**Table 1 ATP8B1 mutation and biochemical parameters in patients enrolled in this study**

| | Sex | Age | *ATP8B1* mutation in allele 1 | *ATP8B1* mutation in allele 2 | AST/ALT/GGT (U/L) | T-Bil/D-Bil/BA (μM) | Pruritus score |
|---|---|---|---|---|---|---|---|
| Patient 1 | M | 2 | c. 3033-3034del (Frame shift) | Not found | 83/60/36 | 4.8/4.6/301.3 | 4 |
| Patient 2 | M | 6 | c.1585-1587TTCdel (p.F529del) | Not found | 81/15/27 | 3.4/3.2/214.1 | 4 |
| Patient 3 | M | 16 | c.234C > G (p.H78Q) c.2021T > C (p.M674T) | c.1729A > G (p.I577V) | 63/51/19 | 0.6/0.3/54.6 | 4 |

was increased to 350 mg/kg/day, in patient 2 one week after the dosage was increased to 500 mg/kg/day, and in patient 3 four weeks after the dosage of 4PB was increased to 350 mg/kg/day. The itching score declined from 4 to 2 in all patients (Figure 3A, B). Although there were multiple dark erosions in the skin of all patients and elephantiasis in patient 3 due to intense and continual scratching, after the onset of 4PB treatment at the dosage of 350 or 500 mg/kg/day, the frequency and intensity of skin scratching was markedly decreased, leading to diminished skin erosion and hemorrhage and improved skin appearance (Figure 3C). At the end of the therapy, hemorrhage and eschar on the patients' skin were diminished and areas of fresh normal skin were evident. The parents of the patients noted an improvement in sleep disturbance during the night and in their child's skin condition. In contrast to the relief of the itching, the serum levels of bile acids and ATX and of ATX activity, all of which have been proposed as potential pruritogens in cholestasis [24], were not decreased by 4PB therapy in any of the patients (Figures 2B, 3A, B). The itch remained unchanged for 6, 4, and 6 weeks after cessation of 4PB therapy in patients 1, 2, and 3, respectively, but then gradually exacerbated, resulting in

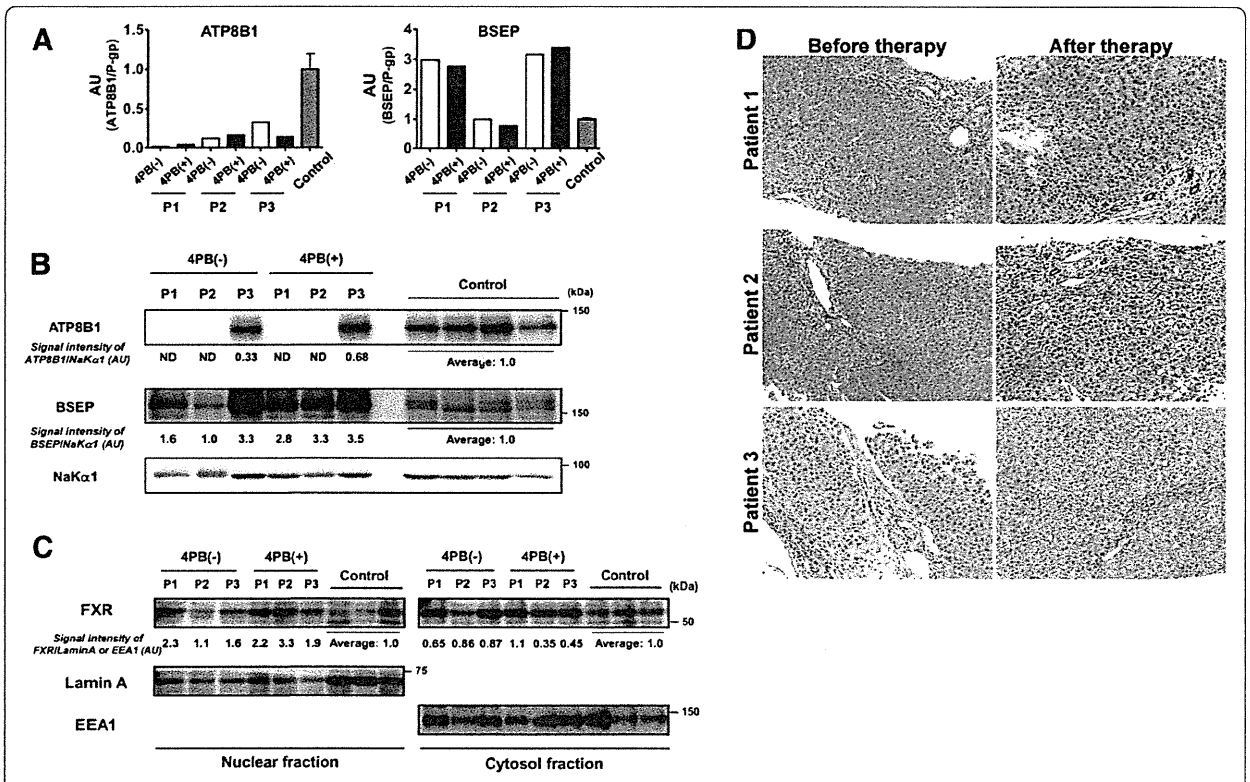


**Figure 1 Studies of liver biopsy specimens obtained from the patients before and after 4PB therapy. (D)** Histological characteristics of liver sections. Liver sections prepared from the liver biopsy specimens of the patients before and after the 4PB therapy were subjected to HE staining as described in Patients and Methods. A typical image under each condition is shown. Original magnification; 200×. **(A-C)** Analysis of mRNA and protein expression levels. cDNA **(A)**, crude membrane fractions **(B)**, and nuclear and cytosolic fractions **(C)** were prepared from liver biopsy specimens from the patients and then analyzed by qPCR **(A)** and immunoblotting **(B and C)** as described in Materials and Methods. In **(A)**, the data were obtained from triplicate determination. Each bar represents the mean ± SE of individual specimens. In **(B and C)**, the signal intensity of ATP8B1, BSEP, and FXR relative to that of NaKα1, LaminA, and EEA1 is presented below each panel. AU, arbitrary unit; P-gp, P-glycoprotein; P1, patient 1; P2, patient 2; P3, patient 3; 4PB(−), before 4PB treatment; 4PB(+), after 4PB treatment.

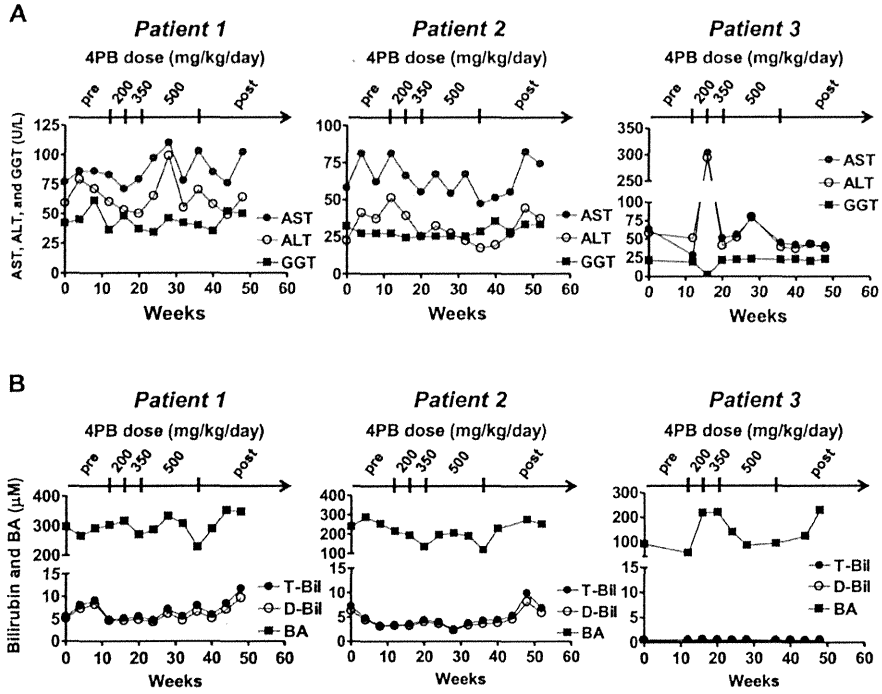


**Figure 2 Liver function testing before, during and after the course of 4PB therapy in the patients.** Serum AST, ALT, GGT **(A)**, total bilirubin, direct bilirubin, and bile acids **(B)** levels were monitored before, during and after the 4PB therapy. T-Bil, total bilirubin; D-Bil, direct bilirubin; BA, bile acids.

regeneration of erosion and hemorrhage again because of intense scratching. In all the patients, 8 weeks after the end of 4PB therapy, the itching score returned to values equal to those before the treatment (Figure 3A, B). Because of the bad taste of 4PB, all patients had difficulty taking the doses at the beginning of the therapy. However, their parents noted the improvement in their child's sleep during the night and in the skin conditions, and encouraged their child to continue the therapy. No patients dropped out of this study. No severe side effects were observed during and after the 4PB therapy. The temporary elevation of AST and ALT concentrations after patient 3 began 4PB treatment at 200 mg/kg/day was thought to be caused by adenovirus infection and not by any adverse effect of the 4PB therapy, because both markers increased promptly after the adenovirus infection appeared and decreased to the basal levels concurrently with recovery from the infection (Figure 2A).

