行政院國家科學委員會補助專題研究計畫成果報告

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執行單位：台大醫院小兒科

中 華 民 國 90 年 10 月 29 日

行政院國家科學委員會專題研究計畫成果報告
BSEP 及 FIC1 基因在新生兒肝炎及正常嬰兒之變異及表現
Genetic alterations of BSEP gene and FIC1 gene and its expression in neonatal hepatitis and normal infants

計畫編號：NSC 90-2314-B-002-401
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主持人：陳慧玲　台大醫院小兒部

一、中文摘要

背景：BSEP及FIC1基因異常近年來被發現是進行性家族性肝內膽汁滯留的致病原因，這些基因的變異在亞洲兒童並未被報告過，在前一年的研究中我們首先發現了台灣兒童之散發性進行性肝內膽汁滯留，約有80%是由BSEP及FIC1造成。本計畫針對新生兒肝炎病患，探討BSEP及FIC1gene的角色，同時了解在不同年齡，膽小管傳送蛋白的表現差異情形。以釐清BSEP及FIC1在嬰兒進一步的致病角色。

進行方法 :本計畫第第一部分部分探討新生兒特別容易產生黃疸的原因，可能是因發育上與成人有異而造成，因此利用胎兒，嬰兒，與成人的肝臟組織切片，以半定量RT-PCR法，比較在不同年齡發育上的差異，同時分析新生兒血液中膽酸濃度。第二部分對新生兒肝炎病人之cDNA做BSEP及FIC1 基因序列分析，以找出基因變異在新生兒肝炎之角色。

結果：本實驗發現 BSEP 及 FIC1 等肝細胞傳送蛋白的表現，在人類胚胎發育的第 16 周已有明顯表現，在 20 周以後到達明顯的程度，與胎兒開始分泌膽汁的時期配合。而在出生後的新生嬰兒，約在四個月到達最高程度，成人的表現量比胎兒低。第二部分測定新生兒肝炎全段序列，共完成五例 BSEP 及三例 FIC1 序列鑑定，均未發現 mutation。新生兒肝炎病例之 BSEP 免疫染色均為陽性，顯示在嬰兒兒肝內膽汁滯留中 BSEP 的表現為陽性或加強。

討論：本研究提供了在人類胎兒及新生兒至成人的肝內傳送蛋白的發育情形，證實 BSEP 等基因在胎兒已有表現，並且在出生後迅速增加。而在一般的新生兒肝炎病患，亦有 BSEP 的表現，而大多沒有 BSEP 的基因變異。

關鍵詞：膽汁滯留，基因變異，兒童肝病，胚胎發育

Abstract

Background/Aim: *FIC1 and BSEP* mutations have recently been found to cause progressive familial intrahepatic cholestasis (PFIC). Mutations in both genes have not been reported in Asian children. We had identified novel mutations in *FIC1* and *BSEP* genes in 80% of Taiwanese infants with sporadic progressive intrahepatic cholestasis. This study is to elucidate the different expression levels of BSEP and other canalicular transporters in different fetal and postnatal ages, and to determine the role of *FIC1* and *BSEP* mutations in children with neonatal hepatitis in Taiwan.

Methods and Results: In the first part we examined the expression levels of fetal, neonatal, and adult livers by semi-quantitative RT-PCR. We found that BSEP and other transporters were expressed as early as 16 weeks of gestational age, and became strongly expressed after 20 weeks. This finding is compatible with the time when bile starts to flow from liver to intestine. We also determined the serum bile acid levels in normal newborn infants and found that normal newborn have serum bile acid below 94μmole/L, much higher that adults, which was below 10 μmole/L. In the second part we sequenced the entire coding sequences of BSEP in 5 patients and FIC1 in 2 patients with neonatal hepatitis. We found no mutation in these patients. Immunohistochemical staining in the liver tissue of neonatal hepatitis patients showed positive results.

