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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中　華　民　國　89　年　10　月　23　日

行政院國家科學委員會專題研究計畫成果報告
兒童之肝內膽汁滯留及 BSEP 基因分析
Intrahepatic Cholestasis in Children and the Role of BSEP gene

計畫編號：NSC 89-2314-B-002-040
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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, 本研究的目的在於釐清 BSEP 及 FIC1 基因在本地兒童慢性膽汁滯留的角色。
方法及結果：在 1980 至 1999 年，301 名肝內膽汁滯留病童中，共有 52 名慢性膽汁滯留，其中 31 名不明原因，其中四名經膽汁酸診斷為先天性膽汁酸合成缺陷，另外 27 名，在其中篩選出 18 名 GGT 值低下者，13 名進行 BSEP 及 FIC1 之基因變異分析。發現在 13 名病人中明顯的分為兩種表現型，第一組病人（五名）組織學只有膽汁滯留的變化，第二組病人（八名）組織學有明顯的巨細胞變化，在七名病人之基因分析中，第一組之四名均有 FIC1 基因突變，第二組之三名中，兩名有 BSEP 之突變，且這些突變均與西方國家之報告相異。並且發現 AFP 之值與基因變異有相關性。
結論：BSEP 及 FIC1 變異，以及膽酸合成缺陷，是本地兒童慢性肝內膽汁滯留重要的原因，結合 GGT 及 AFP 及組織學診斷，對於早期診斷這些基因變異有很大的幫助。

關鍵詞：膽汁滯留，基因變異，兒童肝病

Abstract

Background/Aim: Genetic defects of *FIC1 and BSEP* have recently been found to cause progressive familial intrahepatic cholestasis (PFIC). Mutations in both genes have not been reported in Asian children, and the roles of these genetic disorders in non-familial infantile cholestasis are unclear. This study is to elucidate the role of *FIC1* and *BSEP* mutations in children with infantile onset chronic cholestasis in Taiwan.
Methods and Results: We examed 301 children admitted for intrahepatic cholestasis during 1980 to 1999 in Taiwan. Fifty-two had chronic intrahepatic cholestasis, including 18 with Alagille syndrome, 2 with neonatal Dubin-Johnson syndrome, 1 with tyrosinemia, and 31 of unknown etiology. These 31 patients were sub-grouped according to γ-glutamyltranspeptidase (GGT) levels: low GGT (18 cases), high GGT (8 cases), and unknown GGT levels (5 cases). Inborn errors of bile acid synthesis were identified in four patients by serum bile acid analysis. In the low GGT patients, two distinct phenotypes could be defined: group 1 (5 patients) was characterized by bland cholestasis and group 2 (8 patients) by giant cell transformations. Group 2 patients were associated with higher transaminase levels, α-fetoprotein levels, and early mortality (p=0.02). Reverse transcription-polymerase chain reaction and cDNA sequencing were performed in 7 infants with low GGT levels for mutation analyses of *FIC1 and BSEP* genes. *FIC1* mutations were found in all 4 patients in group 1 but none in the 3 patients in group 2. Whereas *BSEP* mutations were found in 2 of the 3 patients in group 2. The *FIC1* mutations comprised of two deletions and three missense mutations. The *BSEP* mutations included two missense mutations and 1 deletion. All the mutations were novel.
Conclusion: FIC1, BSEP mutations and inborn errors of bile acid synthesis are important etiologies in infants with non-familial chronic intrahepatic cholestasis in Taiwan. Combination of serum GGT, AFP levels and histology is useful in differentiating patients of *FIC1* from *BSEP*

mutation, and the phenotype strongly correlated with genetic diagnosis and prognosis.