### Effect of 4PB therapy on liver histology and BSEP expression in PFIC1 patients

A liver biopsy was performed 6 months after the initiation of 4PB therapy and compared with the specimens obtained 1 day before onset of 4PB therapy. The specimens of the age-matched control subjects were obtained from OTCD patients without administration of 4PB when they underwent liver transplantation. qPCR and immunoblot analysis demonstrated that even in the specimens taken after the 4PB therapy, the mRNA expression of ATP8B1 was still much lower in all the patients than that of age-matched control subjects, and ATP8B1 protein expression in the membrane fraction was undetectable in patients 1 and 2. In patient 3, ATP8B1 expression was increased about 2-fold by 4PB therapy, but still lower than that in age-matched control subjects (Figure 1B, C). This result was consistent with the lack of change in ATP8B1–FLAG $^{F529del}$ and a 2.1- and 1.3-fold increase in ATP8B1$^{H78Q+M674T}$–FLAG and ATP8B1$^{I577V}$–FLAG in UPS-1 cells after treatment with 4PB at a clinically relevant concentration (1 mM) (Additional file 3: Figure S2). BSEP protein expression was increased after 4PB therapy without affecting its mRNA expression as is the case in patients with PFIC2 and OTCD (Figure 1A, B) [14,16]. The amount of FXR in the nuclear fraction of cells from the PFIC1 patients was nearly equal to or a little higher than that of age-matched control subjects, and was not significantly affected by 4PB therapy except for patient 2 (Figure 1C). In this patient, the amount of FXR was increased 3-fold after 4PB therapy. Histological analysis showed that in the specimens obtained from the PFIC1 patients before this study, the portal area was enlarged and had progressed to fibrosis with mild inflammation and partly bridging fibrosis and

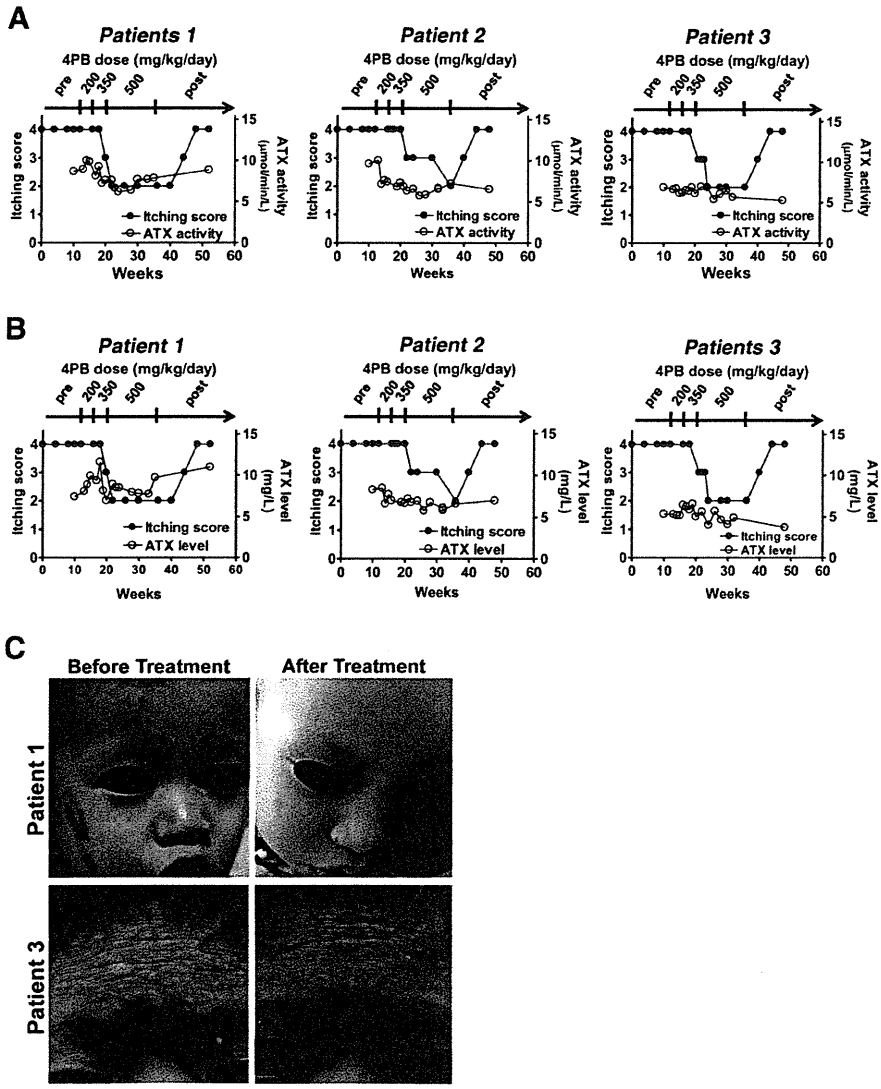


**Figure 3 Itching intensity in the patients before, during, and after the course of 4PB therapy. (A, B)** Correlation diagram of itching scores for the PFIC1 patients with serum ATX activity **(A)** and ATX level **(B)** in the patients before, during, and after 4PB therapy. Pruritus severity was scored ranging from 0 (no pruritus) to 4 (cutaneous mutilation, with bleeding and scarring) as described in Patients and Methods. **(C)** Skin of the patients before and after the 4PB therapy.

that 4PB therapy caused no remarkable change in these features (Figure 1D).

## Discussion

The main complaint in the clinical course of PFIC1 is often the intractable itching, which significantly disrupts the patients' activities of daily living, work productivity, and ability to sleep, and thereby decreases the quality of life for them and their families [2]. Topical steroids, antihistamine agents, and rifampicin are the only prescribed drugs currently available for the cholestatic pruritus in PFIC1 patients, but these medications are often ineffective, as was the case for the patients enrolled in this study [3]. The principal finding of our current study is that 4PB therapy at a clinically relevant dosage used in OTCD patients markedly relieved intractable cholestatic pruritus in PFIC1 patients. At the end of the therapy, hemorrhage and eschar on the patients' skin were diminished, and areas of fresh normal skin appeared as the frequency and intensity of skin scratching decreased. Parents of the patients noted that 4PB therapy made it easier for their child to get to sleep and markedly reduced their child's sleep disturbance during the night. Thus, favorable outcomes of 4PB therapy were observed, and these were dose-dependent, although the patients and their families in this clinical study were not

informed of the detailed protocol. The facts that the patients and their families were not informed of this aspect and that the drugs prescribed for 1.5, 5, and 15 years in patient 1, 2, and 3, respectively, before this study were ineffective in improving the sustained intractable itch suggest that the relief of refractory cholestatic itching in these PFIC1 patients occurred because of the 4PB therapy and not because of a placebo effect or an effect of other medications.

The visual analog scale (VAS) is the most general method for assessing pruritus, but is not easily applied to younger children because VAS is a graphic method based on the patients' subjective rating of symptoms [25]. Therefore, in this study, the patients' itching was evaluated on the basis of cutaneous findings and scored according to the method used in a previous report [21]. A potential concern when using this method is that the improvement in itch may be underestimated. The severity of skin erosion, thickness, and cicatrices depends on the duration of the itch and can vary in the patients scored as 4. Therefore, there may have been a time lag in the improvements in skin appearance even if the frequency and intensity of scratching decreased to the same degree in all patients. In this study, the improvement of pruritus in patient 3, who experienced intractable pruritus for a longer period than patients 1 and 2, might have been underestimated compared with that in patients 1 and 2. To overcome this limitation, it might be better to apply the 5-D itch scale, a recently developed method that assesses the subjective symptoms of itching in patients from five dimensions: degree, duration, direction, disability and distribution [25]. In the 5-D itch scale, but not in the VAS, the subjective symptoms reported by the patient can be supported by his/her family. Therefore, in future clinical studies, the change in chronic pruritus in younger patients should be explored using the 5-D itch scale as well as the method used in this study. This should allow a more accurate determination of the clinical outcomes and the beneficial effects of 4PB therapy on cholestatic pruritus in PFIC1 patients.

In contrast to the relief of intractable cholestatic itching, beneficial effects of 4PB therapy were not observed in liver function tests and liver histology despite an increase in BSEP expression in liver membrane fractions as reported previously (Figure 1B, D and 2) [14,16]. This could be because a two- to three-fold increase in BSEP expression may be insufficient to improve intrahepatic cholestasis in PFIC1 patients. The other possible reason is that the transport activity of BSEP is lost completely in PFIC1 patients because of the disrupted lipid asymmetry of the CM [6,11] and, consequently, the increase in BSEP expression by 4PB therapy cannot compensate for the reduced capacity of bile salt excretion into bile. Although no remarkable improvement in liver function was observed in the patients in our study, 4PB therapy might have therapeutic potency for specific PFIC1 patients with mutations in *ATP8B1* that attenuate ATP8B1 expression, but do not affect its protein activity. An *in vitro* analysis has shown that treatment with 4PB partially restored the decreased expression of ATP8B1 caused by p.G308V, p.D454G, and p.D544N, all of which are naturally occurring mutations [26]. Future clinical studies should validate the therapeutic effect of 4PB and its safety for use in PFIC1 patients who carry mutations that attenuate ATP8B1 expression but do not affect its protein activity.