Discussion: Our study provided the first data on the expression levels of liver canalicular transporters in human fetal and postnatal periods. This data helps to elucidate the neonatal bile physiology. In addition, we found no evidence that genetic alternations occurred in benign neonatal hepatitis.

Keywords: cholestasis, gene mutation, childhood liver disease, fetal development

二、計畫緣由與目的

Backgaround

Intrahepatic cholestasis, resulted from inherited, metabolic, infectious, and toxic insults, is one of the most common and devastating liver disease in infants. Intrahepatic cholestasis within the first 2 months of age is common in Chinese infants. Evaluation of the cholestatic infants remains a difficult task, owing to the diversity of cholestatic syndromes and to the obscure pathogenesis of many of these disorders. Only about half of the cases have identifiable causes. Recently studies in the identification and characterization of genes that are mutated in inherited forms of cholestasis have progressed rapidly and provided the best opportunity to elucidate these processes of bile physiology. These forms of genetic diseases were termed progressive familial intrahepatic cholestasis (PFIC), which is a group of autosomal recessive disorders with jaundice onset in infancy. Patient progressed to early liver cirrhosis and hepatic failure before 10 years of age. One of the most important genes identified is the bile salt export pump (BSEP); its defects resulted in PFIC type 2. BSEP confers canalicular export of bile salts, the rate-limiting step of bile secretion. In addition, FIC1 and MDR3 gene, responsible for PFIC type 1 and 3, also have important physiological roles.

There has been no report of PFIC patients in Chinese children. Our previous studies have identified 27 patients with phenotypes of PFIC. Novel mutations in FIC1 and BSEP have been determined in some of the patients. PFIC accounts for about 15% in our patients with initially diagnosed neonatal hepatitis. It is apparent that PFIC and its genetic defects play important roles in our patients. Neonatal hepatitis, with the initial clinical and pathological presentation indistinguishable from PFIC, may represent milder form of genetic alterations of PFIC or dysfunction of canalicular proteins. In addition, newborns and infants are prone to cholestasis secondary to various insults such as drugs, sepsis, and ischemia. This may be due to the immature expression of canalicular transporters. We continue the works to elucidate the roles of genetic defects and regulations of BSEP and FIC1 in neonatal hepatitis and in normal developmental physiology.

三、結果與討論

Fetal and postnatal expression of canalicular transporters. We examined the expression levels canalicular transporters of fetal, neonatal, and adult livers by semi-quantitative RT-PCR. Primers deduced from coding sequences of *BSEP, FIC1, MDR3, MRP2, NTCP* were used. Human fetal livers were obtained from legal abortions with gestational weeks 14 to 23. Postnatal livers aged 1 month, 4 months, 1 year and from adult patients were obtained during surgery of non-cholestatic liver disease. We found that BSEP and other transporters were expressed as early as 16 weeks of gestational age, and became strongly expressed after 18 weeks. (Fig 1) This finding is compatible with the time when bile starts to flow from liver to intestine at about fetal age 20 weeks. After birth, transporters were expressed at lower level in the first few months and increased to higher lever at 4 months, and then decreased to a lower level in adults. (Fig 2) This was in contrast to previous belief that expression levels of canalicular transporters are highest in adults than in infants. Our finding indicate that after birth, expression of canalicular transporters increased rapidly to cope with the increased bile pool and bile flow. We also determined the serum bile acid levels in 100 normal infants aged below 1 year. We found that normal newborn have serum bile acid below 94 μmole/L, much higher that adults, which was below 10 μmole/L.

BSEP and FIC1 sequences in patients with neonatal hepatitis. Primers deduced form the entire coding sequences of BSEP and FIC1 were used. We sequenced the coding sequences of BSEP in 5 patients with and FIC1 in 2 patients with neonatal hepatitis. We found no mutation in these patients. Immunohistochemical staining using polyclonal antibody in the liver tissue of neonatal hepatitis patients showed positive results.

