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P- Reviewer: Bradley EL, Neri V, Zerem E **S- Editor**: Wen LL **L- Editor**: A **E- Editor**: Wang CH

9. 代謝

Wilson病

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Wilson病の治療は，銅キレート薬（D-ペニシラミンまたは塩酸トリエンチン）あるいは亜鉛薬（酢酸亜鉛）の内服と低銅食療法である．銅キレート薬と酢酸亜鉛は併用も可能である．薬剤の選択は症例の臨床症状と重症度による．

診断のポイント

1. 臨床症状・所見 肝硬変，錐体外路症状ならびにKayser-Fleischer角膜輪が本症の三主徴である．幼児期以降の肝障害ならびに学童期以降に発症した神経症状をみたときには，本症を鑑別する必要がある．神経症状としては，構音障害，振戦ならびに歩行障害などが高い頻度でみられる．その他，精神症状（とくに思春期以降）や血尿などがみられることもある．

2. 臨床検査所見 特徴的生化学検査所見は，血清セルロプラスミン値の低下と尿中銅排泄量の増加である．しかし，血清セルロプラスミン値正常例が約5%の頻度で存在する点，4歳以下の年少例における尿中銅排泄量は正常対照群と比し有意差が認められない点に注意が必要である．多くの場合，血清銅値は低値であるが，溶血を伴う症例では高値となる．

3. 診断 血清セルロプラスミン値の低下と尿中銅排泄量の増加（100 μg/日，1.5 μg/kg/日または0.2 μg/mg creatinine）を認めれば本症と診断できる[1]．確定診断法としては，肝銅含量の測定がもっとも信頼性が高い．肝組織中の銅含量が200 μg/g wet tissueあるいは250 μg/g dry tissue以上であればWilson病と診断できる．

重症度評価

重症度判定には，肝障害の程度と神経障害の程度をそれぞれ評価する．肝障害は，肝酵素の上昇のみ，急性・慢性肝炎，代償性肝硬変，非代償性肝硬変，そして肝不全と重症化していく．これらのうちのどの状態であるかを評価する．最重症型は，意識障害と溶血を伴い急速に肝不全が進行する劇症肝炎型であり，全症例の4～7%にみられる．死亡する危険が高く，肝移植の適応となる．神経症状を呈する症例の場合は，症状の進行速度と治療への反応に注意が必要である．重症例では症状が急速かつ治療抵抗性に進行し，発症後1～3か月程度で寝たきり状態になることもある．とくに，神経症状としてジストニアが強い症例にその傾向がある．

基本病態

病態の中心は，肝臓から胆汁中への銅の排泄障害による肝細胞内への銅の蓄積である．また，肝細胞内における活性型（ホロ型）セルロプラスミン合成も障害される．蓄積した銅は，当初メタロチオネインと結合し（MT-Cu），無毒化されて貯蔵される．しかし貯蔵閾値を超えたとき，銅イオンとヒドロキシラジカルなどのフリーラジカルが出現し，スーパーオキシドジスムターゼ(super oxide dismustase：SOD)などの活性酸素消去能を超えると細胞障害を生じ，肝細胞壊死がもたらされる．さらに，血中に放出された銅（非セルロプラスミン銅）は全身諸臓器，とくに大脳基底部，角膜および腎臓などに蓄積し，それらの臓器障害をひきおこす．

治療の実際

薬物治療には，銅キレート薬による銅排泄促進あるいは亜鉛薬による銅吸収阻害の2種類の方法がある．これらは単独あるいは併用にて用いられる．また，銅の摂取を制限する低銅食療法も必要となる．銅キレート薬にはD-ペニシラミン（メタルカプターゼ®）と塩酸トリエンチン（メタライト®250）の2種類があり，亜鉛薬は酢酸亜鉛(ノベルジン®)がある．

1. D-ペニシラミン わが国におけるWilson病治療の第一選択薬である．投与方法は，15～25 mg/kg/日（最大量：1,400 mg/日）を食間空腹時に2～3回に分けて内服する．服薬時のポイントは，必ず空腹時（食前1時間もしくは食後2時間以上あけて）に内服させることである．本薬剤の最大の問題点は，副作用の出現頻度が20～25%と高いことである[2]．アレルギー反応などの場合はD-ペニシラミン使用を継続できることが多いが，自己免疫疾患の出現や骨髄抑制などの重篤な副作用が出現した場合には使

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用を断念せざるをえない．また，神経症状を有する症例に対しては，一過性にその神経症状を増悪させる可能性があるため注意が必要である．

2．塩酸トリエンチン D-ペニシラミンが副作用などにより使用できない例に用いられる．また，神経症状に対する治療効果が高いとの報告があるため，神経症状がみられる症例に対してははじめから使用することもある．投与方法は，40〜50 mg/kg/日（最大量：2,500 mg/日）をD-ペニシラミンと同様，食間空腹時に分2〜3にて内服する．本薬剤は副作用がほとんどないことが利点である．

3．酢酸亜鉛 わが国では2008年4月に販売が開始された，最新のWilson病治療薬である．投与方法は，成人（16歳以上）では75〜150 mg/日 分3，6〜15歳は75 mg/日 分3，そして5歳以下は50 mg/日 分2を食前1時間もしくは食後2時間に内服する（投与量はいずれも亜鉛として）．単剤での治療のみならず，銅キレート薬との併用も可能である．なお，銅キレート薬と亜鉛薬を併用する場合は，銅キレート薬と亜鉛が消化管内にて結合するのを防ぐため，服薬時間を最低でも1時間以上ずらす必要がある．

4．低銅食療法 薬物療法とともに，食事からの銅の摂取を制限する「低銅食療法」を行う．銅の摂取量は，治療開始時には1.0 mg/日（乳幼児は0.5 mg/日）以下に制限する．治療により症状や検査値が改善し安定すれば，やや制限をゆるめて1.5 mg/日まで摂取可能とする．なお，亜鉛製剤を内服しているときは，銅キレート薬のみにて治療を行っているときほど厳密な銅の摂取制限は必要ないと考えられている．

最新ガイドライン/エビデンス

日本先天代謝異常学会のホームページ（URL：http://square.umin.ac.jp/JSIMD/）に，「先天代謝異常症の診療指針」が掲載されている．Wilson病に関しても，主要症状および臨床所見，検査所見，診断基準ならびに鑑別診断が示されている．

近年のトピックス

現在のWilson病治療のkey drugは酢酸亜鉛（ノベルジン®）である．本薬剤は，発症前の症例と治療によってすでに症状と検査所見が安定している症例に対する治療効果が確認されている[3]．しかし，米国などでは亜鉛薬が第一次選択薬として幅広く用いられている[4]．**私の治療方針**で述べたように，明らかな肝障害があったとしても，本薬剤単剤で治療可能な症例が多く存在すると考えられる．

> **私の治療方針**
>
> Wilson病と診断された時点での臨床症状と重症度によって，初期治療に用いる薬剤を選択する．肝障害のみの場合は，代償性肝硬変までであれば酢酸亜鉛単剤にて治療を行う．非代償性肝硬変あるいはそれ以上の重症例は，塩酸トリエンチンと酢酸亜鉛の併用にて治療を開始する．神経症状がみられる場合は，（神経症状が）軽〜中等度であれば塩酸トリエンチン単剤で治療を行い，重度の場合は塩酸トリエンチンと酢酸亜鉛の併用を行う．発症前の症例に対しては，酢酸亜鉛を用いる．

ピットフォールと対策

銅キレート薬，亜鉛薬ともに，その内服時間と食事との関係には十分注意する．とくに，銅キレート薬を使用する場合は「食間空腹時内服」を厳守する必要がある．せっかく早期に診断され治療が開始されたにもかかわらず，銅キレート薬を食後に内服していたため，症状が悪化して不可逆的な障害を残してしまった症例が時折みられる．また，D-ペニシラミンの服用により神経症状の悪化をみたときには，D-ペニシラミンを減量するか，塩酸トリエンチンもしくは塩酸トリエンチンと酢酸亜鉛の併用に切り替えることが望ましい．

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Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2

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To examine the effects of 4-phenylbutyrate (4PB) therapy in a patient with progressive familial intrahepatic cholestasis type 2. A homozygous c.3692G>A (p.R1231Q) mutation was identified in *ABCB11*. In vitro studies showed that this mutation decreased the cell-surface expression of bile salt export pump (BSEP), but not its transport activity, and that 4PB treatment partially restored the decreased expression of BSEP. Therapy with 4PB had no beneficial effect for 1 month at 200 mg/kg/day and the next month at 350 mg/kg/day but partially restored BSEP expression at the canalicular membrane and significantly improved liver tests and pruritus at a dosage of 500 mg/kg/day. We conclude that 4PB therapy would have a therapeutic effect in patients with progressive familial intrahepatic cholestasis type 2 who retain transport activity of BSEP per se. *(J Pediatr 2014;164:1219-27).*

Progressive familial intrahepatic cholestasis type 2 (PFIC2), an inherited autosomal-recessive liver disease caused by mutations in *ABCB11* encoding the bile salt export pump (BSEP), is characterized by cholestasis and jaundice in the first year of life.[1] This disease progresses to severe cholestasis with sustained intractable itching, jaundice, diarrhea, and failure to thrive, leading to liver failure and death before adulthood. No medical therapy has been established for PFIC2.[1,2] BSEP is an adenosine triphosphate (ATP)-binding cassette transmembrane transporter located on the canalicular membrane of hepatocytes that mediates the biliary excretion of monovalent bile salts.[3-6] Therefore, in patients with PFIC2, biliary bile salt secretion is impaired, bile salts accumulate in hepatocytes, and consequently the hepatocytes are damaged.

