As shown in Table 1, the most common mutation was I172N(22.8%), followed by In2(21.1%), deletion or large gene conversion(14%) and R356W(8.8%). One patient had uncharacterized bands of exon 9 on SSCP gel. Sequencing revealed an insertion of 41 bp from intron 6 (nt. No. 1520-1560) at codon 396 and deletion of 13bp from codon 396 to codon 420 of exon 9. The mutation causes a stop codon (TAG) 15 amino acid downstream the insertion site.

Table 1 Mutation frqeuencies on affected alleles

Mutation allele	Number of chromosomes	Percentage(%)
P30L	1	0.9
In2	24	21.1
P30L, In2, Ex3	7	6.1
In2, Ex3	7	6.1
I172N	26	22.8
P30L, In2, Ex3, I172N	1	0.9
Ex6 cluster	1	0.9
Q318X	5	4.4
R356W	10	8.8
Q318X, R356W	4	3.5
cdn483 GG→C	3	2.6
R483P	1	0.9
Ex9, insdels	1	0.9
Deletion or large conversion	16	14.0
Unknown	7	6.1
Total	114	100%

The correlation between the genotype and phenotype was also studied. All patients with null alleles were salt-loser. Patients with In2 as the determining allele were all salt losers except one simple virilizer. All patients carrying I172N were simple virilizers. Both the patients with the Cdn483 GG→C/R483P and Cdn483 GG→C/del or LGC were salt-loser. The patient with I172N/ex9, insertion and deletion mutation was a simple-virilizer. One patient with P30L, In2, Ex3, I172N/P30L mutation was a simple virilizer rather than the predicted nonclassic phenotype.

三、Discussion

Allele-specific PCR is a simple and rapid method to detect known mutations [9]. Using this approach, we found 101 chromosomes (among 114 chromosomes) carrying the 8 common mutations: P30L, IVS656(In2), Exon3 deletion (Ex3), I172N, Exon 6 cluster (Ex6), V28IL, Q318X, R356W. The mutation frequencies were I172N (22.8%), In2(21.1%), deletion or LGC(14%), and R356W(8.85), which was similar as the previous report [7]. We found 19 chromosomes(16.7%) having more than one mutation: In2, Ex3(6.1%), P30L, In2, Ex3(6.1%), P30L, In2, Ex3, I172N(0.9%), and Q318X, R356W(3.5%)(Table 1). All these mutations are located in tandem on the chromosome. This further supports that the mutations are caused by a conversion event involving a segment of the chromosome.

We detect a novel mutation in exon 9. A 13-bp fragment from codon 396 to codon 400 (G GTC TGG GAG AGG) was deleted and a 41-bp fragment (CACTCTGTACT-CCTCTCCCCAGGCCAGCCGCTCAGCC-CGCT) was inserted at the deleted region. The mutation was inherited from the mother. Because the inserted sequence is derived from the intron 6 (nt. No. 1520-1560), this may also be an event of unequal crossing over. The mutation generates a premature stop codon 15 amino acid downstream of the insertion. This should result in a non-functional P450c21 because the heme-binding domain (codon 421 to 440) is deleted [10]. However, the patient was a simple-virilizer because she also had an I172N mutation that has residual enzyme activity.

The correlation between phenotype and genotype of our patients was good except in two cases. One patient with the genotype of P30L, In2, Ex3, I172N/P30L was a simple virilizer rather than a nonclassic patient. We suspect that the promoter region of both alleles was also converted to the pseudogene sequence because we could not amplify the full-length of CYP21 using a specific primer (nt. no. -122 -103) annealing to the promoter sequence. It has been shown in *in vitro* expression studies that the transcription rate of the pseudogene promoter was three to five fold less efficient than CYP21 [11,12]. Reports of biochemical data of subjects with heterozygous or homozygous gene conversions in the promoter region suggest that these gene conversions in the promoter region could be related to mild nonclassic form of CAH [13]. Our simple-virilizing patient carrying both the conversion of promotoer region and P30L suggests that the function of 21-hydroxylase is further impaired as compared to each single mutation. In the genotype of In2/R356W, one patient was salt wasting and the other was a simple-virilizer. The phenotype variation of In2 in combination with a null allele has also been reported.

四、計劃成果自評

本研究內容達成原計劃預期的目標。將可提供小兒內分泌醫師處理懷疑先天性腎上腺增生患童時，確定診斷的工具，並可提供婦產科醫師對高危險性家庭孕婦的產前診斷、產前治療的根據。

本研究亦發現未曾見於國人的先天性腎上腺增生的基因突變及其臨床表徵，可提供給從事相關研究人員的參考。

本研究成果將於學術期刊發表。

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