台灣先天雙側無輸精管病人纖維囊腫基因之突變圖像 Mutation spectrum of the CFTR gene in Taiwanese patients with congenital bilateral absence of the vas deferens

中文摘要

先天性兩側無輸精管（congenital bilateral absence of vas deferens; CBAVD），是男性不孕症的原因之一，在歐美國家的報告中，約發生於 1~2% 的不孕男性。在高加索(Caucasian)民族常見的一種稱做纖維囊腫(cystic fibrosis, CF)的遺傳疾病中，發現絕大部分的個案都有 CBAVD 的臨床表徵。CF 是高加索民族中最爲常見的遺傳疾病之一，屬於體染色體隱性遺傳(autosomal recessive)，其發生率約爲 1:2,500 個活產嬰兒，而攜帶頻率(carrier frequency)約爲 1:25。纖維囊腫的病因爲纖維囊腫基因的突變造成。纖維囊腫基因於 1989 年被確認並定序，稱爲 cystic fibrosis transmembrane conductance regulator gene (CFTR)，位於第七對染色體的長臂上(q31~q32)，由 250Kb 的 DNA 構成，其所 encode 出來的 CFTR 蛋白質（1480 個氨基酸）形成分泌性上皮細胞上的氯離子通道(chloride channel)，調控氯離子進出細胞。纖維囊腫基因的變異使得 CFTR 蛋白質的功能失常，造成分泌性上皮細胞的氯離子濃度異常，而導致這些分泌性上皮細胞所形成的管腔閉鎖(atresia)或甚而發育不全。有超過 800 種的 CFTR 突變被發現，這些不同的基因突變決定了不同的生物表徵(phenotype)。

歐美國家的研究中顯示，60 – 90% 的 CBAVD 病人至少帶有一種 CFTR 基因的突變。另外一個在 CFTR 基因上位於 intron 8 3’端的 thymidine (T)鹽基的序列(ISV8-poly T)，也被發現與形成 CBAVD 有很大關聯。CF 在台灣屬於一種罕見疾病，但是在我們臨床執業中，卻也不乏 CBAVD 的病人前來尋求人工生殖的幫忙。爲了瞭解台灣 CBAVD 病人的 CFTR 突變情形，我們初步針對高加索民族常見的突變基因進行篩檢，結果顯示，27 位台灣 CBAVD 的病人，都沒有檢測出高加索民族所常見的∆F508 及 R117H 的突變，而 5T 出現的的頻率（44.4%）則比高加索民族高出許多。台灣在文獻上有報告的 CF 病例非常稀少，在最近的一篇文獻中，報告了兩個個案，發現了兩種新的突變型(E7X 和 989-992insA)。因此我們強烈地懷疑，台灣 CBAVD 病人的 CFTR 突變基因圖像(mutation spectrum)與高加索民族是不同的。有鑑於此，我們針對 36 位台灣 CBAVD 的病人，以溫度梯度膠體電泳（Temporal temperature gradient gel electrophoresis, TTGE）及基因定序（sequencing）的方法進行全面性的 CFTR 突變基因的篩檢。結果顯示，分別在五位病人的 DNA 定序中，發現到五種不同的 CFTR 基因突變：p.V201M、p.N287K、c.-8G>C (125G>C)、p.M469I 和 p.S895N。其中 p.N287K 發生在 CFTR 基因的第一組 transmembrane spanning domain，p.M469I 發生於第一組 ATP binding domain，以及 p.S895N 發生於第二組 transmembrane spanning domain，是新發現的 novel mutation 或是 polymorphism。除此之外，在 IVS8-Tn 的定序結果顯示有 7 位病人帶有 5T/5T，另有 7 位病人則是 5T/7T 的表現。因此，台灣 CBAVD 病人的 CFTR 基因突變的整體出現頻率爲(7x2+7+5)/72=36%。從這個結果看來，台灣的 CBAVD 病人所帶的 CFTR 突變基因圖像與高加索民族是截然不同的，CFTR 的突變也無法解釋大多數台灣 CBAVD