We have published experimental evidence that 4-phenylbutyrate (4PB), a drug used to treat ornithine transcarbamylase deficiency (OTCD), has an additional pharmacologic effect to increase the hepatocanalicular expression of BSEP and the biliary excretion capacity of bile salts when given at a clinically relevant concentration in patients with OTCD.[7-9] Greater BSEP expression levels in liver specimens from patients with OTCD after 4PB therapy compared with before 4PB therapy suggest that 4PB treatment increases BSEP expression in humans.[10] These results indicate the possibility that 4PB may be a potential therapeutic compound for patients with PFIC2 who show a reduced BSEP expression at the canalicular membrane but who have retained transport activity of BSEP per se. To test this hypothesis, we investigated the effects of 4PB therapy in a patient with PFIC2 carrying a homozygous c.3692G>A (p.R1231Q) mutation in *ABCB11*.

Abbreviation	Definition
[^{3}H]-TC	[^{3}H]-Taurocholate
4PB	4-Phenylbutyrate
ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
ATP	Adenosine triphosphate
BSEP	Bile salt export pump
cDNA	Complementary DNA
D-Bil	Direct bilirubin
EV	Empty vector
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GGT	Gamma-glutamyl transferase
GPCR	G protein-coupled receptor
HA	Hemagglutinin
mRNA	Messenger RNA
OTCD	Ornithine transcarbamylase deficiency
PCR	Polymerase chain reaction
PFIC2	Progressive familial intrahepatic cholestasis type 2
P-gp	P-glycoprotein
qPCR	Quantitative polymerase chain reaction
T-Bil	Total bilirubin
TfR	Transferrin receptor
UDCA	Ursodeoxycholic acid
WT	Wild type

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Supported by a Grant-in-Aid for Young Scientists (B) (21790146) and Astellas Foundation for Research on Metabolic Disorders (to H.H.). H.H. and Y.S. have applied for a US patent on the effect of 4-phenylbutyrate on bile salt export pump (13/299 989). The other authors declare no conflicts of interest.

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Methods

We obtained approval for the study from the institutional ethics review boards. Informed consent was provided by the patient's parents before assessment because the patient was younger than 18 years of age. A detailed description of the materials and methods is presented in the Appendix (available at www.jpeds.com). All materials and methods used standard techniques and commercially available reagents.

The patient, a girl who is now 1 year of age, was born to parents of Pakistani descent. She developed hepatocellular cholestasis and jaundice with normal serum gamma-glutamyl transferase (GGT) activity at the age of 2 months and was diagnosed with PFIC2 by the presence of the c.3692G>A (p.R1231Q) mutation in both alleles of *ABCB11* and no detectable immunosignal for BSEP at the canalicular membrane of a liver section sample (Figure 1, A and B). Despite treatment with ursodeoxycholic acid (UDCA; 120 mg/day), she continued to have severe cholestasis with sustained intractable itch, jaundice, diarrhea, and failure to thrive. 4PB therapy was started at the age of 4 months. The administration of UDCA was maintained during and after the course of 4PB treatment.

Sequence Analysis of *ATP8B1* and *ABCB11*

Genomic DNA was isolated from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin), and all exons of *ATP8B1* and *ABCB11* and flanking intron–exon boundaries were amplified by polymerase chain reaction (PCR). Primer sequences and PCR conditions were designed according to previous reports.[11,12] Both strands were analyzed using BigDye Direct Cycle Sequencing Kit (Applied Biosystems, Foster City, California).

In Vitro Studies

pShuttle (Clontech, Palo Alto, California) containing complementary DNA (cDNA) of human BSEP with a hemagglutinin (HA) tag at the N-terminus (HA-BSEP$^{wild\ type\ [WT]}$) and that of HA-BSEP^{WT} with the c.3692G>A (p.R1231Q) or p.T1210P mutation (HA-BSEP^{R1231Q} and HA-BSEP^{T1210P}) were used for this study.[8] The c.3692G>A (p.R1231Q) and p.T1210P mutations were introduced into pShuttle containing HA-BSEP^{WT} cDNA by site-directed mutagenesis as described previously.[9] HEK293T cells and McA-RH7777 cells transfected with pShuttle containing HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P} cDNA, or empty vector (EV) (HA-BSEP^{WT}, HA-BSEP^{R1231Q}, HA-BSEP^{T1210P}, or EV HEK293T cells and HA-BSEP^{WT}, HA-BSEP^{R1231Q}, HA-BSEP^{T1210P}, or EV McA-RH7777 cells) were subjected to analysis of quantitative PCR (qPCR), cell surface biotinylation, immunofluorescence, and transport. All in vitro experiments were performed as described previously,[7,9] and a detailed description of the experiments is presented in the Appendix. The cells were treated with 1 mM 4PB for 24 hours before the in vitro experiments, which were designed to examine the effects of 4PB on HA-BSEP^{WT}, HA-BSEP^{R1231Q}, and HA-BSEP^{T1210P}.

Treatment of the Patient with PFIC2 with 4PB

Oral administration of 4PB (AMMONAPS; Swedish Orphan Inter AB, Stockholm, Sweden) was started at a daily dosage of 200 mg/kg/day divided into 4 doses a day. After 1 month, the dosage was increased to 350 mg/kg/day, which was maintained for an additional month. Because neither a therapeutic effect nor any side effects were observed, the dosage was increased up to 500 mg/kg/day, which is a clinically relevant dosage for OTCD, and this dosage was maintained for the next 4 months.

A liver biopsy sample was collected before and after the course of 4PB treatment. A part of the sample was preserved in RNAlater (QIAGEN, Hilden, Germany) for RNA preparation and stored at −20°C. Another portion was fixed in 4% formaldehyde at room temperature for histological analysis, and the remaining portion was snap-frozen in liquid nitrogen for preparation of membrane fractions and stored at −70°C in a deep freezer. Serum was collected before, during, and after the course of 4PB treatment. Liver tests were performed using standard methods immediately after collection, and the remaining specimens were preserved at −70°C for further analysis.

The severity of pruritus was scored according to a previous report[13]: 0, none; 1, mild scratching when undistracted; 2, active scratching without abrasion; 3, abrasions; or 4, cutaneous mutilation, with bleeding and scarring. Serum concentrations of total bile acids, histamine, and tryptase were measured by an enzyme cycling method (Wako Pure Chemicals, Osaka, Japan), a competitive enzyme immunoassay (Bertin Pharma, Montigny le Bretonneux, France), and a fluoroenzyme immunoassay (USCN Life Science Inc, Hubei, People's Republic of China), according to the manufacturer's instruction. The concentration and activity of autotaxin in serum were assessed using a specific two-site enzyme immunoassay and the measurement of choline liberation from the substrate lysophosphatidylcholine.[14]

Histologic Analysis of Human Liver Specimens

Liver biopsies were fixed in 10% formalin and embedded in paraffin. Then, 4-μm thick sections from the liver specimens were prepared by a Microm HM340E (Microm International GmbH, Walldorf, Germany), adhered to the glass coverslips, and subjected to hematoxylin and eosin staining and immunohistochemistry followed by microscopic analysis with an Olympus CX41 (Olympus, Tokyo, Japan) to evaluate the degree of cholestasis, giant cell transformation, inflammation in the liver tissues, and BSEP expression at the canalicular membrane.

Statistical Analyses

The data in the Figures are presented as the mean ± SE. The significance of differences between 2 variables and multiple variables was calculated at the 95% confidence level by Student *t* test and by one-way analysis of variance with Tukey test, respectively, using Prism software (GraphPad Software, Inc, La Jolla, California).