At present, the mechanism underlying the relief of cholestatic pruritus by 4PB therapy remains to be elucidated. No decrease in the factors suspected to be causally associated with cholestatic pruritus (Figures 2, 3A, B) are consistent with the observation in PFIC2 patient during 4PB therapy [16]. 4PB and/or its metabolites may modulate the local concentrations of these pruritogens, which may not have been detected by systemic measurements. The physiological function of ATX, an enzyme secreted extracellularly that generates lysophosphatidic acid, is thought to be mediated predominantly by activation of G protein-coupled receptors (GPCRs) [27]. TGR5, a GPCR activated by bile salts, in sensory nerves could contribute to bile salt-induced itching [28]; if so, 4PB and/or its metabolites might antagonize the GPCRs responsible for itch signaling and therefore attenuate the activation of sensory neurons. Alternatively, 4PB therapy may disrupt pruriceptive projections to the brain through distribution of 4PB and/or its metabolites into the brain [29] or may affect pruritogens or antipruritogens that have not been identified yet. Further studies to test these possibilities will provide a better understanding of the mechanisms responsible for the effects of 4PB therapy on cholestatic pruritus and thereby the molecular mechanism of cholestatic pruritus itself. Information obtained from these studies will contribute to the development of new molecular target drugs for cholestatic pruritus, which will hopefully be more effective than 4PB and consequently, improve the clinical application of 4PB.

## Conclusions

Our study has provided clinical evidence that 4PB therapy can relieve the refractory itching in PFIC1 patients, and thereby improved the quality of life of the patients and their families. Future clinical studies with more patients and longer time periods than were possible in this study should be undertaken to confirm the favorable effects of 4PB therapy using the 5-D itch scale and the other method used in this study. If confirmed, 4PB therapy could become the preferred choice, instead of topical steroids, antihistamine agents, and surgical procedures,

for attenuating cholestatic pruritus in patients with PFIC1 and benign intrahepatic cholestasis type 1 (BRIC1), a hereditary disorder characterized by mutations in *ATP8B1* and by recurrent and intermittent episodes of cholestasis with refractory cholestatic pruritus [30]. 4PB therapy might also be effective for intractable pruritus caused by other cholestatic disorders such as Alagille syndrome. Clinical trials will be required to determine the utility and safety of 4PB as a therapy for these diseases.

## Additional files

**Additional file 1: Supplemental information.**

**Additional file 2: Effects of mutations in *ATP8B1* on mRNA and protein expression levels, cellular localization, and function of ATP8B1.**

**Additional file 3: Effect of 4PB on the expression levels of ATP8B1 mutants.**

### Abbreviations

ALT: Alanine amino transaminase; AST: Aspartate aminotransaminase; ATX: Autotaxin; AU: Arbitrary units; BA: Bile acids; BRIC: Benign intrahepatic cholestasis; BSEP: Bile salt export pump; CM: Canalicular membrane; D-Bil: Direct bilirubin; ER: Endoplasmic reticulum; EV: Empty vector; FITC: Fluorescein isothiocyanate; FXR: Farnesoid X receptor; GGT: Gamma-glutamyl transferase; GPCR: G protein-coupled receptor; HA: Hemagglutinin antigen; HE: Hematoxylin-eosin; $Na^+$: $K^+$-ATPase α1 subunit, NaKα1; ND: Not detected because of low expression; OTCD: Ornithine transcarbamylase deficiency; PFIC: Progressive familial intrahepatic cholestasis; PS: Phosphatidylserine; qPCR: Quantitative polymerase chain reaction; SE: Standard error; T-Bil: Total bilirubin; VAS: Visual analog scale; WT: Wild type; 4PB: 4-phenylbutyrate.

### Competing interests

H.H. has applied a patent on the effect of 4-phenylbutyrate on bile salt export pump (US serial No.13/299,989).

### Author contribution

YH recruited and enrolled the patients, collected specimens from the patients, performed the clinical assessment and follow-up, contributed to data interpretation, and drafted the manuscript together with HH. HH directed and supervised all of the research and took a lead role in writing the manuscript. SN performed most of the *in vitro* experiments, contributed to data interpretation, and drafted the manuscript together with HH. HK and KB recruited and enrolled the patients, collected specimens from the patients, carried out clinical assessment and follow-up, contributed to data interpretation, and revised the manuscript for intellectual content. KI measured ATX concentration. KH provided UPS-1 cells. KN, TK, and AK collected specimens from the patients and carried out clinical assessment and follow-up. HN helped to make a diagnosis of the patients, and contributed to data interpretation. YM, KO, and HK contributed to data interpretation and revised the manuscript for intellectual content. All authors approved the manuscript before submission.

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# 胎児・新生児の肝・胆道系の発生

中野　聡　　鈴木光幸　　清水俊明

## はじめに

新生児期に診断される胆汁うっ滞症の中には，胎児期の肝・胆道系の発生異常に起因する疾患が存在し，Alagille症候群，胆道閉鎖症，Caroli病などがこれに該当する。胎生期の肝胆道系の発生を理解することは，これらの疾患の発症機序を理解する上で重要である。

細胞分化の観点からみると，肝臓，胆嚢，胆管などの肝胆道系の各器官は，前腸内胚葉の共通の前駆細胞から分化する。この前駆細胞から肝細胞や肝内胆管への分化にはNotch，TGF-$\beta$，Wnt/$\beta$-cateninなどを介したシグナル伝達系が[1]，胆道系への分化にはNotchシグナルの下流に位置するHes1などの転写因子が重要である[2]。

本稿では，胎内での肝・胆道系の発生機序，および胎生期から出生直後までの肝・胆道系の生理的機能について概説し(表)，肝胆道系の発生異常に起因する主な疾患を提示する。

## 肝・胆道系の発生

### 1．肝臓の発生

肝臓，胆嚢，および胆汁器官は，胎生第4週の初めに前腸(後の十二指腸)の尾方部あるいは遠位部から腹側への突起物(肝憩室)として発生する(図Ⅰ)。第5週目に肝憩室の大きな頭側部は，肝臓の原基(肝細胞索)となり，肝細胞索は肝実質(肝細胞)と胆管上皮細胞に分化し，増殖を繰り返し肝臓の右葉と左葉の原基となる[3](図Ⅱ，Ⅲ)。第5～10週頃には，臍帯静脈からの栄養素や酸素の豊富な血液により，肝臓は上腹腔の大部分(全体重の約10%)を占めるようになるまで急速に発育する。肝の両葉はほぼ同じ大きさであるが，この後右葉がより大きく発育する。肝臓が腹腔内で尾側に突出するまで発育すると，肝臓と前腸，および肝臓と前腹壁の間の中胚葉は膜状となり，小網と肝鎌状間膜を形成する。

胎生期の肝循環は臍帯血流量の約45%が肝実質細胞を灌流し，残りの55%は肝内短絡である静脈管を通り下大静脈へ流れ込み，主として卵円孔を通って脳や心筋へ流入する。

### 2．胆道系の発生

胆道系は肝外胆管系と肝内胆管系に分類でき，肝外と肝内胆管の境界は一般的に左右肝管の第1分枝部である。肝外胆管系はさらに肝外胆管と胆嚢に区分でき，肝外胆管は左右肝管，総肝管，総胆管で構成される[4]。肝内胆管と肝外胆管は発生起源と形成時期が異なるため，項を分けて説明する。

#### 1）肝外胆管系

第5週目に肝憩室の小さな尾側部は胆嚢となり，その茎が胆嚢管を形成し，それらと前腸をつなぐ管が胆管となる(図Ⅱ，Ⅲ)。第5週まで胆管は前腸(十二指腸ループ)の腹側面に接着しているが，第6週目に前腸の回転に伴い，胆管接合部も前腸の背側へ移動する(図Ⅳ)。胆嚢や胆嚢管などの肝外胆汁器官の内部は，当初上皮細胞で閉塞されているが，第7～8週までに細胞変性(空胞化)により総胆管下部から肝側に向かって再疎通が進行する(図Ⅴ)。第10週頃には肝外胆管は管腔構造と

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表　在胎週数と肝・胆道系の発生・発達

| 在胎週数 | 肝臓 | 肝外胆管 | 肝内胆管 |
|---|---|---|---|
| 4 | 肝憩室の発生 | | |
| 5 | 肝憩室頭側部が肝原基へ | 肝憩室尾側部が胆嚢へ | |
| 6 | 造血の開始 | 胆管が十二指腸背側へ移動 | 細胆管の形成 |
| 7 | | | |
| 8 | アルブミン合成 | 細胞変性による空胞化が進行 | 胆管板の形成 |
| 9 | グリコーゲン合成 | | |
| 10 | | 肝外胆管が管腔構造に | 肝外胆管と結合 |
| 11 | | | |
| 12 | | 胆嚢の内腔が完成 | 胆管板の消失<br>胆汁の生成 |
| 13 | | | 胎便は暗緑色へ |
| 20 | Fib，Tf，LDLの産生 | | 未熟な肝内胆管形成 |
| 32 | 造血能の減退 | | |
| 出生 | | | |
| 1〜2年 | | | 肝内胆管の完成 |

Fib：フィブリノゲン
Tf：トランスフェリン
LDL：低比重リポ蛋白(low-density lipoprotein)

