# 行政院國家科學委員會補助專題研究計畫成果報告

## 男性不孕症與DAZ基因之甲基化

計畫類別：■個別型計畫　　□整合型計畫
計畫編號：NSC89－2314－B－038－023
執行期間：88年08月01日至89年07月31日

計畫主持人：江漢聲
共同主持人：魏曉瑞
計畫參與人員：

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## 男性不孕症與 DAZ 基因之甲基化

## Methylation of DAZ Gene in Male Infertility

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主持人：江漢聲　台北醫學大學醫學研究所

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### 一、中文摘要

基因甲基化可影響基因表現，在目前 Y 染色體上有基因的缺損，以確知會影響精子之生成，然而對有些不明原因男性不孕症的病人雖其 Y 染色體上的基因序列正常，然而其基因表現是否與基因控制區域之甲基化有關。本篇著重以 Bisulfite P.C.R. 的方式來偵測基因的 methylation，並以 m-RNA 來檢驗基因表現並相互比較。

**關鍵詞**：Y 染色體、基因缺損、基因表現、基因甲基化

### Abstract

Y chromosome microdeletion is known to cause poor spermatogenesis. There are 10-15% male infertility patients who had Y gene microdeletion. However, still the etiology of most male infertility patients are unknown.

Methylation is now was studied in many genes especially in the protooncogens and was known to cause dysfunction of gene expression without gene derangment.

In this project we set up m-RNA in situ to detect gene expression. Bisulfite PCR is also developed to detect the gene methylation. We compare these patients' gene expression and DAZ gene promoter methylation.

**Keywords**: Y chromosome, gene microdeletion , gene expression, gene methylation

### 二、緣由與目的

男性不孕正在近年來已知與遺傳有極大的關連性，事實上，Cell Cycle Genes 以及 Signal transudation Gene 等等都可能與男性不孕症有關係。但有些基因與精子生成或與精子之功能及結構性有關係。

近來自從 Vogtt 等人在 Lancet 發表 Ygene Microdeletion 與 Spermatogenese 的相關性後，Y Chromosome 已跳脫過去只認為大多為 junk gene 的印象。約有 10-15%的 oligo 或 azoospermia 的生成與 Y chromosome microdeletion 有關。

然而仍有一大半的病人的 etiology 為無解。我們已知目前有許多 Gene 其序列完整但其表現確有差距。尤其在與 Cancer 有關之 protooncogen，而其基因之表現可能與基因控制區之甲基化（Methylation）有關，所以本篇研究計畫著重在男性不孕症的病人其 Y Gene 雖無缺損，但其表現是否與其基因的甲基化有相關性。

### 三、結果與討論

我們共收集了 339 例男性不孕症阻塞型病人除外的病人的 DNA，其中有 Azoospermia 的病人 215 位，Oligoasthenospermia 的病人共 86 位，每位病人除了以 PCR12 對 primers 來偵測有無 Yq11 microdeletion 之外亦均做了染色體檢查。

在染色體異常部分在無精症之病人中佔 17.7%，多半為 XXY。而在精蟲數少的病人中佔 8.1%。而前者病人中有基因缺損者約佔 9.3%，而後者則有 12.8%。我們除

了這些在染色上與 Y Gene 上有異常者尚有 263 位病人可以行 Bisulfite PCR。在無精症病人我們亦收集了他們的睪丸切片，行 m-RNA in situ hybridization 來偵測基因的表現量。由此我們可以確定是否由於基因之甲基化造成一些病人雖無基因之缺損但在基因的表現卻不足。

## 四、計畫成果自評

目前我們已有 DNA bank，而在著手建立 m-RNA in situ hybridization。我們用 nonradionsotop DIG-Labelled 之 C-DNA 來做 Detection，而我們試二種切片，一種為冰凍切片而另一種則為 Paraformldehyde 固定，經臘包埋後切片。

而 Bisulfite PCR 之建立已漸穩定，但人員仍在培訓中，而此二種技術完成後，我們的基因庫與收集的切片即可儘速完成我們的研究。

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