Keywords: cholestasis, gene mutation, childhood liver disease

二、計畫緣由與目的

Backgaround

Intrahepatic cholestasis of infancy consists of a diversity of diseases of different etiologies, including genetic syndromes, inborn errors of metabolism, infection, and toxic insults. Evaluation of these patients is a great challenge for pediatric gastroenterologist. Recent advances in molecular biology have elucidated the genetic basis for progressive familial intrahepatic cholestasis (PFIC) that was originally reported in Amish kindred. Patients were characterized to have early onset cholestasis, progressive liver cirrhosis and hepatic failure in the first or second decade. Three genes causing PFIC have been cloned. Genetic defects in *FIC1*, that mapps to chromosome 18q21 encodes a P-type ATPase, are mutated in patients of PFIC-1 (Byler's disease) and benign recurrent intrahepatic cholesatsis (BRIC). The *bile salt export pump* (*BSEP*) gene that maps to chromosome 2q24 is mutated in patients with PFIC-2. PFIC-1, 2 and BRIC are characteristic in low serum γ - glutamyltranspeptidase (GGT) levels. Mutations in *multidrug-resistant 3 (MDR3/PGY3)* gene are responsible for a distinct type of PFIC with high serum GGT levels.

FIC1 and *BSEP* mutations have been identified in Amish, European, and Arabian patients that were mapped to either chromosome 18q21 or 2q24. However, the frequencies of FIC1 and BSEP mutations in all the patients with PFIC is not clear. In a group of European patients with PFIC phenotype, BSEP mutations were found in 10 of 19 patients. It is also unknown whether these genes cause diseases other than PFIC and BRIC.

Genetic defects of *FIC1* and *BSEP* have not been reported in Asian children. Infantile cholestatic disease is common in Children in Taiwan and Asia. Our previous observations showed that 16% of the patients with neonatal hepatitis had a poor outcome resembling the course of PFIC. The etiologies remained cryptogenic. To elucidate the role *FIC1 and BSEP* in our patients, we studied the genetic alterations in a group of patients with low GGT levels, a characteristic feature for PFIC patients with *FIC1* or *BSEP* defects. We found novel mutations in the *FIC1* and *BSEP* in 86% of the seven cases examined.

三、結果與討論

Results

Phenotype characterization

A total of 52 children with chronic cholestasis were identified among the 301 patients of infantile intrahepatic cholestasis. Eighteen patients had Alagille syndrome, two had Dubin-Johnson syndrome with neonatal onset. Their clinical and pathological pictures had been reported. One patient had tyrosinemia. The remaining 31 patients were originally diagnosed as idiopathic chronic intrahepatic cholestasis. Four were diagnosed to have inborn errors of bile acid synthesis according to low serum bile acid levels.

Fourteen patients with low GGT levels were analyzed. Two phenotypes were identified. Group 1 included six patients with histology of bland cholestasis with minimal lobular disarray. Portal fibrosis was minimal to mild Bile ducts were normal or slightly reduced in number. Group 2 patients included eight patients with evident lobular disarray, marked giant cell transformation, or confluent cell necrosis accompanied by pericellular fibrosis. Group 1 patients had chronic or intermittent cholestasis and pruritus, with mild elevation of transaminase and bilirubin levels. The associated extrahepatic manifestations included diarrhea, rickets, and failure to thrive. Transmission electron microscopic examination was done in three patients. "Byler bile" was identified in two of them.

Group 2 patients had marked elevations of transaminase and bilirubin levels. Peak

bilirubin aspartate aminotransferase and alanine aminotransferase levels were higher than those in group 1 patients, p=0.02, p=0.001, and p=0.001, respectively. Pruritus and failure to thrive were frequent. One patient had chronic diarrhea. The disease progression was rapid. Five of the eight patients (62%) died before the age of 2 years.

Correlation of AFP levels with histology and survival. Serum AFP levels of the 14 patients correlated with their histological diagnosis. Patients with high AFP levels had significantly shorter survival time than those with normal AFP levels (p=0.01, log-rank test).

Genotype characterization

Analyses for FIC1 gene. Eight patients had frozen liver tissues available for analyses of *FIC1* gene alterations. These included 5 patients in group 1 (bland cholestasis) and 3 patients in group 2 (giant cell transformation). Four of the 5 group 1 patients had mutations in *FIC1* gene. Two patients showed homozygous missense mutations, one 886 C →T mutation (R296C) in case 2 and the other 2081 T→A mutation (I694N) in case 3. Both represented putative cytoplasmic domains of P-type ATPase. Case 1 showed heterozygous 73-bp deletion at nucleotide position 556~628 and a heterozygous nonsense mutation 3391 C→T (Q1131X). The deletions resulted in frameshift and protein truncation following codon 185, and an addition of 27 novel amino acids. A heterozygous 98-bp deletion at nucleotide position 185~282 was found in case 6, resulting in protein truncation at codon 62 (Fig.6).