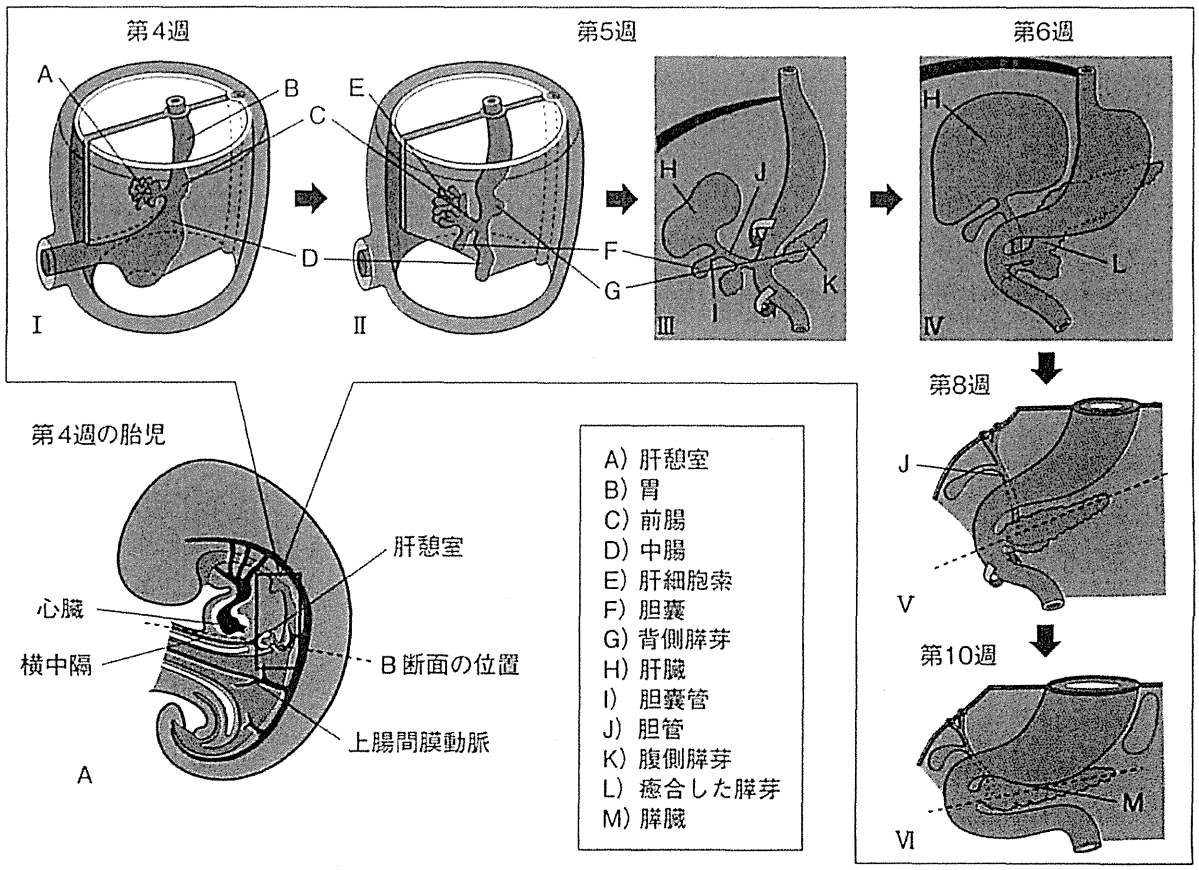


図　肝・胆道系の発生（Mooreら，2011より作成）[3]

なり(図Ⅵ)，また第12週頃に胆嚢の内腔が完成する。

**2）肝内胆管系**

第6週目頃から肝細胞索の小さな空胞として細胆管が形成され肝葉内，肝葉間と合流を繰り返しながら次第に大きな肝内胆管が形成されていく。肝細胞索は肝実質に分化し，胆管の壁を覆い，胆管に接する肝細胞索は胆管上皮細胞に分化し，1～2層の円筒状の細胞からなる胆管板(ductal plate)を形成する。肝内胆管と肝外胆管は第10週に肝門部で結合し(図Ⅵ)，第12週頃に胆管板はリモデリングにより，その多くが消失する。肝外と肝内胆管が接続することで胆道系は完成するが，その明確な機序は不明である。

一部の胆管板は門脈域肝葉組織内へ遊走し，第20週頃に未熟な肝内胆管が形成される。肝内胆管の形成は，肝門部から末梢側へ，大きなグリソン鞘から小さなグリソン鞘へ向かって進み，生後1～2年で完成するといわれている。

## 3．肝・胆道系機能の確立

**1）造血・貯血機能**

肝臓が発生した後の第一の機能は造血である。造血は第6週目から始まり，肝臓は鮮紅色の外観となる。この時期には，造血細胞数は肝細胞数より多い。初期の肝細胞は小さく(成熟期30～35 μmに対して20 μm以下)，含有グリコーゲンも少ない。出生近くになると肝細胞はグリコーゲン含量が増加することで大きくなり，肝細胞集団が優位となる。肝臓の造血機能は第32週頃から次第に減退し，出生時にはほとんど機能を失う。

羊胎仔では肝の血管コンプライアンス(血管内に蓄えられる血液量)は1.0 mL/mmHg/kgと，全身の血管コンプライアンス(3.7 mL/mmHg/kg)の28%に相当し，胎生期において肝は貯血の役割も担っていると考えられている[5]。

**2）蛋白合成**

肝臓では，第7～8週頃にアルブミンの合成が始まり，早期胎児の腫瘍蛋白質であるαフェトプロテインの合成量に反比例して増加する。第20～30週頃までに胎児肝は，フィブリノゲン，トランスフェリンおよび低比重リポ蛋白(low density lipoprotein：LDL)を産生する。この時期の血中の主要蛋白質濃度は成熟児に比して低値を示す。血中リポ蛋白質濃度は生後第1週目に急上昇して思春期と同等になるが，アルブミン濃度が成人レベル(>3.5 g/dL)に達するには生後数カ月を要する。

**3）胆汁分泌**

第12週頃から肝細胞で胆汁生成が始まり，第13週目以降には十二指腸へ胆汁が流入し胎便は暗緑色に変化する。ヒトにおいて胆嚢内胆汁はタウリン抱合またはグリシン抱合型胆汁酸が大部分を占めているが，胎便中には硫酸抱合型胆汁酸が主として認められる。これは硫酸抱合型胆汁酸が尿中に排泄されるため，胎児尿として排出された羊水嚥下の影響に由来すると考えられている。

**4）血糖調節**

肝は血糖値を厳密に調節し，余分な炭水化物をグリコーゲンとして蓄える。グリコーゲンはグルコースの重合体であり，飢餓状態では直ちに加水分解されてグルコースになる。血糖値を維持するために，肝細胞は糖新生またはグリコーゲン分解によってグルコースを産生する。

胎児のグリコーゲン合成は第9週頃から始まり，出生直前にグリコーゲンは最も急速に蓄積され，胎児肝は成人の2～3倍のグリコーゲンを含んだ状態で出生を迎える。この蓄積されたグリコーゲンのほとんどが出生直後に消費され，グリコーゲンが再び蓄積されるのは生後2週頃からで，生後3週頃に成人と同等になる。

糖新生は胎児肝にもみられるが，出生後に急速に活発となる。出生直後の血糖維持は，肝のグリコーゲン分解に依存しているが，その後グリコーゲン分解と糖新生の両者が可能となる。早期産児の血糖値が変動する理由の一つとして，効率的なグリコーゲンの合成，蓄積および分解の制御が満期(第40週頃)に完成することによる[6]。

## 肝胆道系の発生異常に起因する疾患

### 1．Alagille症候群(Alagille syndrome：AGLS)

1975年にAlagilleらは，肺動脈狭窄症，特異的顔貌(広い額，落ち窪んだ眼，とがった顎，鞍状ないし真っ直ぐな鼻)，椎骨奇形，成長障害，性腺機

能不全などを合併する肝内胆管低形成症を報告した。1997年にAlagille症候群(AGLS)の原因としてNotch受容体のリガンドである*Jagged-1*遺伝子が発見された[7,8]。Notchとはショウジョウバエの発生関連遺伝子として発見され，ハエの翅の切れ目(ノッチ)に由来する。その後の研究で，ヒトにおいても細胞間の情報交換のシステムとしてNotchシグナルが，多臓器の発生過程で繰り返し使用されていることが明らかになった[9]。

AGLSの諸症状は，胎児期の*Jagged-1*遺伝子変異に代表されるNotchシグナルの伝達障害による臓器の発生異常が原因であり，実際に胎児期の*Jagged-1*発現部位はAGLSの障害臓器に一致している。胆道系において*Jagged-1*遺伝子は，門脈周囲に存在する肝芽細胞のNotch2受容体を活性化させて，胆管上皮細胞に分化させる役割を担っており，この反応は生後1～2年の肝内胆管完成まで続く。AGLSでは肝内胆管の低形成(小葉間胆管の減少：paucity)が特徴的であるが，この所見は*Jagged-1*遺伝子異常により肝芽細胞から胆管上皮細胞への分化が誘導されないことに起因する。

## 2. 胆道閉鎖症(Biliary atresia：BA)

胆道閉鎖症(BA)は，生後早期に肝外胆管に不可逆的な完全閉塞をきたす疾患である。進行すると胆汁性肝硬変から肝不全へ至るため，救命には手術が不可欠である。BAの病因には，胎生期の発生異常説，レオウイルス属などのウイルス感染説，マイクロキメリズム説などのさまざまな仮説が提唱されてきたが，決定的なものはまだない。多くの症例では，出生後に一旦形成された肝外胆管に何らかの原因で炎症が生じ，その修復機転で胆管閉塞をきたす。一方で，他の臓器の発生異常とともに本疾患が発見されることもあり，一部の症例では胎生期から肝外胆管の閉塞をきたした発生異常と考えられている。