的成因，這與 CF 在亞洲／台灣的低發生率是一致的。

我們的研究，雖不至於牽涉到 CFTR 與 CBAVD 之間相關的機轉，但是針對 CBAVD 病人，乃至於他們的配偶所做的 CFTR 突變基因的篩檢，提供作爲不孕症遺傳諮商的參考，尤其在異國婚姻愈趨普遍的今天，是相當重要而且必要的。

英文摘要

Congenital bilateral absence of the vas deferens (CBAVD) is a relatively frequent cause of male infertility accounting for 1-2% of all cases. It is a common finding in males with cystic fibrosis (CF), and some, if not all, otherwise healthy men with CBAVD reflect a newly recognized, primarily genital phenotype of CF after genetic screening. CF, which is the most common autosomal recessive disorder in Caucasians, has an estimated frequency of one in 2,500, and one in 25 is carrier of the disease. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR gene, identified and cloned in 1989, contains 27 exons encompassing approximately 250 kb of DNA on chromosome 7q31.2. CFTR, a chloride channel protein, contains 1480 amino acids and regulates chloride ion influx/efflux across epithelial cell membrane. Mutations in the CFTR gene disrupt CFTR function, resulting in the changes of chloride concentration in the epithelial cells, leading to atresia or even hypoplasia of the lumen that formed by these epithelial cell. More than 800 disease-associated mutations have been discovered in the gene, resulting in variable phenotypes.

The study in Caucasian population showed that 60-90% infertile males with CBAVD carry mutations in CFTR. Besides, the poly-T tract in intron 8 (IVS8-Tn) of CFTR were also noted to be related with the formation of CBAVD. CF is a rare disease in Taiwan, nevertheless in our clinical practice, there are CBAVD patients visiting and searching for the help of artificial reproductive techniques. In order to understand the involvement of the CFTR gene, we first screened for the most common mutations of CFTR gene and looked for clinical correlations in 27 patients with clinically diagnosed CBAVD. The clinical results showed that none of the 27 patients had CF symptoms. We did not detect any definite renal anomaly ultrasonographically. No mutations of F508 or R117H were identified in any of the samples analyzed. In the screening of IVS8-Tn, the frequency of 5T alleles was 44.4%, which was significantly higher than in the 46 normal fertile males for which there was a 5T frequency of 5.4%. The 5T frequency of Taiwanese CBAVD patients was also much higher than that of Caucasian CBAVD patients. There was little CF case report in Taiwan, however in a recent document, two novel CF mutations (E7X

and 989-992insA) were reported. Therefore we strongly suspected that the mutation spectrum of CFTR mutation in Taiwanese patients with CBAVD is different from that of Caucasian population. In order to test the involvement of the CFTR gene in the etiology of Taiwanese male infertility, we have screened the entirety of the CFTR gene by TTGE (temporal temperature gradient gel electrophoresis) mutation analysis followed by direct DNA sequencing in 36 infertile males with the anomalies of the vas deferens. Five mutations: p.V201M, p.N287K, c.-8G>C (125G>C), p.M469I, and p.S895N, were found in five of the patients. p.N287K occurred in the first transmembrane spanning domain, p.M469I in the first ATP binding domain, and p.S895N in the second transmembrane spanning domain, were novel. In addition, 7 homozygous and 7 heterozygous 5T alleles in intron 8 polyT tract were found. The overall frequency of CFTR mutant alleles in Taiwanese CBAVD males was (7x2+7+5)/72=36% . Unlike the Caucasian patients, the CFTR mutations cannot account for the majority of Taiwanese CBAVD. This is consistent with the low incidence of CF in Asian/Taiwanese population. Furthermore the mutation spectrum of CFTR in CBAVD patients does not overlap with that of Caucasian CFTR mutation spectrum.

Our study, although not involved in the mechanism about the relationship between CFTR mutation and CBAVD, it is most important and necessary that comprehensive analysis of the CFTR gene in its entirety for both the infertile male and his partner is essential for those who are considered for the IVF procedure.