Analyses for BSEP gene. cDNA sequencing was performed in 3 patients. A homozygous 850G → C(V284L), and a heterozygous 1-bp deletion at position 1145 was found in patient 15. The deletion resulted in frameshift after codon 382 followed by 15 novel amino acids and protein truncation. A homozygous missense mutation 3137 G→A (G1004D) was found in case 14.

All the above 6 patients who carried mutated *FIC1* or *BSEP* were Chinese descents. There were two aborigine patients of Polynesian ethnicity in group 2, but liver specimens were not available for genetic analysis.

Discussion

In our study, genetic cholestasis disease was found in 86% (6/7) infantile onset chronic intrahepatic cholestasis with low GGT levels. And all the mutations were different from that reported from the Amish, European, or Arabic descents. It is a significant finding since the frequency of genetic diseases can vary greatly in people of different ethnic background. For example, alpha-1-antitrypsin deficiency, tyrosinemia, and cystic fibrosis were rarely seen in Asian children whereas Alagille syndrome is frequent in our children. The finding prompt a specific direction of clinical awareness and research effort toward Asian patients of infantile cholestasis in the future. In the nomenclature, the diagnosis of specific genetic defect is preferred, since PFIC is often confusing in these patients without a family history.

We found that the phenotypic diagnosis, based on clinical, serologic, and histologic features, correlated very well with genetic changes in chronic cholestatic patients with serum low-GGT levels. Two groups of patients were identified. Group 1 was characterized by normal AFP level and bland cholestasis in histology, and high frequency of *FIC1* mutation (4 of 4 patients examined). In contrast, group 2 was characterized by high AFP level, giant cell transformation with lobular disarray, and high frequency of *BSEP* mutations (2 of 3 patients examined). Our results support the observations by Bull et al who had suggested different clinical and histological features in PFIC patients mapped to 18q21 and elsewhere. The former had bland intracanalicular cholestasis and coarsely granular bile. The latter, proved to be patients mapped to 2q24, had moderately to markedly elevated transaminase levels with cholestasis and background of "neonatal hepatitis" in histology. We suggest that phenotypic characterization is a simple method to identify non-familial patients suspected to have *FIC1* and *BSEP* mutations. Subsequent cDNA sequencing is useful to

confirm the genetic diagnosis, and serve as a basis for genetic counseling or future gene therapy.

Furthermore, the phenotypic classification has a prognostic significance. Group 2 patients, with giant cell transformation and high AFP levels, had a shorter survival time than group 1 patients. AFP is elevated in a variety of liver diseases in infancy and correlates with the severity of giant cell transformation.

The five mutations in *FIC1* detected from four patients and 3 *BSEP* mutations detected from two patients in our study were all novel. Two *FIC1* missense mutations (case 2 and 3), R296C and I694N, that occurred in the cytoplasmic domain, were similar to those of common Amish mutation G308V and common I661T mutation in patients from Faeroe Islands. No common mutations were shared among our patients, indicating that these mutations did not come from a common origin. This finding limit the development of rapid diagnostic methods in our patients.

In conclusion, genetic cholestatic disease is an important but under-recognized clinical entity in Asian countries. In non- familial cases of chronic cholestasis, combination of GGT and AFP provide clues for early diagnosis of *FIC1* and *BSEP* defects and may have prognostic significance. A clinical awareness and more aggressive treatment for these patients such as ursodeoxycholic acid, biliary diversion surgery, or preparation for liver transplantation will benefit these patients.

四、計畫成果自評

The original aim of this study is well accomplished. Our results elucidate that genetic defects are important causes in childhood chronic cholestatic liver diseases that were previously idiopathic. And this is the first reports of Asian children with *BSEP* and *FIC1* mutations. This finding provide great information in pediatricians to diagnose and treat our patients in this very difficult field, because previously many patients were diagnosed to be idiopathic. The diagnosis and further researches on the pathophysiology of these cholestatic liver disease may provide newer therapeutic approaches to these children. The above findings have been submitted for publication.