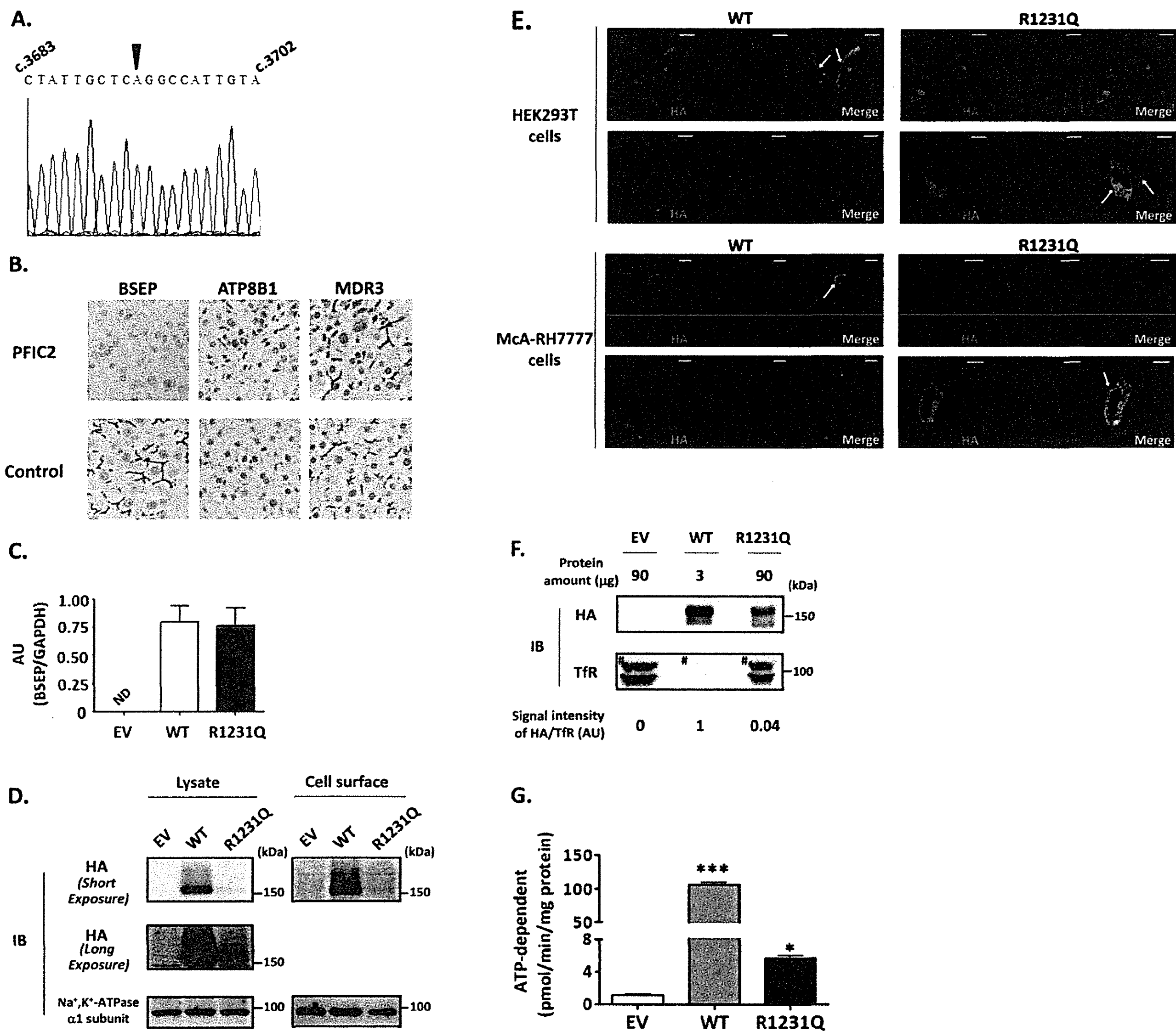

Figure 1. A, B, Diagnosis of PFIC2 in a patient who developed intrahepatic cholestasis with normal GGT activity. **A,** Genetic sequencing analysis. The homozygous c.3692G>A (p.R1231Q) mutation in *ABCB11* identified is shown by the *arrowhead*. **B,** Immunohistochemistry. Liver sections prepared from the liver biopsy specimens of the patient with PFIC2 and of control subjects were subjected to immunohistochemistry as described in the Methods. A typical image under each condition is shown. Original magnification, ×400. **C, D,** Determination of mRNA and protein expression levels of $BSEP^{R1231Q}$. EV, $HA\text{-}BSEP^{WT}$, or $HA\text{-}BSEP^{R1231Q}$ HEK293T cells were subjected to **C,** qPCR and **D,** cell surface biotinylation and then analyzed as described in the Methods. **E,** Cellular localization of $BSEP^{R1231Q}$. HEK293T cells (*top*) and McA-RH7777 cells (*bottom*) expressing $HA\text{-}BSEP^{WT}$ (WT; *left*) or $HA\text{-}BSEP^{R1231Q}$ (R1231Q, *right*) were analyzed by confocal immunofluorescence microscopy as described in the Methods. *Arrows* in the merged images indicate colocalization. *Scale bar*: 10 μm. **F, G,** $BSEP^{R1231Q}$-mediated uptake of [^{3}H]-TC. Membrane vesicles (**F,** 3 or 90 μg in and **G,** 10 μg in prepared from EV, $HA\text{-}BSEP^{WT}$, and $HA\text{-}BSEP^{R1231Q}$ HEK293T cells were subjected to **F,** immunoblotting and **G,** an uptake assay. In **C-G**, a representative result of 2-3 independent experiments is shown. Bars represent the mean ± SE of each experiment in triplicate. $^{*}P < .05$, $^{***}P < .001$, *AU*, arbitrary units; *IB*, immunoblotting; *ND*, not detected because of low expression levels.

Results

Progressive familial intrahepatic cholestasis type 1 is caused by mutations in *ATP8B1* encoding a membrane protein that is expressed on the canalicular membrane of hepatocytes and that translocates phosphatidylserine from the outer leaflet to the inner leaflet. Patients with progressive familial intrahepatic cholestasis type 1 present with similar clinical

symptoms and biologic variables as patients with PFIC2. Therefore, all encoding exons and flanking areas of both *ATP8B1* and *ABCB11* were sequenced, and a homozygous c.3692G>A (p.R1231Q) mutation in *ABCB11* was identified, which has been reported previously in European white patients with PFIC2 (Figure 1, A).[12] This result, combined with the immunosignal of ATP8B1, but not of BSEP at the canalicular membrane of liver sections (Figure 1, B), was the basis of the diagnosis of PFIC2.

To characterize the effect of the c.3692G>A (p.R1231Q) mutation on BSEP, HA-BSEP^{WT} and HA-BSEP^{R1231Q} were expressed ectopically in HEK293T cells and McA-RH7777 cells, a rat hepatoma cell line that develops canalicular membranes through the formation of couplets as hepatocytes. qPCR analysis showed that the BSEP messenger RNA (mRNA) expression level was similar between HA-BSEP^{WT} and HA-BSEP^{R1231Q} in HEK293T cells (Figure 1, C). In contrast, a cell-surface biotinylation study showed much lower cell surface expression of HA-BSEP^{R1231Q} than HA-BSEP^{WT} in HEK293T cells (Figure 1, D). Immunofluorescence analysis showed no colocalization of HA-BSEP^{R1231Q} with the plasma membrane marker, Na^+, K^+-ATPase $\alpha 1$ subunit, in HEK293T cells or with the canalicular membrane marker, MRP2, in McA-RH7777 cells, which was consistent with the results of immunohistochemistry (Figure 1, B and E). HA-BSEP^{R1231Q} was localized predominantly in the endoplasmic reticulum (Figure 1, E), suggesting that this mutation induces incomplete folding of BSEP molecules retained in the endoplasmic reticulum followed by proteasomal degradation, as has been reported for CFTRΔF508,[15] resulting in a decrease in HA-BSEP^{R1231Q} expression at the cell surface. The ATP-dependent uptake of [^{3}H]-taurocholate ([^{3}H]-TC), into membrane vesicles isolated from HA-BSEP^{WT} and HA-BSEP^{R1231Q} HEK293T cells was almost linear up to 5 minutes (data not shown). Its uptake value per minute was about 97- and 4.8-fold greater in vesicles from HA-BSEP^{WT} and HA-BSEP^{R1231Q} HEK293T cells, respectively, than in vesicles from EV-HEK293T cells (Figure 1, G). Normalizing the BSEP expression levels in the membrane vesicles based on the results of the immunoblotting, in which the expression of HA-BSEP^{WT} was 25-fold greater than that of HA-BSEP^{R1231Q} (Figure 1, F), showed that the transport activity of [^{3}H]-TC mediated per unit mass of HA-BSEP^{R1231Q} molecules did not differ significantly from that mediated by HA-BSEP^{WT}.

Effect of 4PB Treatment on the Cell-Surface Expression and Transport Capacity of HA-BSEP^{R1231Q}

qPCR analysis and the cell-surface biotinylation study demonstrated that 4PB treatment for 24 hours at 1 mM, a clinically relevant concentration in patients with OTCD,[7,16] increased the cell surface expression of HA-BSEP^{WT} and HA-BSEP^{R1231Q} by 2.2- and 2.0-fold, respectively, in HEK293T cells without significantly changing the expression level of each mRNA (Figure 2, A and B). The expression level of HA-BSEP^{WT} and HA-BSEP^{R1231Q} and of their mRNAs was evaluated after normalization by that of the transferrin receptor (TfR) and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, respectively, which were not affected by 4PB treatment (data not shown). Consistent with the cell surface biotinylation study, immunofluorescence analysis showed that HA-BSEP^{R1231Q} colocalized partially with the canalicular membrane marker, MRP2, in McA-RH7777 cells in the 4PB-treated condition (Figure 2, C). Treatment with 4PB also increased the expression of HA-BSEP^{WT} and HA-BSEP^{R1231Q} by 1.7 and 2.2 times, respectively, in membrane vesicles prepared from HEK293T cells expressing each form of BSEP (Figure 2, D). This was accompanied by an increase in the ATP-dependent uptake of [^{3}H]-TC. The HA-BSEP^{WT}- and HA-BSEP^{R1231Q}–mediated uptake of [^{3}H]-TC, which was calculated by subtracting the ATP-dependent uptake of [^{3}H]-TC into membrane vesicles of EV HEK293T cells from that of HA-BSEP^{WT} and HA-BSEP^{R1231Q} HEK293T cells, was increased by 1.6- and 2.0-fold, respectively (Figure 2, E). These results suggest that 4PB treatment at a clinically relevant dosage for humans could increase BSEP expression at the canalicular membrane in patients with PFIC2 with the c.3692G>A (p.R1231Q) mutation in *ABCB11* and, consequently, expand the capacity to secrete bile salt into bile.

Treatment with 4PB increased the cell surface expression of HA-BSEP^{R1231Q} in HEK293T cells cultured at 30°C to the same extent as in HEK293T cells cultured at 37°C (Figure 2, F). Considering that a low culture temperature around 30°C is capable of correcting the trafficking to the plasma membrane of the mutated misfolded protein,[7,17,18] this finding indicates that the process that modulates the trafficking of BSEP to the plasma membrane is unlikely to contribute to the mechanism underlying the increase in the cell surface expression of HA-BSEP^{R1231Q} with 4PB treatment.