## 3. Caroli病

①肝内胆管の多発，部分的な拡張，②結石，胆管炎，肝膿瘍の頻発，③肝硬変，門脈圧亢進症の欠如，④嚢胞性腎疾患，尿細管拡張を伴うものをCaroli病(純型)とし，進行性の門脈域線維化を示す先天性肝線維症を合併するものをCaroli症候群(線維化合併型)と定義する。

Caroli症候群は常染色体劣性嚢胞性腎疾患(autosomal recessive polycystic kidney disease：ARPKD)の肝胆道病変として知られ，ARPKDの責任遺伝子としてPKHD1(polycystic kidney and hepatic disease1)がクローニングされ，この遺伝子がコードする蛋白質としてfibrocystin/polyductinが同定されている。Fibrocystin/polyductinは胆管細胞の増殖・分泌を制御するprimary cilia(胆管細胞管腔側の線毛)に発現している。Fibrocystin/polyductinが欠如すると，primary ciliaを介したシグナル伝達異常により胆管細胞の増殖亢進と分泌促進が生じる。その結果，胆管板のリモデリング異常(ductal plate malformation：DPM)が胎生肝で起こり，肝内胆管の多発性，進行性の嚢状拡張をきたす。実際にCaroli病の動物モデルであるpolycystic kidney(PCK)ラットでは，胎生期の胆管細胞における増殖亢進とアポトーシスの不均衡により胆管板の遺残と拡張が発生する。生後も細胞増殖が制御できず肝内胆管拡張が進行する[10]。

## おわりに

胎児・新生児の肝・胆道系の発生を述べ，胎児肝が果たす役割および発生異常に起因する疾患群について概説した。肝・胆道系の発生は胎生4～12週頃までの極初期の現象であるため，ヒト胎児での検討は十分ではなく，他動物からの類推事柄も多い。近年，器官分化を決定する分子機構の解明とともに胆道系の発生にかかわる種々のシグナル伝達系・転写因子が明らかとなった。一部の疾患では分子メカニズムレベルでの病態解明にまで飛躍しており，新しい診断法や治療法への発展が期待されている。

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TOPIC HIGHLIGHT

WJGP 5th Anniversary Special Issues (3): Pancreatitis

# Acute pancreatitis in children and adolescents

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## Abstract

In this Topic Highlight, the causes, diagnosis, and treatment of acute pancreatitis in children are discussed. Acute pancreatitis should be considered during the differential diagnosis of abdominal pain in children and requires prompt treatment because it may become life-threatening. The etiology, clinical manifestations, and course of acute pancreatitis in children are often different than in adults. Therefore, the specific features of acute pancreatitis in children must be considered. The etiology of acute pancreatitis in children is often drugs, infections, trauma, or anatomic abnormalities. Diagnosis is based on clinical symptoms (such as abdominal pain and vomiting), serum pancreatic enzyme levels, and imaging studies. Several scoring systems have been proposed for the assessment of severity, which is useful for selecting treatments and predicting prognosis. The basic pathogenesis of acute pancreatitis does not greatly differ between adults and children, and the treatments for adults and children are similar. In large part, our understanding of the pathology, optimal treatment, assessment of severity, and outcome of acute pancreatitis in children is taken from the adult literature. However, we often find that the common management of adult pancreatitis is difficult to apply to children. With advances in diagnostic techniques and treatment methods, severe acute pancreatitis in children is becoming better understood and more controllable.



**Key words:** Acute pancreatitis; Children; Pathophysiology; Etiology; Diagnosis; Treatment

**Core tip:** The etiology, manifestations, and course of acute pancreatitis in children are often different than in adults, and these differences should be highlighted. The etiology of acute pancreatitis in children is drugs, infections, trauma, or anatomic abnormalities. The diagnosis of acute pancreatitis is based on clinical symptoms, serum pancreatic enzyme levels, and imaging studies. Treatments in adults and children are similar. With advances in diagnostic techniques and treatments, severe acute pancreatitis in children is becoming better understood and more controllable.

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## INTRODUCTION

Acute pancreatitis is not necessarily a rare disease, even in children and adolescents (hereinafter referred to as "children"), and may be life-threatening if it is severe[1,2]. Therefore, acute pancreatitis should always be considered during the differential diagnosis of abdominal pain in children, and appropriate treatment should be started promptly when necessary. However, many treatment regimens are based on consensus conferences and evidence in adults, so a search for the cause and appropriate treatment in children is often difficult[3,4]. This paper discusses the causes, diagnosis, and treatment of acute pancreatitis in children, including a review based on our own experiences.

**Table 1 Etiology of childhood acute pancreatitis**

| |
|---|
| Congenital anomalies, periampullary obstruction |
| Choledochal cyst, abnormal union of the pancreaticobiliary junction, gallstone, cholecystitis, pancreatic divisum, tumor, ascaris aberrant |
| Infectious |
| Mumps, measles, coxsackie, echo, lota, influenza, epstein-barr virus, Mycoplasma, salmonella, gram-negative bacteria |
| Drugs |
| L-asparaginase, steroid, valproic acid, azathioprine, Mercaptopurine, mesalazine, Cytarabine, Salicylic acid, indomethacin, tetracycline, chlorothiazide, isoniazid, anticoagulant drug, borate, alcohol |
| Trauma |
| Blunt injury, child abuse, ERCR, After surgery |
| Systemic disease |
| Reye syndrom, systemic lupus erythematosus, polyarteritis nodosa, Juvenile rheumatoid arthritis, sepsis, multiple organ failure, Organ transplantation, hemolytic-uremic syndrome, henoch-schoenlein purpura, kawasaki disease, inflammatory bowel disease, chronic intestinal pseudo-obstruction, gastric ulcer, anorexia nervosa, food allergy, cystic fibrosis |
| Metabolic |
| Hyperlipoproteinemia (I, IV, V), hypercalcemia, diabetes, α1 antitrypsin deficiency |
| Nutrition |
| Malnutrition, high-calorie infusion, vitamin A and D deficiency |
| Others |
| Familial, idiopathic |

ERCP: Endoscopic retrograde cholangiopancreatography.

**Table 2 Cause of acute pancreatitis in children and adolescents**

| Ref. | Location | Cases | Etiology (%) Systemic | Biliary[1] | Anatomic[2] | Trauma | Familial | Metabolic[3] | Drugs | Others[4] | Idiopathic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lopez[50] | United States | 274 | 48 | 10 | NA | 19 | NA | 0.7 | 5 | 0.4 | 17 |
| DeBanto *et al*[1] | United States | 301 | 3.5 | 10.5 | 1.5 | 13.5 | 5.5 | 4 | 11 | 16.5 | 34 |
| Werlin *et al*[8] | United States | 180 | 14 | 12 | 7.5 | 14 | 3 | 5.5 | 12 | 24 | 8 |
| Nydegger *et al*[4] | Australia | 279 | 22.2 | 5.4 | NA | 36.3 | NA | 5.8 | 3.2 | 2.2 | 25.1 |
| Suzuki *et al*[19] | Japan | 135 | 8.9 | 30.4 | 25.9 | 9.6 | NA | NA | 11.1 | 3.7 | 10.4 |
| Lantz *et al*[2] | United States | 211 | 3.3 | 11.8 | 5.2 | 7.6 | 0.9 | 6.2 | 19.9 | 13.8 | 31.3 |

All studies contained more than 100 cases. NA: Not available. [1]Gallstone, biliary sludge, choledochal cyst; [2]Abnormal union of the pancreaticobiliary junction, pancreatic divisum; [3]Diabetic acidosis, hyperlipidemia, organic acidemias, hypercalcemia; [4]Associated viral infection, postendoscopic retrograde cholangiopancreatography, alcohol, autoimmune, cystic fibrosis, post-surgery.

## ETIOLOGY

Alcohol and gallstones are the etiology of acute pancreatitis in many adults, and although some differences exist based on sex and ethnicity, these two etiologies account for more than 60% of cases of acute pancreatitis in adults[5,6]. However, the etiology in children is often drugs, infections, trauma, and anatomic anomalies such as choledochal cysts and abnormal union of the pancreatobiliary junction (Table 1)[1,4,7,8]. Table 2 shows the incidence of acute pancreatitis by etiology. There is a considerable difference in the etiology of acute pancreatitis in Western and Asian children[9].

### Drugs

Among drugs used in childhood and adolescence, L-asparaginase (ASNase), steroids, and valproic acid often cause pancreatitis as an adverse reaction. In particular, ASNase, a key drug used in treatment of childhood leukemia, is associated with a higher incidence of pancreatitis as compared to other drugs, ranging from 2%-16% when mild cases are included[10-12]. A characteristic of pancreatitis associated with ASNase, in addition to clinical symptoms of abdominal pain and tenderness, is the early absence of elevated serum amylase levels in about half of patients[13,14]. This phenomenon is attributed to inhibition of protein synthesis by ASNase[14]. Therefore, when acute pancreatitis is suspected based on clinical findings, even in the absence of serum amylase elevation, acute pancreatitis must always be considered in the differential diagnosis, and it is important not to miss the opportunity for early treatment. Azathioprine and mesalazine can also cause pancreatic toxicity, so if serum pancreatic enzyme levels increase during the treatment of inflammatory bowel disease, drug-related pancreatitis must also be considered[15].