There has been no report in the expression levels of canalicular transporters in fetal and neonatal livers. Physiological studies have revealed that neonatal liver is immature in bile physiology. The bile acid pool is smaller, and serum bile acid is higher in neonatal liver. These factors contribute to the susceptibility of neonates and infants in cholestatic liver diseases. Animal studies suggested that neonates lack bile-salts independent bile flow, which was thought to be mediated by MRP2. The molecular mechanisms for the immature bile physiology are not known. Our study provided the first data on the expression levels of canalicular transporters in human fetal and postnatal livers. We found that BSEP, MDR3 and MRP2 expression was shown as early as 14~16 weeks, and became apparent in 20 weeks. These transporters are important in the bile secretion in human fetus. On the other hands, FIC1 and NTCP were not expressed as high as the above transporters. This data helps to elucidate the neonatal bile physiology.

In addition, we found no evidence that genetic alterations occurred in benign neonatal hepatitis. The results were limited by the small case numbers we have sequenced. Because there had no hot spot mutations in the BSEP and FIC1 genes, we have to do the complete sequence in coding region, which exceed 3 kb in both genes. More cases should be sequenced before we could draw a conclusion in this issue.

In conclusion, we found that expression of canalicular transporters was evident in human fetal liver before the bile flow started. After delivery, the neonatal liver has lower levels of canalicular transporters, and reaching higher levels before

weaning age. In sporadic neonatal hepatitis with benign course, genetic alterations in BSEP and FIC1 were not found.

四、計畫成果自評

The original aim of this study is accomplished. Our study elucidated important results on the development of fetal liver bile flow. This finding provide information for pediatricians to understand the fetal and neonatal bile physiology. Our study also established a genetic diagnosis method in neonatal hepatitis. The above findings have been submitted for publication.

五、參考文獻

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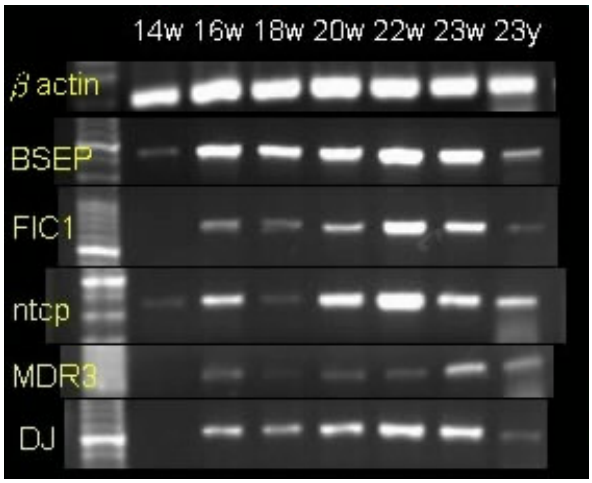

Fig. 1 Semiquantitative RT- PCR of canalicular transporters in the developmental fetal livers. (w: weeks of gestation, y; years of age)

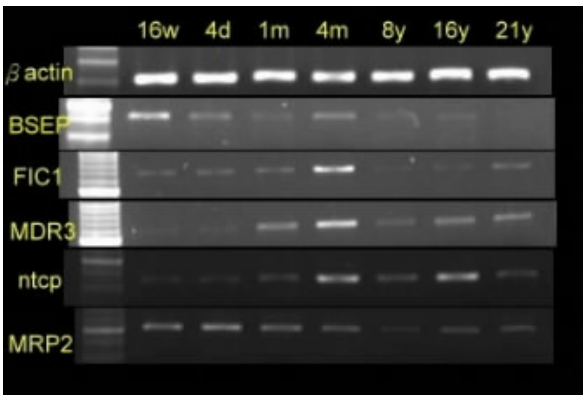

Fig 2. Semiquantitative RT- PCR of canalicular transporter in liver after birth. (w: weeks of fetal gestation; d, m, y: days, months, and years of postnatal age)