Therapeutic Effect of 4PB in the Patient with PFIC2 with the c.3692G>A (p.R1231Q) Mutation in *ABCB11*

Serum liver tests and the itching score did not improve during the period of 4PB treatment at the dosages of 200 and 350 mg/kg/day. However, the serum level of aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) started to decrease when the dosage was increased to 500 mg/kg/day. Their values finally reached the range of 70-85 U/L, which is close to the reference range (AST, <55 U/L; ALT, <40 U/L; Figure 3, A). Consistent with the decrease in AST and ALT levels, the concentrations of total bilirubin (T-Bil) and direct bilirubin (D-Bil) decreased after the start of 4PB treatment at 500 mg/kg/day. One month after this dosage was started, both measurements were normalized to within the reference range (T-Bil, $<18\ \mu M$; D-Bil, $<5\ \mu M$; Figure 3, B). In contrast, the concentration of serum bile acids remained above the reference range ($<10\ \mu M$) during the period of 4PB treatment, probably as the result of the coadministration of UDCA. The itching score was reduced concomitantly with

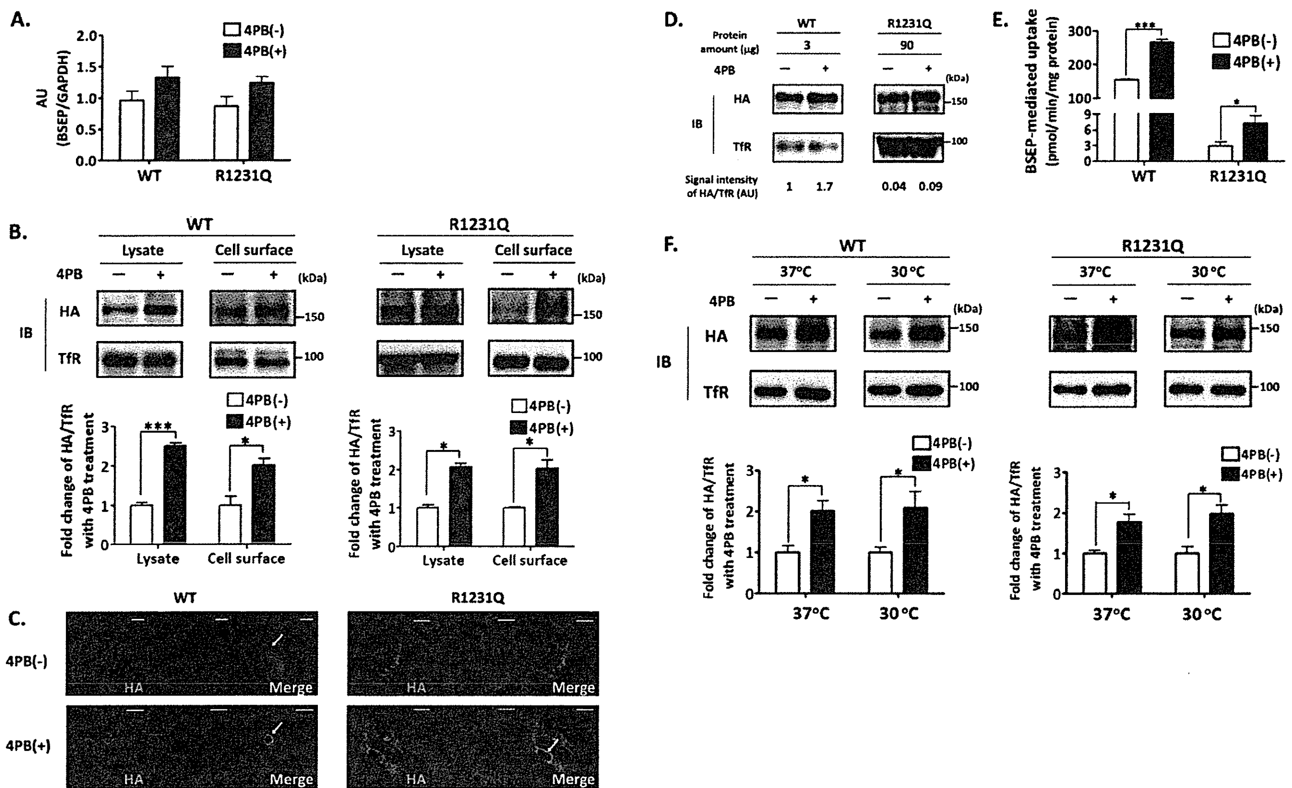

Figure 2. **A, B,** Effects of 4PB treatment on mRNA and protein expression levels of BSEP^{R1231Q}. HA-BSEP^{WT} (WT) and HA-BSEP^{R1231Q} (R1231Q) HEK293T cells were treated with or without 1 mM 4PB for 24 hour and subjected to **A,** qPCR and **B,** cell surface biotinylation and analyzed as described in Methods. **C,** Effect of 4PB on cellular localization of BSEP^{R1231Q}. McA-RH7777 cells expressing HA-BSEP^{WT} (WT, *left*) or HA-BSEP^{R1231Q} (R1231Q, *right*) were treated with or without 1 mM 4PB for 24 hours and then subjected to confocal immunofluorescence microscopy as described in **Figure 1, E.** *Arrows* in the merged images indicate co-localization. *Scale bar*: 10 μm. **D, E,** Effect of 4PB on transport of [^{3}H]-TC by BSEP^{R1231Q}. Membrane vesicles (**E,** 3 or 90 μg in and **D,** 10 μg in prepared from HA-BSEP^{WT} and HA-BSEP^{R1231Q} HEK293T cells after treatment with or without 1 mM 4PB were subjected to immunoblotting **D,** and an **E,** uptake assay as described in Effect of 4PB on BSEP^{R1231Q} expression at a low temperature. *Top*, HA-BSEP^{R1231Q} HEK293T cells were cultured for 24 hours at 37°C or 30°C in the presence or absence of 1 mM 4PB, biotinylated, and analyzed as described in the Methods. In **A-F,** a representative result from 2 or 3 independent experiments is shown. *Bars* represent the mean ± SE of each experiment in triplicate. $^*P < .05$, $^{***}P < .001$.

the improved results of liver tests (Figure 3, C-E). Almost complete and persistent relief of pruritus helped improve the child's ability to sleep and thus increased her quality of life. However, the itching score did not correlate significantly with the serum levels of autotaxin, bile acids, or autotaxin activity (Figure 3, C-E), all of which have been proposed as potential pruritogens in cholestasis.[19] No decrease in other possible pruritogens, the serum levels of histamine and tryptase, was observed as well (data not shown).

A liver biopsy was performed 4 months after the onset of 4PB therapy at the dosage of 500 mg/kg/day and studied with the specimens isolated for the diagnosis of PFIC2 before the enrollment in this intervention study. Histologic analysis showed that in the specimens obtained after 4PB therapy, giant cell transformation in the hepatic lobuli and the portal and lobular inflammation were relieved, compared with those before the therapy, and cholestasis almost disappeared (**Figure** 4, A). qPCR and immunoblot analysis demonstrated that in the patients with PFIC2, the liver *BSEP* mRNA expression before 4PB therapy in the patient with PFIC2 was almost the same as that in age-matched and adult control subjects, whereas the protein expression in the membrane fraction was approximately 2.5% of that in age-matched control subjects. 4PB therapy partly restored the reduced BSEP expression without increase in the mRNA expression, although the expression level of BSEP was still 12%-13% of that in age-matched control subjects (**Figure** 4, B and C). The specimens of the age-matched and adult control subjects were obtained from the pediatric patients without cholestasis when they underwent liver transplantation and the healthy organ donors. The expression level of BSEP mRNA and BSEP was evaluated after normalization by that of P-glycoprotein (P-gp)

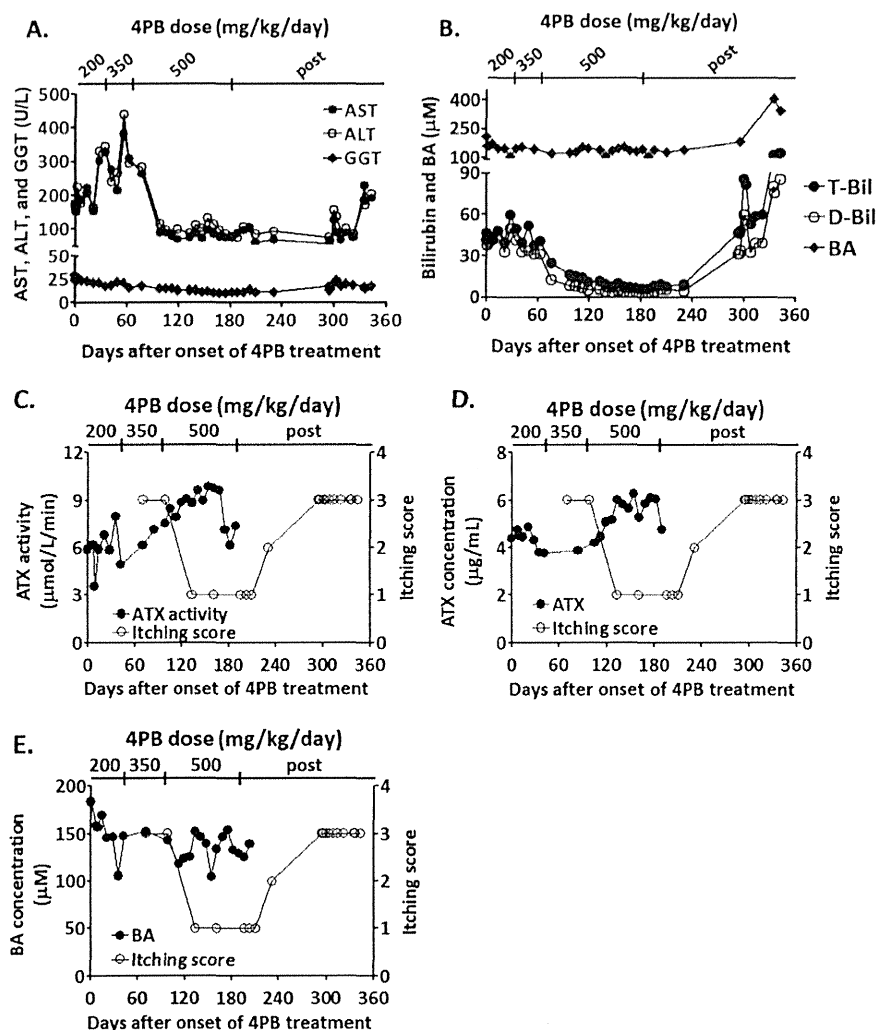

Figure 3. **A, B,** Levels of **A,** serum AST, ALT, GGT and **B,** T-Bil, D-Bil, and bile acid were monitored during and after the period of 4PB therapy. **C-E,** Correlation diagram of itching scores for the patient with PFIC2 with **C,** autotaxin activity and **D,** serum concentrations of autotaxin and **E,** bile acid in the patient during and after 4PB therapy. Pruritus severity was scored ranging from 0 (no pruritus) to 4 (cutaneous mutilation, with bleeding and scarring). *ATX*, autotoxin; *BA*, bile acid.

mRNA and of TfR, respectively, which were not affected by the treatment with 4PB.[7,10,16]

The liver tests and itching score remained unchanged 1.5 months after the end of 4PB therapy but gradually returned to values almost equal to those before her enrollment in this intervention study (**Figure 3**). During and up to 1.5 months after the end of 4PB therapy, the patient's height and weight continued to increase. No severe side effects were observed during or after 4PB therapy.