### Infectious disease

Mumps is often encountered in daily clinical practice, but few patients develop pancreatitis that requires additional treatment. Pancreatitis as a complication is reported in 0.3%-15% of patients when mild cases are included[16]. Abdominal symptoms such as pain and tenderness may occur before the clinical onset of mumps (4-8 d after viral infection) and often spontaneously resolve in about 1 wk. In addition, pancreatitis may occur without parotid

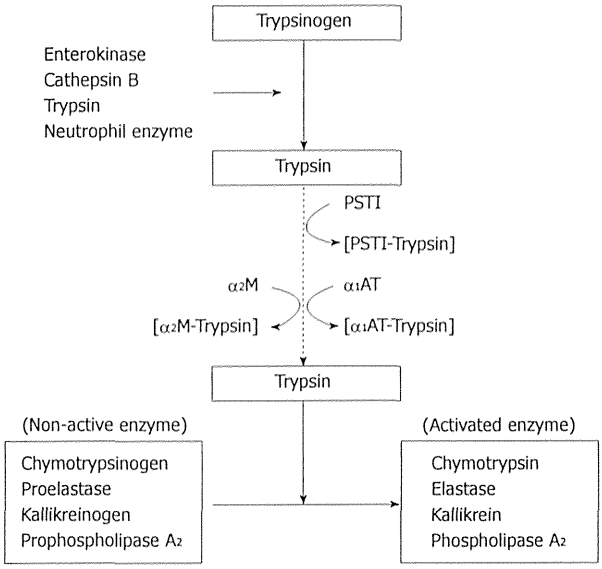


**Figure 1 Suppression mechanisms for pancreatic enzyme activation.** PSTI: Pancreatic secretory trypsin inhibitor; $\alpha_2$M: $\alpha_2$-macroglobulin; $\alpha_1$AT: $\alpha_1$-antitrypsin.

gland swelling in a few patients. When pancreatitis of unknown etiology occurs, testing for the mumps virus is recommended. Two deaths have been reported to date, so although rare, possible serious infection must be kept in mind[17].

Pancreatitis associated with mycoplasma infection is broadly classified into two types: early onset type during early infection (days 1-3) and late-onset type after respiratory tract symptoms have occurred (days 7-14). The mechanism in the former is thought to be direct invasion of mycoplasma into the pancreas, and in the latter, pancreatic injury caused by autoantibodies to acinar cells[18]. The prognosis in pancreatitis due to mycoplasma is generally good.

### *Congenital anomalies*

Among anomalies of the pancreatobiliary system, choledochal cyst is the most common cause of acute pancreatitis[1,2,4,19]. In fact, many choledochal cysts are discovered because of symptoms of acute pancreatitis. In children with acute pancreatitis in whom the etiology is unclear, ultrasonography, endoscopic retrograde cholangiopancreatography (ERCP), or magnetic resonance cholangiopancreatography (MRCP) should be performed[20,21]. Most choledochal cysts, with the exception of Todani classification type II (bile duct diverticulum) and type III (choledochocele), are associated with abnormal union[22]. The sphincter of Oddi is usually most thickened in the duodenal muscularis mucosa; however, in abnormal union, because this sphincter surrounds a common channel after union of the main pancreatic duct and common bile duct, there is communication between the ducts during sphincter contraction[23]. Therefore, reflux of bile into the pancreatic duct, a protein plug in the common channel, or gallstone impaction is probably involved in the onset of pancreatitis.

## PANCREATITIS CAUSED BY GENETIC MUTATIONS

Hereditary pancreatitis is due to autosomal dominant inheritance with about 80% penetrance. A relationship between a mutation in the cationic trypsinogen gene (protease serine 1, *PRSS1*) and hereditary pancreatitis was identified in 1996[24]. In 2000, a mutation in the serine protease inhibitor gene (*Kazal* type 1: *SPINK1*) was reported to be related to chronic idiopathic pancreatitis of unknown cause[25]. Patients with hereditary pancreatitis due to a *PRSS1* gene mutation or relapsing pancreatitis due to a *SPINK1* gene mutation can develop pancreatic exocrine insufficiency and diabetes in the future, and they are a high-risk group for pancreatic cancer[26-28]. The cause of these complications like cancer, as in chronic pancreatitis due to other etiologies, involves hyperplasia and metaplasia of the pancreatic duct epithelium due to recurrent or chronic inflammation. *K-ras* gene mutations also play a role[29]. Diabetes or pancreatic cancer developing in childhood cases has not been reported.

Recently, variants in *CPA1*, which encodes carboxypeptidase A1, were implicated in early onset pancreatitis in children up to 10 years old. The mechanism by which *CPA1* variants confer increased pancreatitis risk may involve misfolding-induced endoplasmic reticulum stress rather than elevated trypsin activity[30].

### *Other causes*

In malignant lymphoma, lymphoma invasion near the head of the pancreas may compress the pancreatic duct and lead to acute pancreatitis[31]. In addition, in solid pseudopapillary neoplasms, intratumoral hemorrhage due to trauma can cause transient tumor enlargement, leading to pancreatic duct obstruction and acute pancreatitis[32].

## PATHOPHYSIOLOGY

To understand the pathophysiology of acute pancreatitis, knowledge about the inhibitory mechanisms of activation of pancreatic enzymes under physiological conditions is necessary. In normal pancreatic acinar cells, lysosomes containing cathepsin B, which are involved in intracellular and extracellular digestion, and zymogen granules containing digestive proenzymes, such as trypsinogen, are released; and these inactive proenzymes remain inactivated[33,34]. In addition, even if trypsin is aberrantly activated in the pancreas for some reason, its activity is blocked by pancreatic secretory trypsin inhibitor (PSTI). Moreover, if trypsin leaks into the blood, the endogenous trypsin inhibitors $\alpha_1$-antitrypsin ($\alpha_1$AT) and $\alpha_2$-macroglobulin ($\alpha_2$M) bind to trypsin and suppress its activity (Figure 1)[35]. Anatomically, the sphincter of Oddi located in the duodenal ampulla of Vater prevents reflux of duodenal fluid into the pancreatic duct. Pancreatic duct pressure is also usually higher than bile duct pressure, so there is no bile reflux into the pancreatic duct[23].

Excessive stimulation of pancreatic exocrine secre-

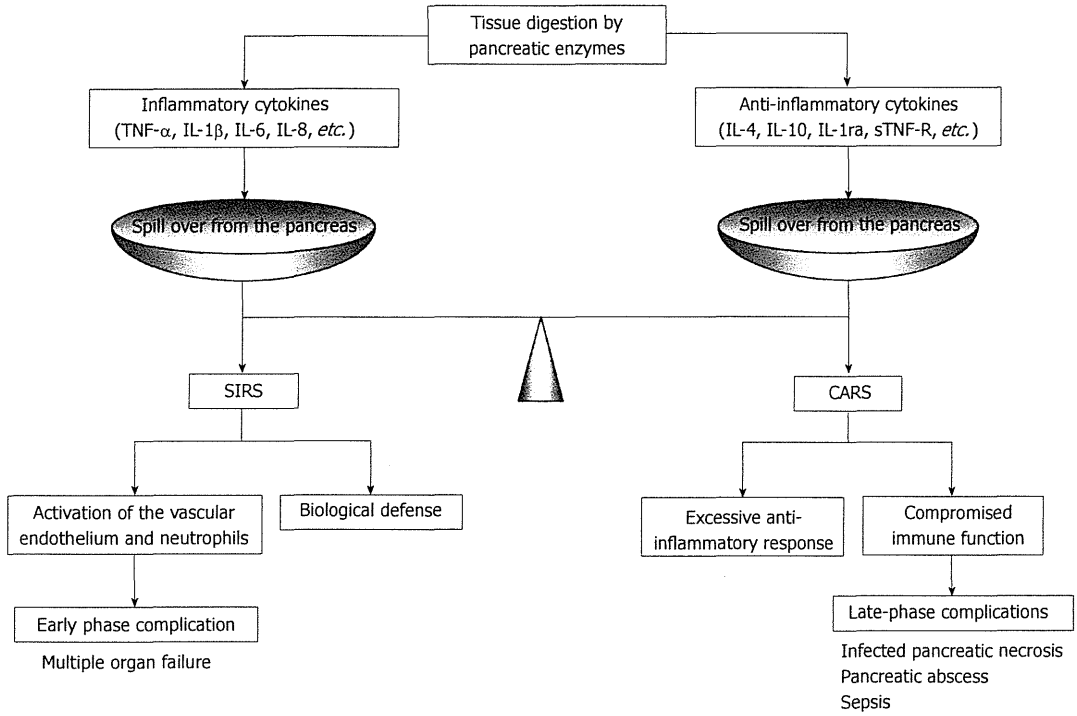


**Figure 2 Compensatory anti-inflammatory response syndrome and systemic inflammatory response syndrome during acute pancreatitis.** TNF: Tumor necrosis factor; IL: Interleukin; sTNF-R: Soluble tumor necrosis factor receptor; CARS: Compensatory anti-inflammatory response syndrome; SIRS: Systemic inflammatory response syndrome.

tions can cause reflux of pancreatic juices and enterokinase, pancreatic duct obstruction, and inflammation. These conditions can disrupt the above-mentioned defense mechanisms, activate trypsin beyond the ability for trypsin inactivation, and increase attacking factors, thus leading to acute pancreatitis[36]. Enterokinase is the most efficient activator, but trypsin itself, lysosomal enzymes (cathepsin B) in pancreatic acinar cells, and neutrophilic enzymes are also activators[34,36]. In experimental models of early acute pancreatitis, blockage of secretion has been suggested as the initiating event, leading to the accumulation of zymogen granules within acinar cells. This event is followed by a co-localization of digestive enzymes and lysosomal enzymes within vacuoles and, finally, an activation of enzymes that cause acute intracellular injury[37]. The activation of zymogen protease in pancreatic acinar cells is thought to play an important role in the development of acute pancreatitis[36,38].

Mild pancreatitis mainly involves the pancreas and local surrounding lesions. It is generally reversible, and about 6 mo after clinical remission, the pancreas recovers its normal morphology and function. In severe pancreatitis, vasoactive substances such as histamine and bradykinin are produced in large amounts with trypsin activation. As this vasoactive process increases, third spacing of fluids and shock due to hypovolemia may occur. In addition, leakage of activated enzymes from the pancreas causes secondary cytokine production. These cytokines trigger the systemic inflammatory response syndrome (SIRS)[39,40]. SIRS results in hyperactivation of macrophages and neutrophils throughout the body and the release of tissue injury mediators; multiorgan failure, including shock, circulatory failure, and acute respiratory distress syndrome (ARDS), may occur[41-43].

Meanwhile, as a biological defense response, anti-inflammatory cytokines and cytokine antagonists are induced to prevent prolongation of SIRS. This predominance of cytokine antagonists is called compensatory anti-inflammatory response syndrome (CARS)[44]. Because CARS inhibits new cytokine production, susceptibility to infection is increased, and infection of vital organs can occur. As a result of infection, endotoxins in the blood stimulate neutrophil aggregation in distal organs, tissue injury mediators are released, and distal organ failure occurs (Figure 2).

## CLINICAL DIAGNOSIS AND ASSESSMENT OF SEVERITY

The diagnosis of acute pancreatitis is in principle based on clinical findings, biochemical tests, and imaging studies. Both a differential diagnosis and assessment of severity are necessary. The etiology of acute pancreatitis in children often differs from that in adults, and differences in the clinical manifestations and course may occur. Therefore, the diagnosis should be made keeping in mind specific features of the disease in children and after obtaining a past medical and family medical history (Figure 3).

### *Clinical manifestations*

More than 90% of adults with acute pancreatitis report

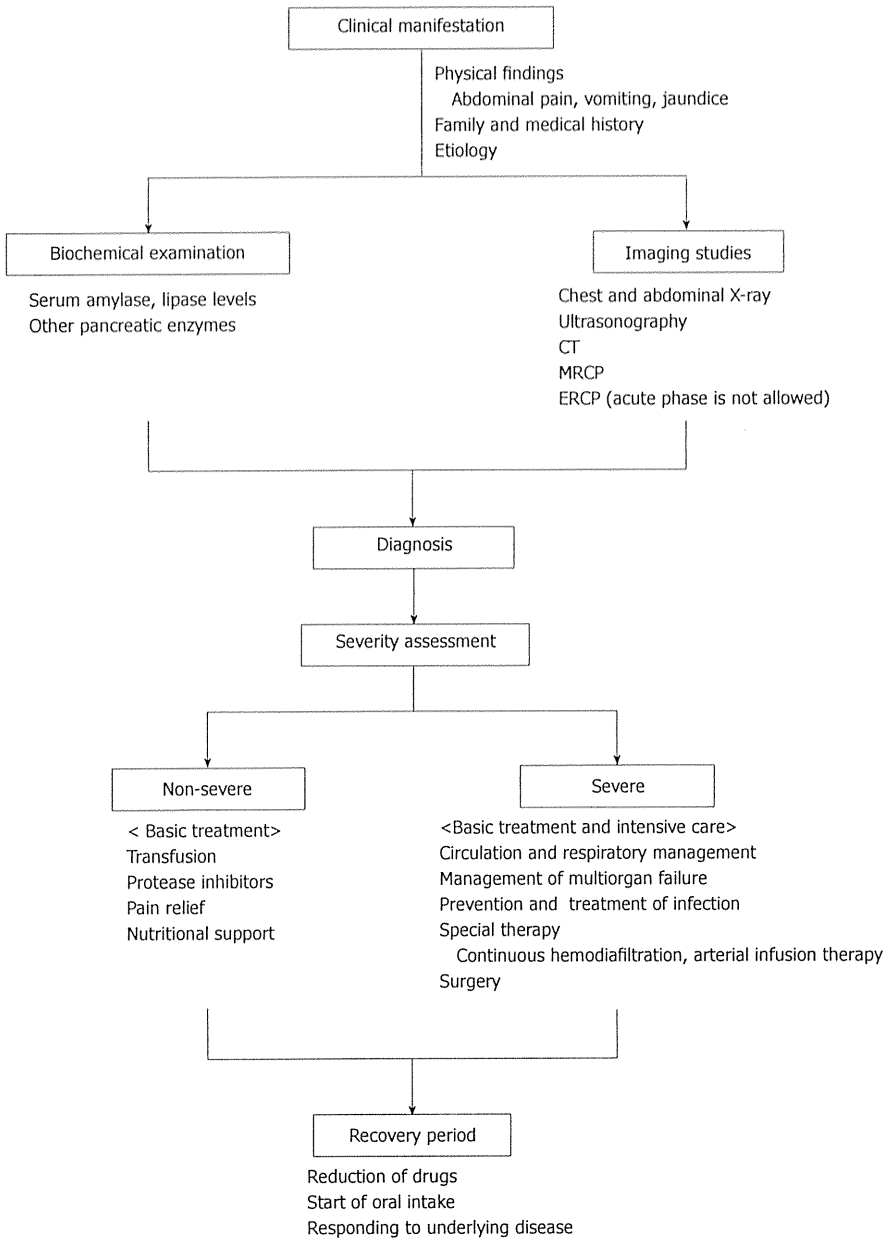


**Figure 3 Clinical diagnosis of acute pancreatitis.** CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; MRCP: Magnetic resonance cholangiopancreatography.

abdominal pain[45,46]. Abdominal pain is also an important early symptom in children. Weizman *et al*[47] reported that all 61 of their pediatric patients with acute pancreatitis initially had abdominal pain. Ziegler *et al*[48] also reported abdominal pain in 40 of 49 patients (82%). Table 3 shows the initial symptoms by age in our series of 135 children with acute pancreatitis[19]. In older children, the frequency of abdominal pain as a first symptom was similar to that in adults, whereas in younger children, vomiting was an important clinical symptom[49]. However, very young children and those with mild pancreatitis sometimes have non-specific abdominal pain. The location, characteristics, and triggers of abdominal pain, as well as physical examination of the abdomen, are important clues in the diagnosis of acute pancreatitis.

Other symptoms may include jaundice, fever, diarrhea, back pain, irritability, and lethargy. Jaundice and clay-colored stools suggest an abnormality of the biliary system such as a choledochal cyst, and there may be a palpable abdominal mass[8]. Infants and toddlers cannot verbalize abdominal pain, but vomiting, irritability, and lethargy are common[48]. In severe acute pancreatitis, children may initially present with shock, followed by symptoms of multiorgan failure, including dyspnea, oliguria, hemorrhage, and mental status changes[1].

### *Laboratory investigations*

The prompt measurement of serum amylase is useful for

**Table 3 First symptoms and chief complaints by age *n* (%)**

| | Age, yr | | | |
|---|---|---|---|---|
| | 1-5 (*n* = 53) | 6-10 (*n* = 47) | 11-17 (*n* = 35) | Total (*n* = 135) |
| Abdominal pain | 46 (86.8) | 39 (83.0) | 32 (91.4) | 116 (85.9) |
| Fever | 21 (39.6) | 21 (44.7) | 10 (28.6) | 52 (38.5) |
| Vomiting | 29 (54.7) | 16 (34) | 6 (17.1) | 51 (37.8) |
| Jaundice | 9 (17) | 2 (4.3) | 0 | 11 (8.1) |
| Back pain | 0 | 1 (2.1) | 5 (14.3) | 6 (4.4) |
| Pale stool | 3 (5.7) | 1 (2.1) | 0 | 4 (3) |
| Diarrhea | 0 | 1 (2.1) | 2 (5.7) | 3 (2.2) |
| Loss of consciousness | 1 (1.9) | 1 (2.1) | 1 (2.0) | 3 (2.2) |
| Others | 5 (9.5) | 2 (4.2) | 2 (5.8) | 9 (6.6) |

a diagnosis of acute pancreatitis[50]. However, elevated levels are also seen in gastrointestinal diseases such as pancreatobiliary tract obstruction and perforative peritonitis, as well as in salivary gland disease and renal failure. Therefore, low disease specificity is a problem. Serum lipase has a sensitivity of 86.5%-100% and specificity of 84.7%-99.0% for diagnosing acute pancreatitis[51]. Thus, its sensitivity is higher compared to serum amylase. In severe pancreatitis, serum lipase levels 7 times higher than normal have been reported within 24 h after onset of pancreatitis[52]. The degree of elevation and serial changes, however, generally do not correlate with disease severity[53]. In acute pancreatitis due to ASNase or valproic acid, which is fairly common in children, serum amylase may not be elevated[13]. Therefore, other serum pancreatic enzymes should also be measured.