Discussion

PFIC2 is an autosomal-recessive, inherited liver disease resulting from mutations in *ABCB11*. Currently, partial external biliary diversion and liver transplantation are the most commonly used therapeutic procedures. Herein, we provide clinical evidence that 4PB therapy at a dosage of 500 mg/kg/day, a clinically relevant dosage used in patients with OTCD, biochemically and histologically improved liver functions and persistently relieved pruritus in a patient with PFIC2 who had decreased BSEP expression at the canalicular membrane but retained transport activity of BSEP per se. These findings suggest that 4PB therapy could help improve the quality of life of patients with this type of mutation in *ABCB11* and their parents. This beneficial effect of 4PB is supported further by our experimental evidence of the effect of 4PB on expression and transport activity of BSEP (**Figure 5**; available at www.jpeds.com). Gonzales et al[20] demonstrated that 4PB therapy improved liver tests in a patient with PFIC2 with a homozygous p.T1210P mutation in BSEP ($BSEP^{T1210P}$),[20] which markedly reduced BSEP

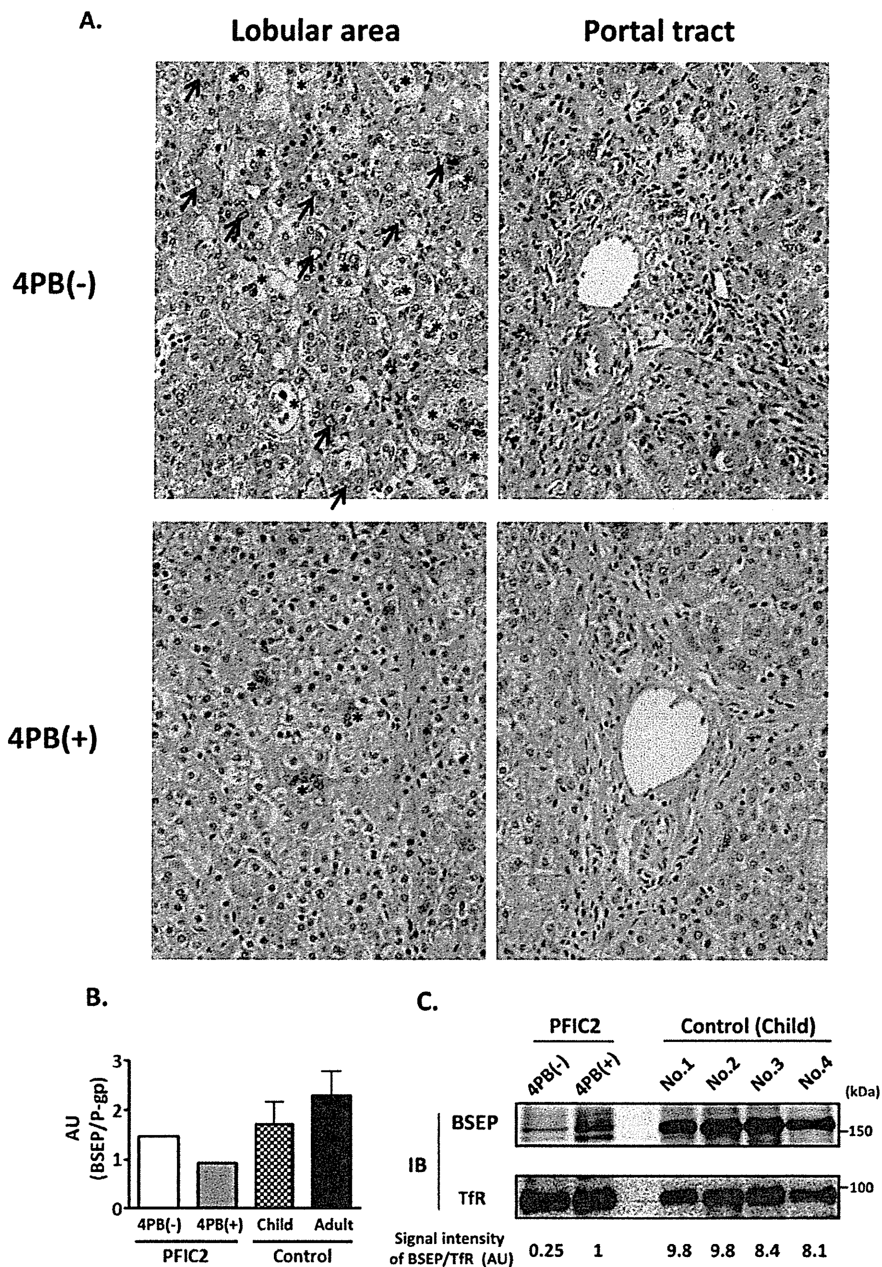

Figure 4. A, Histologic characteristics of liver sections. Liver sections prepared from the liver biopsy specimens of the patient with PFIC2 before and after the 4PB therapy were subjected to hematoxylin and eosin staining as described in the Methods. A typical image under each condition is shown. *Arrows* and *asterisks* indicate cholestasis and giant hepatocytes, respectively. Original magnification, ×200. **B, C,** mRNA and protein expression levels of BSEP. Liver biopsy specimens from the patient with PFIC2 before and after the 4PB therapy and from the control subjects were subjected to **B,** qPCR and **C,** immunoblotting and analyzed as described in the Methods. In **B,** the data were obtained from triplicate determination. Each *bar* represents the mean ± SE of individual specimens. *4PB(−)*, before 4 PB treatment; *4PB(+)*, after 4PB treatment.

expression at the canalicular membrane but did not abolish its transport activity (**Figure 5**).

The observed increase in BSEP expression after 4PB therapy in patients with PFIC2 with $BSEP^{R1231Q}$ or $BSEP^{T1210P230}$ suggests that the 4PB treatment restored biliary excretion of bile salts, relieved bile salt accumulation in hepatocytes, and thereby improved liver tests in these patients. The BSEP expression in the membrane fractions from liver specimens from the patient with PFIC2 with $BSEP^{R1231Q}$ and in the canalicular membrane of liver sections from the patient with PFIC2 with $BSEP^{T1210P}$ was still lower than that found in specimens of age-matched control patients even after treatment

with 4PB. It is conceivable that partial restoration of BSEP expression at the canalicular membrane is sufficient to fulfill the physiologic role of BSEP, leading to improvement in liver functions in the patients with PFIC2 with $BSEP^{R1231Q}$ or $BSEP^{T1210P}$. This finding is consistent with experimental evidence showing that liver pathology in mice with *mdr2* deficiency, which completely diminishes the biliary excretion of phosphatidylcholine, is markedly corrected by the relatively little expression of *MDR3*, a human orthologue of *mdr2*.[21] Alternatively, 4PB may have other as-yet-identified roles in combating intrahepatic cholestasis in addition to the increase in BSEP expression at the canalicular membrane.

We have provided experimental evidence suggesting that treatment with 4PB retards degradation of the canalicular membrane-resident forms of $BSEP^{WT}$, $BSEP^{E297G}$, and $BSEP^{D482G}$, the mutation found most frequently in patients with PFIC2, by interrupting the internalization process, which increases their expression at the canalicular membrane.[7,8,10] In this study, although we could not measure the degradation rate of cell surface-resident $HA\text{-}BSEP^{R1231Q}$ because of its low expression levels, treatment with 4PB had no significant effect on the level of mRNA expression of $BSEP^{R1231Q}$ and $BSEP^{T1210P}$ and was less likely to affect the trafficking to the plasma membrane of $BSEP^{R1231Q}$ (**Figure 2**, A, F; **Figure 4**, B; and **Figure 5**, A, E). Given that modulation of mRNA expression and trafficking to the plasma membrane is a mechanism that increases cell surface expression of both types of mutated BSEP, these results strongly suggest that degradation of $BSEP^{R1231Q}$ and $BSEP^{T1210P}$ trafficked to the canalicular membrane was inhibited with 4PB treatment, as is the case for $BSEP^{WT}$, $BSEP^{E297G}$, and $BSEP^{D482G}$, resulting in an increase in BSEP expression at the canalicular membrane in patients with PFIC2 with the respective mutations. Analysis using an in vitro minigene system has suggested that c.3692G>A (p.R1231Q) in *ABCB11* causes aberrant *ABCB11* splicing.[22] However, because BSEP was detected around 160 kDa, which is identical to the value in the control patients (**Figure 4**, C) and to the reported molecular weight,[9] it is likely that the correct splicing occurred to some degree in our patient despite having c.3692G>A (p.R1231Q) in *ABCB11* and its resultant protein product on the canalicular membrane was prevented from degradation by 4PB therapy. At present, we do not know the reason for the presence of some WT splicing in our patient, but this might be explained by the balance in the expression levels of splicing factors; for example, because the aberrant splice product associated with some missense mutation in *ABCB11* is abolished completely by transduction of SC35, but not of the other splicing factors assessed.[22]

In our patient with PFIC2, the pruritus disappeared concomitantly with the improvement in liver tests after the onset of 4PB therapy at 500 mg/kg/day. However, we observed no decrease in the factors suspected to be implicated in causing the itch (**Figure 3**, C-E). 4PB and/or its metabolites may modulate the local concentrations of these pruritogens, which may not have been detected by systemic measurements. The physiologic function of autotaxin, an enzyme secreted extracellularly that generates lysophosphatidic acid, is thought to be mediated predominantly by activation of G protein-coupled receptors (GPCRs).[23] TGR5, a GPCR activated by bile salts, on sensory nerves could contribute to bile salt-induced itching[24]; if so, 4PB and/or its metabolites might antagonize the GPCRs responsible for itch signaling and therefore attenuate activation of sensory neurons. Alternatively, 4PB therapy may affect unidentified pruritogens or antipruritogens.

In conclusion, our study demonstrated that 4PB therapy provides a favorable outcome—improvement in liver function and relief of refractory itching—in a patient with PFIC2 with decreased BSEP expression at the canalicular membrane but with retained BSEP transport activity. This therapeutic effect of 4PB and its safety should be validated by future clinical studies with more patients and for longer periods than was possible in this study. ■

We thank Larissa Kogleck for advice on the manuscript and Drs Sumito Ito and Tomoko Kito for experimental assistance (both from the University of Tokyo).

Submitted for publication Sep 4, 2013; last revision received Oct 31, 2013; accepted Dec 13, 2013.

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Appendix

4PB for the in vitro studies was purchased from Sigma-Aldrich (St Louis, Missouri). AMMONAPS, a pharmaceutical grade of 4PB for the treatment of a PFIC2 patient, was purchased from Swedish Orphan Inter AB. [^{3}H]-TC (2 Ci/mmol) was obtained from American Radiolabeled Chemicals (St Louis, Missouri). Anti-HA antibody was obtained from Roche Diagnostics (Mannheim, Germany). Antibodies against BSEP for immunoblotting, Na^+, K^+-ATPase α1 subunit, and calnexin were purchased from Abcam (Cambridge, United Kingdom). Antibodies against TfR and Alexa Fluor secondary antibodies were obtained from Invitrogen (Carlsbad, California). Anti-BSEP, ATP8B1, and MDR3 antibodies for immunohistochemistry were purchased from Santa Cruz Biotech (Dallas, Texas), Sigma-Aldrich, and Enzo Life Sciences (Plymouth Meeting, Pennsylvania), respectively. All other chemicals were of analytical grade.

Cell Culture. HEK293 T cells and McA-RH7777 cells were purchased from the American Type Culture Collection (ATCC number: CRL-11268 [HEK293 T] and CRL-1601 [McA-RH7777], Manassas, Virginia). HEK293 T cells and McA-RH7777 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2 and 95% humidity.

Measurement of BSEP mRNA Level. HEK293 T cells were seeded in 24-well plates at a density of 2.5 × 10^5 cells per well; transfected with pShuttle containing HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P} cDNA or EV using FuGENE HD (Promega); and treated with or without 1 mM 4PB for 24 hours. Total RNA was isolated using Isogen II (NIPPON GENE, Tokyo, Japan) according to the manufacturer's instructions. Total RNA from the liver specimens of humans was isolated using an RNeasy Mini Kit (QIAGEN).

Reverse transcription was performed using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. BSEP, P-gp, and GAPDH mRNA levels were measured by qPCR using a LightCycler instrument and the appropriate software (Ver. 3.53; Roche Diagnostics) as described previously.[1] qPCR was performed using the following primers: 5′-TGCCCAGTGC ATCATGTTTA-3′ and 5′-CCCTGGAAGTTGTCCCATTT-3′ (BSEP), 5′-GGCCAACATACATGCCTTCATCGAG-3′ and 5′-TGTCCAGGGCTTCTTGGACAACC-3′ (P-gp), and 5′-GGGGAGCCAAAAGGGTCATCATCT-3′ and 5′-GACGCC TGCTTCACCACCTTCTTG-3′ (GAPDH). *BSEP* gene expression in each reaction was normalized by the expression of *GAPDH* in HEK293T cells or *P-gp* in human liver specimens as appropriate.

Cell Surface Biotinylation. HEK293T cells were seeded at a density of 5.0 × 10^5 cells per well in 6-well plates coated with poly-L-lysine and poly-L-ornithine; transfected with pShuttle containing HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P} cDNA or EV using FuGENE HD; and treated with or without 1 mM 4PB for 24 hours. Forty-eight hours after the transfection, cell surface biotinylation was performed to investigate the expression on the plasma membrane as described previously.[2] The isolated biotinylated proteins were subjected to immunoblotting.

Preparation of and Transport Assays with Membrane Vesicles. HEK293T cells were seeded in a 15-cm dish at a density of 6.0 × 10^6 cells; transfected with pShuttle containing BSEP^{WT}, BSEP^{R1231Q}, or BSEP^{T1210P} cDNA or EV using FuGENE HD; and treated with or without 1 mM 4PB for 24 hours. The membrane vesicles were prepared 48 hours after transfection as described previously.[3] The isolated membrane vesicles were subjected to immunoblotting and transport assays. Transport assays were performed using the rapid filtration method reported previously.[3] Membrane vesicles were incubated at 37°C with [^{3}H]-TC (1 mM) for 5 min in the presence of 5 mmol/L ATP or AMP. ATP-dependent uptake of ligand was obtained by subtracting the value in the absence of ATP from that in its presence. To calculate BSEP-mediated uptake of [^{3}H]-TC, the value of its ATP-dependent uptake by the membrane vesicles of EV HEK293T cells was subtracted from that of HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P} HEK293T cells.

Preparation of Crude Membrane Fractions from Human Liver. Human liver specimens were homogenized in hypotonic buffer (1 mM EDTA, 5 mM sodium phosphate, pH 7.0) supplemented with protease inhibitor cocktail using QIAshredder (QIAGEN), and then centrifuged at 800 × *g* for 10 minutes at 4°C. The supernatant was ultracentrifuged at 100 000 × *g* for 1 hour at 4°C, and the pellet was dissolved in lysis buffer comprising hypotonic buffer with 1% Triton-X.

Immunoblotting. Specimens were loaded into each well of a 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis plate with a 3.75% stacking gel and subjected to immunoblotting as described previously.[2] Immunoreactivity was detected with an ECL Advance Western Blotting Detection Kit (Amersham Biosciences, Piscataway, New Jersey). The intensity of the band was quantified using Multi Gauge software (ver. 2.0; Fuji Film). Expression of BSEP and each form of HA-BSEP was normalized by the expression of TfR or Na^+, K^+-ATPase a1 subunit as appropriate.

Immunocytochemistry. HEK293T cells and McA-RH7777 cells were transfected with pShuttle containing HA-BSEP^{WT}, HA-BSEP^{R1231Q}, or HA-BSEP^{T1210P}; seeded on glass coverslips (Matsunami Glass Ind Ltd, Osaka, Japan) in 12-well plates at a density of 2.5 × 10^5 cells per well; and treated with or without 1 mM 4PB for 24 hours. The cells were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) for 10 minutes, permeabilized in 0.1% saponin/PBS for 10 minutes, blocked with 3% bovine serum

albumin/PBS for 30 minutes, and stained with anti-HA and anticalnexin (endoplasmic reticulum marker) or anti-Na^+, K^+-ATPase α1-subunit (HEK293T cells)/anti-MRP2 (McA-RH7777 cells; plasma membrane marker) for 2 hours. The cells were then stained with Alexa Fluor 488 donkey anti-rat immunoglobulin G and Alexa Fluor 546 donkey anti-rabbit immunoglobulin G or Alexa Fluor 546 donkey antimouse immunoglobulin G for 1 hour. These staining procedures were performed at room temperature. The cells were mounted onto glass slides with VECTASHIELD mounting medium (Vector Laboratories Inc, Burlingame, California) and then visualized by confocal microscopy using a Leica TCS SP5 II laser scanning confocal microscope (Leica, Solms, Germany).

Immunohistochemistry. Four-micrometer-thick sections embedded in paraffin were adhered to coated glass slides and stained with VENTANA BenchMark ULTRA fully automated staining instrument (Ventata Medical Systems, Inc, Tucson, Arizona) with anti-BSEP antibody and Leica BOND-III (Leica), the fully automated immunohistochemistry and in situ hybridization staining system, with antibody against MDR3 or ATP8B1 according to the manufacturer's instructions.

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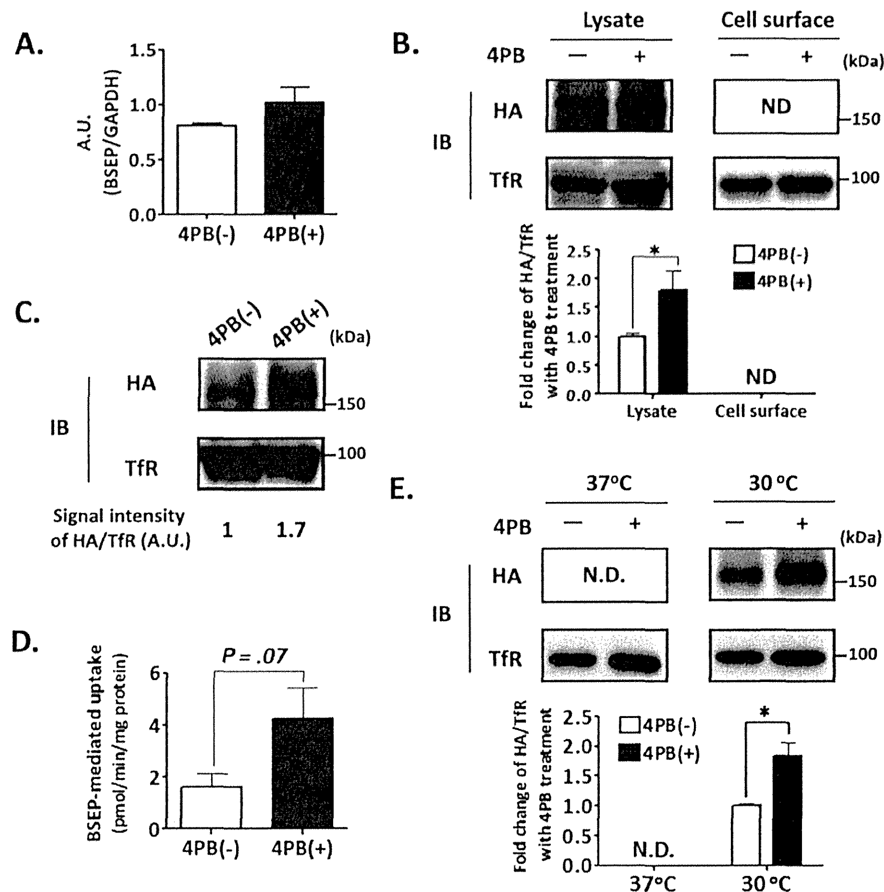

Figure 5. **A, B,** Effects of 4PB treatment on mRNA and protein expression of BSEP^{T1210P}. HA-BSEP^{T1210P} HEK293T cells were treated with or without 1 mM 4PB for 24 hours and subjected to **A,** qPCR and **B,** cell-surface biotinylation and then analyzed as described in **Figure 2**, A and B. **C, D,** Effect of 4PB on transport of [^{3}H]-TC by BSEP^{T1210P}. Membrane vesicles (**C,** 90 μg in and **D,** 10 μg in were prepared from HA-BSEP^{T1210P} HEK293T cells after treatment with or without 1 mM 4PB and were subjected to **C,** immunoblotting and **D,** the uptake assay as described in **Figure 2**, D and E. **E,** Effect of 4PB on BSEP^{T1210P} expression at a low temperature. Top, HA-BSEP^{T1210P} HEK293T cells were cultured for 24 hours at 37°C or 30°C, with or without 1 mM 4PB, biotinylated, and analyzed as described in **Figure 2**, F. In **A-E,** a representative result from 2 independent experiments is shown. *Bars* represent the mean ± SE of each experiment performed in triplicate. $^*P < .05$.

学術集会記録

第50回日本小児外科学会学術集会

会長講演

エビデンスのもたらすもの：臨床医学の発展と医療行政への発信

岩中　督[1]

I. はじめに

日本小児外科学会機関誌委員会から，第50回日本小児外科学会学術集会会長講演を機関誌に収載する旨の指示が届いた．本稿では，当日お話しさせていただいた内容から，自身の履歴，教室の歴史を削除し，さらに謝辞の一部も省略させていただいて，当日の講演部分を口語体でお届けする．また，当日使用したスライドを一部改変して図表として添付させていただくこととした．

II. 会長講演

皆様こんにちは．濵田吉則先生，過分なご紹介ありがとうございました．与えられております時間に制約がありますので，早速会長講演を始めたいと存じます．

今日私がお話しさせていただこうと考えておりますのは，手術データベースのお話です．今回の学術集会の主題は「エビデンスにもとづいた小児外科医療の継承：新たなる50年へのアプローチ」とさせていただきました．35年間，自分が小児外科医としてやってきた，臨床・研究・教育のお話をさせていただくのが本来の会長講演であるとは思っておりましたが，今回50回目の，半世紀の節目の学術集会を担当させていただき，自分の外科医としての集大成の経験を話すより，これからの50年へ向けて，これからの小児外科医に是非取り組んでいただきたい，と思っている臨床研究の一つのあり方についてお話しさせていただきます．

1. 我が国の小児外科臨床研究の現状

さて，まず我が国の小児外科領域の臨床研究の状況を俯瞰したいと思います（図1）．少子化のためにほとんどの疾患で個々の患者数が減っていることは間違いありません．一方，小児外科学会の認定施設や教育関連施設は多く，狭い国土であっても施設の棲み分けや集約は進んでいません．規模の小さな施設でも，重症の外科疾患を扱うことは日常行われています．それゆえ，小児外科医の労務環境が悪く，診療が精一杯で臨床研究を行うゆとりがありません．また個々の施設の患者数が少なく，前向きの臨床研究はほとんど行うことができません．このような現状を打破し，我が国から最新の臨床研究を発信するためには，多施設共同研究を行う，前向きコホートの臨床研究を何とか工夫する必要があるわけですが，その方法論として最も簡便なのが，大規模臨床データベースをうまく活用する，という方法です．

2. National Clinical Database の構築

2010年に日本外科学会が基盤となって立ち上げた手術症例データベース，いわゆるNational Clinical Databaseで，何を知ることができて，その結果何ができるか，を今日は述べたいと考えます（図2）．まず，少数の入力項目で，我が国全体の外科医療の把握，特に手術の実施状況や専門医制度の現状を知ることができます．さらに入力情報を積み上げると，各施設や個々の医師の医療水準評価，すなわちベンチマーキングができ，その結果を各施設へフィードバックできるようになります．さらにそのデータベース上に介入研究を積み上げることで，様々な前向きの臨床研究が可能となります[1]．

このデータベースの仕組みをシェーマにしますと，このスライドのようになります（図3）[2]．1階部分が，日本外科学会が中心になって分析する基本的な外科診療情報です．各学会がそれぞれ内容を決めて登録する2階部分で，各学会が知りたいと考えている様々な情報を知り得るだけでなく，個々の施設や医師に医療水準をフィードバックします．3階部分はその上に積み上げた臨床研究部分を示します．RCTに近い介入研究，がん登録，領域横断的な臨床研究も可能です．小児外科領域は現在までに中2階くらいのデータ集積しかしていませんので，本日は今の小児外科のデータ集積から何ができるか，他の領域ではどのようなことをしているか，小児外科領域が同じことをしたいのであれば，今より何をしなければならないのか，をご紹介したいと思います[3-5]．

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登録が開始された2011年のデータを2012年4月に締め切りました．日本外科学会系のすべての領域で，約5,000診療科が参加し，16,000名が入力のための登録をして，133万件のデータを集めました（図4）．2012年のデータも加えますと，今年の4月の時点で267万件の情報が集積されました．皆さんご存じのように，このデータベースでは，関係するすべての学会の医師の情報を，医籍番号を使って1箇所に統合しましたので，どの手術をどの専門医が行っているかどうかすべてわかります．これほど大規模に，専門医制度と連動した臨床データベースは国内外において前例はありません．

図5は，2011年の小児外科手術の一覧です．このデータベースは悉皆性を求め全数把握に努めていますので，新生児疾患などは，5年に一度の学会が行う集計よりやや多めの症例が登録されています．ただ，再手術なども含まれていますので，患者数とは一致しません．あくまでも手術数となります．

図6は，2011年の小児手術の一部を示しています．食道閉鎖症はほぼ全例に小児外科医が関わっていますが，鼠径ヘルニアでは約7割，急性虫垂炎に至っては，小児外科医以外の医師によって実施されている手術がかなりの部分を占めています．これらの数字から，今の日本の小児外科医療の現状，小児外科専門医が果たしている役割などを推測しますと，新生児外科領域の治療は，専門的な知識のみならず，それなりの医療環境が必要ですので，ほとんどが小児外科の認定施設で，小児外科専門医の手によって行われています．乳児手術領域は，鼠径ヘルニアや腸重積症のように，それほど高い技術度を必要としない手術であっても，入院・手術・周術期管理にそれなりの人手や環境の整備が必要ですので，小児施設の小児外科医がそれなりにしっかり絡んでいる，というところでしょうか．一方で，成人にも同様の手術があり，患児の大半が学童以上の虫垂切除術などでは，広く地域の病院で小児外科医以外の外科医によって行われている，ということがよくわかります．

本来なら，15歳以下の小児手術は小児外科専門医が行うべきでしょう．そのためには，現在の約500名の小児外科専門医では少なすぎます．また小さな施設でも，小児であれば何でも頑張ってやっていこう，というのでは劣悪な労務環境になりますので，設置母体が違っても，小児施設間での連携を良くして，施設の棲み分けや集約なども考えていくべきではなかろうかと思います．一方で，小児外科領域の疾患は，本当に小児外科専門医がやれば成績はよくなるのでしょうか？手術時間も短く，出血量も少なく，かつ術後合併症は本当に少なくなっているのでしょうか？小児外科学会の認定施設以外の情報を使わねばできないこのような検証は，今まで一度もなされたことはありません．自分たちで，良いに決まっていると信じている，あるいは信じたいと思っているだけではないでしょうか？もしも，小児外科専門医が絡んでいなくても，手術内容や術後結果に優位性がないのであれば，その手術は小児外科専門医への申請資格にすべきではないと思いますし，手術実績で評価する専門医の資格審査・診療実績に，そのような手術は入れるべきではないのではないか，という警鐘が鳴らされたと考えるべきではないでしょうか．ただ，現在小児外科領域が行っているNational Clinical Databaseの入力項目は，疫学的な分析は可能ですが，術者別・施設別の医療水準評価，専門医別の医療水準評価などはできません．

3. NCDのデータ分析

現時点で，このような医療水準評価を行うことができるデータ入力を義務化している，消化器外科領域，心臓血管外科領域の現状を皆様にお示しし，大規模臨床データベースの威力の一端をご紹介したいと思います[6][7]．図7は消化器外科領域のデータ分析結果を示しています．消化器外科領域に包含されている術式は112術式ですが，そのうちここに挙げた8術式に関しては，患者さんの術前リスクなどをきちんとadjustした百数十項目の入力を義務づけています．消化器外科領域全60万件のデータのうち，これら8術式手術が約12万件を占めています．他の術式では，小児外科領域の入力よりやや多い34項目です．資料にお示ししている8術式で，緊急性の高い汎発性腹膜炎に対する対応能力も含めて，ほぼ消化器外科技術の総合力が評価できるのではないか，と思われます．肝切除術は，すべての術式をあわせて，7,821件登録されています．その肝切除術を例に使って，データベースの効力をお示しします．34歳の女性で術前リスクがほとんどない患者さんの肝左葉切除術を行うとします（図8）．この患者さんではリスク因子はほとんどありません．実施手術はS2，3，4のいわゆる肝左葉切除術です．同じような手術のデータが全国で多数集積されていますので，この様な患者さんに同手術を実施したとすると，術後30日死亡は0.1%，外科手術部位感染が4.2%，胆汁瘻が3.9%というのが全国の平均値ということになります．この図8の左側の患者情報を術前に入力すると，リアルタイムでこの右側の情報がフィードバックされますので，術前の説明の際に患者さんに「全国平均ではこのような結果になります」というと同時に，数年分のデータがたまってきた時点で，「うちの成績はこうですからもっと安全ですよ」というような情報

提供が可能になっているはずです．

一方で，高リスクの患者さんではどうでしょうか．図9は，76歳の男性で，喫煙歴があり，ASA3という米国麻酔学会基準でかなり全身状態に問題ありという患者さんに，赤字で示したような尾状葉合併肝右葉切除術を行うときのリスクを示しています．術後30日死亡が7.2%，手術関連死亡をみると4人に1人はなくなる，SSIは21.4%と高率，敗血症に14.1%罹患する可能性がある，というのが全国平均，という答えが，術前にわかります．

図10は，ある施設における8術式すべての総合評価を，その施設にフィードバックするときの資料です．この施設では，術後死亡が全国平均より低いですが，術後の消化管出血がやや多く，術後肺炎は非常に多い，という結果が出ました．施設は，この結果を真剣に検証し，どうすれば術後合併症を少なくすることができるのか，を考えねばなりません．

その時に使用されるのが，臨床プロセス指標の利用です．図11は，心臓血管外科領域のバイパス手術における入力結果を分析したものですが，このデータベースでは術前・術後の様々な臨床プロセスの入力も義務づけています．たとえば自施設の術後合併症が多いときに，全国の施設では術前術後にどのようなことを行っているのが標準なのか，というこのような情報と自施設のプロセスとを比較検討し「うちは，これをしていないから成績が悪いのかもしれない」というような，分析を可能とします．これもデータベースの大きな効果の一つです．

図12に，アウトカム分析と各施設の症例数との相関について示します．経験症例数が多いとアウトカムは本当によいのか，もしよいのであれば，患者さんをHigh-volume Centerに集約すべきか，もし集約したとしたら患者の通院に不利益は生じないか，20 km以上通わねばならない患者がどれくらい増えるのか，成績は本当に上がるのか，専門医をもっと育てやすくなるのか，外科医の労務環境はよくなるのか，などの分析が可能になります．患者住所の7桁の郵便番号登録が，患者の集約などの分析に非常に威力を発揮します．

それでは，小児外科領域で同様の評価を行うには，何をせねばならないのでしょうか（図13）．まずは，risk-adjustされた入力フォーマットを作成することになりますが，すべての手術を入力するのではなく，施設や専門医の医療水準評価のみを行うのであれば，それぞれの領域で，数術式の詳細入力をすれば全体像が予測できるようになります．ただrisk-adjustの定義はしっかり決めておく必要があります．たとえば成人では，仮に糖尿病合併と言っても，食餌療法のみ，経口糖尿病薬の服用，インシュリンの自己注射，糖尿病はあるものの治療無し，など程度は様々です．これらをきちんと区別して入力しないと，risk-adjustされたことになりません．小児外科領域では，たとえば心疾患の合併は，予後などに大きく作用しますので，本来の小児外科手術がうまくいっても予後は悪い，ということはままあります．Waterstonの分類やSpitzの分類はレトロスペクティブな検討で提唱されたものであり，科学的ではありません．たとえば心疾患の合併でも，ここにあげたように，たとえば下部心臓型総肺静脈還流異常症のように生後数日以内に心内修復が必要な疾患，姑息手術が必要な疾患，乳児期早期に心内修復が必要な疾患，等々のように，合併する疾患の程度がわかるような術前入力が，非常に重要になります．

医療水準評価に利用する実際の入力フォーマットを示します（図14）．対象疾患としては，主たる新生児疾患をいくつか，主たる後天性疾患もいくつか，小児がんのいくつか，あたりでよいと考えます．小児外科疾患はそれほど多くありませんので，かなり項目数の多い入力フォーマットを作成しても，大きな負担はありません．かつ，このデータベースでは追跡はしませんので，せいぜい術後90日目のアウトカムで十分です．

この2階部分のデータの上に3階部分を載せて，臨床研究を行うことも可能です（図15）．我々が目指しているもの，今日私がお話ししたいことは，この臨床研究です．小児用の新しい機器や新薬の開発は，あまり企業の関心を引きませんが，市販後調査，特に小児における適応拡大などには，大きな威力を発揮します．小児外科疾患は数が少なく，施設単位では前向きコホートの研究は不可能です．前向きの研究をする場合には，まず2階部分のrisk-adjusted dataをきちんと作成します．同一疾患に対する術式別のアウトカムの違いの検証などは容易です．たとえば葛西手術の肝門部の剥離範囲は狭い方がよいか可及的に広い方がよいか，これらは諸先輩が自施設の術式とその結果を積み重ねて，今まで繰り返しレトロスペクティブに議論をしてきましたが，まだ科学的な結論は出ていません．この研究を前向き多施設で行って，初めてscientificな検証ができる，と考えています．具体的には，ある一定期間，たとえば2014年，2015年の2年間，すべての認定施設で，胆道閉鎖症のrisk-adjustされた2階部分の臨床データの上に，自施設ではこちらの術式を行っている，という記載を加えれば，2011年の登録実績が160例でしたので，2年間で320例の前向き研究ができます．これを，事務局で「今回は狭く」「今回は拡大で」と，ランダムに振り分けることができれ

ばほぼRCTに近い結果を出すことができます．1年後の減黄率で評価するというprimary endpointをおけば，2017年には我が国のお家芸の葛西手術の科学的評価をお示しすることができましょう．同様に新しい術式の工夫や開発にも威力を発揮します．

III. まとめ

本日の会長講演のまとめになります（図16）．まだ生まれて間もないNational Clinical Databaseです．現時点でも産みの苦しみで様々なトラブルシューティングを行っています．初年度は約9万件のクレームメールをいただきました．ただ，うまく利活用できれば，非常に有益なデータベースであることは間違いありません．世界初の，外科医情報とリンクした小児外科大規模手術データベースへ発展していっていただきたいと思っています[8]．そして，日本の小児外科技術の優秀さを世界に発信しましょう．米国のAmerican College of Surgeonsの同様のNSQIP-Pediatricと比較すれば，お互いに切磋琢磨できます．多施設共同研究を様々な角度で行うことで，我が国の小児外科施設の連帯感も生まれます．これからの世代の方々に是非期待したいと思っています．

IV. 謝辞

冒頭で述べさせていただきましたように，本日の会長講演で，専門としてきた小児内視鏡外科の話をさせていただくか，このデータベースの話をさせていただくかは，結構悩みました．小児内視鏡手術は諸先輩の頑張りで，黎明期，発展期を終わり，そろそろ成熟期にさしかかろうとしています．私は発展期と成熟期の入り口あたりを支えてきたのかな，とも思っています．本日の会長講演に至る道のりでは，友人である多くの外国の小児外科医たちにお世話になりました．時にはメンターとして私をしっかり指導してくれました（図17）．また我が国の小児外科医の多くの先生方にもご指導いただきました．今回の学術集会は，50年の節目でもあり，主題にも掲げさせていただきましたように，これからの発展をどうすればよいか，を考える学術集会にしたい，と思ってきました．それゆえ，自分が運営委員長として活動しているNational Clinical Databaseが，小児外科領域でまだまだ未熟な状況でとどまっていますので，この会長講演の機会を借りて，これからの若い方々にメッセージを発信させていただきたいと考えました．図18は2年前，私が理事長を終えるときの記念写真です．本当に皆様には公私共々お世話になりました．

一方，今日の会長講演でお話しできませんでしたが，小児内視鏡手術の仲間たちには（図19），この15年本当にお世話になりました．今年の6月，小児内視鏡手術のトレーニングと技術審査の話を北京の国際小児内視鏡外科学会（International Pediatric Endosurgery Group: IPEG）で，理事長講演として話させていただきます（図20）．

また私の活動を，公的にも私的にも支えてくれた楽しい仲間たち，現在の施設でともに働いている教室員にもお礼を申し上げたいと思います．また，今回の学会主催に際しまして，一切目立たないように，しかししっかりと支えてくださいました同門会の皆様にも心より感謝申し上げます．最後に，小児外科医になって35年，ほとんど家族を顧みることなく，やりたい放題のことをして参りました．自分のやりたいことも我慢して，育児や私の面倒を見てくれた妻麗子，ならびに疲れている時にいつも癒やしてくれた子どもたちに，この場を借りてお礼を言いたいと思います．本当にありがとう．

夢を語る会長講演をさせていただきました．これからの我が国の小児外科医療の発展を祈念して会長講演を終わりたいと思います．ご清聴ありがとうございました．

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