### Imaging

When acute pancreatitis is suspected, plain chest and abdominal X-rays are essential. A plain chest X-ray may show a pleural effusion, ARDS, or pneumonia. Although these findings are not specific for acute pancreatitis, they are important for the assessment of disease severity. A plain abdominal X-ray may show an ileus, colon cut-off sign, sentinel loop sign, calcified gallstones, pancreatic stones, or retroperitoneal gas. This information is important in assessing the clinical course of acute pancreatitis and is necessary for a differential diagnosis to rule out other diseases such as gastrointestinal perforation[54,55].

Ultrasonography is a convenient and non-invasive test. It is the test of first choice for screening to diagnose acute pancreatitis in children and for following the clinical course. The ultrasound diagnosis of acute pancreatitis is based on pancreatic morphology, appearance of the pancreatic parenchyma and pancreatic duct, and extrapancreatic findings[56,57].

CT scanning together with ultrasonography is essential for diagnosing acute pancreatitis. CT is useful to evaluate any extrapancreatic lesions, monitor the clinical course, and assess severity. In particular, CT is superior for early assessment of acute pancreatitis when ultrasound findings are nonspecific because of abdominal gas[56,58].

Pancreatitis in children is often caused by pancreatobiliary tract anomalies such as a choledochal cyst or abnormal union of the pancreatobiliary junction. Therefore, ERCP should be performed in pancreatitis of unknown cause. MRCP imaging has also improved and is useful in searching for a cause of acute pancreatitis in children[59]. In particular, MRCP should be performed before ERCP to detect any pancreatobiliary tract disease in children with initial onset of acute pancreatitis of unknown cause. However, in younger children, abnormal union of the pancreatobiliary junction is often difficult to delineate[21].

### Severity assessment

Rapid and accurate assessment of severity is useful for selecting appropriate initial treatment and predicting the prognosis. In 2002, DeBanto *et al*[1] were the first to suggest a scoring system for predicting the severity of acute pancreatitis in children. This system is modified from the Ranson and Glasgow systems and consists of the following eight parameters: age (< 7 years old), weight (< 23 kg), white blood cell count at admission (> 18500 cells/μL), lactic dehydrogenase at admission (> 2000 U/L), 48-h trough $Ca^{2+}$ (< 8.3 mg/dL), 48-h trough albumin (< 2.6 g/dL), 48-h fluid sequestration (> 75 mL/kg per 48 h), and 48-h rise in blood urea nitrogen (> 5 mg/dL). They set the cutoff for predicting a severe outcome at three criteria. However, this scoring system is not exact for Asian children[18]. Lautz *et al*[2] also reported that DeBanto pediatric scores have limited ability to predict acute pancreatitis severity in children and adolescents in the United States. Recently, we reported the usefulness of a new severity assessment that modified the acute pancreatitis severity scoring system of the Ministry of Health, Labour and Welfare of Japan (JPN score) for use in children[60,61]. The parameters of the pediatric JPN score were as follows: (1) base excess ≤ -3 mEq or shock (systolic blood pressure cutoffs according to age group); (2) $PaO_2$ ≤ 60 mmHg (room air) or respiratory failure; (3) blood urea nitrogen ≥ 40 mg/dL [or creatinine (Cr) ≥ 2.0 mg/dL] or oliguria (< 0.5 mL/kg per h); (4) lactate dehydrogenase ≥ 2 × the value of the upper limits; (5) platelet count ≤ $1 \times 10^5/mm^3$; (6) calcium ≤ 7.5 mg/dL; (7) C-reactive protein ≥ 15 mg/dL; (8) number of positive measures in pediatric SIRS score ≥ 3; and (9) age < 7 years old or/and weight < 23 kg. The cutoff for predicting a severe outcome was set at three criteria.

The CT severity index has proven to be very useful in adults[62]. Recently, Lautz *et al*[58] also reported that the CT severity index was superior to a clinical scoring system for identifying children with acute pancreatitis at heightened risk for developing serious complications.

## TREATMENT

The initial treatment for acute pancreatitis is to withhold oral intake of food or fluid to allow the pancreas to rest (*i.e.*, prevent stimulation of pancreatic exocrine secretions). Fluid and electrolyte supplementation, enzyme inhibition therapy, and treatment to relieve pain and

prevent infection are provided. It is important to gradually permit liquid and food intake at a suitable time while continuing treatment. This treatment strategy is based on a consensus conference and evidence accumulated in adult patients. The basic pathogenesis of acute pancreatitis does not greatly differ between adults and children, and the treatment selected for children should be similar to that in adults.

### *Infusion of extracellular fluid*

Because fluid leaks into the surrounding tissue due to inflammation associated with acute pancreatitis, adequate infusion to supplement extracellular fluid is needed during initial treatment. In severe cases, increased vascular permeability and decreased colloid osmotic pressure causes extravasation of extracellular fluids into the surrounding tissue and retroperitoneum and then into the peritoneal cavity and pleural cavity, thus leading to large losses in circulating plasma volume[63]. This acute circulatory impairment causes a rapidly deteriorating condition in early acute pancreatitis.

## DRUG THERAPY

### *Analgesics*

Pain in acute pancreatitis is often intense and persistent, and pain control is required. Appropriate use of analgesics can effectively reduce pain, but this should not interfere with making a diagnosis or providing other treatments[64-66]. The analgesics used include pentazocine, metamizole, and morphine.

### *Antibiotics*

In mild cases of acute pancreatitis, the incidence of infectious complications and mortality rates are low, and prophylactic antibiotics are usually not necessary. However, even in mild cases, antibiotics should be considered if severity increases or complications like cholangitis develop. In severe cases, antibiotics can reduce infectious pancreatitis complications and improve the prognosis[67]. Drugs should be selected with good tissue distribution to the pancreas.

### *Pancreatic protease inhibitors and octreotide*

The Santorini Consensus Conference in 1997 concluded that gabexate mesilate did not contribute to reduced mortality rates in acute pancreatitis[68]. However, in severe acute pancreatitis, continuous infusion of large doses of gabexate mesilate may decrease complications and mortality rates[69]. Similar efficacy in children has been reported, but no clear evidence exists[70]. Protease inhibitors may be a part of combined modality therapy (especially to improve hemodynamic status), but judicious administration is advised in severe cases.

Octreotide was introduced in the early 1980s and offers several advantages over somatostatin, such as a much longer half-life and the option for either subcutaneous or intravenous administration[71]. Octreotide is a powerful inhibitor of exocrine pancreatic secretion and cholecystokinin production[72]. Several studies have evaluated the effect of octreotide on the incidence of clinical pancreatitis after ERCP and postoperative complications such as pancreatic duct fistula following pancreaticoduodenectomy and pancreatic transplantation[73,74]. Effectiveness in reducing complications in acute pancreatitis has not been demonstrated[75]. However, at the case report level, octreotide has been effective in treating pancreatic pseudocysts as a complication in acute pancreatitis and in preventing and treating drug-related pancreatitis due to ASNase, a key drug used to treat lymphocytic leukemia in children[76-78]. As a somatostatin derivative, the most common adverse effect of octreotide is abdominal distention, but adverse effects such as failure to thrive are unlikely if octreotide is given for only 2-6 wk.

## NUTRITIONAL SUPPORT

In severe pancreatitis, the early initiation of enteral nutrition reduces the incidence of infections and leads to shorter hospital stays[79]. An enteral feeding tube is placed in the duodenum or in the jejunum past the ligament of Treitz[80]. This type of nutrition is recommended to reduce stimulation of exocrine pancreatic secretion.

Control of abdominal pain and serum pancreatic enzyme levels should be considered in deciding when to resume oral intake. If serum pancreatic enzymes are decreasing, overall status is good, and abdominal pain has subsided, liquid intake can be started. If serum amylase and lipase levels are approximately less than two times the upper normal limits, a fat-restricted diet should be started[81]. Energy and fat intake can gradually be increased with careful monitoring.

### *Specific treatment for severe pancreatitis*

In patients with infected pancreatic necrosis, surgical drainage and pancreatectomy may be indicated. Specific treatments such as continuous hemodiafiltration to remove humoral mediators and continuous regional arterial infusion of a protease inhibitor and antibiotics have been effective in adults[82,83]. These specific treatments have also been effective and lifesaving in children[84,85]. Although there is no universally acceptable scoring system for predicting the severity of childhood acute pancreatitis, consideration should be given to early transfer of severe patients to a medical center where intensive treatment is available.

### *Endoscopic treatment and surgery*

Anatomic anomalies such as abnormal union of the pancreatobiliary junction are an indication for surgery. In patients with outflow tract obstruction of pancreatic juices caused by ampulla of Vater anomalies or pancreatic divisum, endoscopic sphincterotomy is effective.

Infectious complications should be clinically suspected if fever or signs of inflammation recur during the course of acute pancreatitis. Symptoms often become

prominent 2 wk or more after the onset of pancreatitis. The definitive diagnosis of infected pancreatic necrosis can be made by CT- or ultrasound-guided local fine-needle aspiration and bacteriologic cultures[86,87]. However, this procedure may be difficult in children. Therefore, worsening blood test results, positive blood cultures, positive blood endotoxins, elevated serum procalcitonin levels, and CT findings of the pancreas may serve as clues to a diagnosis of infected pancreatic necrosis[88].

Patients whose general condition is stable can be conservatively treated with antibiotics and observed, but if their condition does not improve, a necrosectomy is required. Necrosectomy early in pancreatitis is associated with a high mortality rate, so it should ideally be performed after the patient's hemodynamic status and general condition have stabilized[89]. Percutaneous necrosectomy, endoscopic transgastric necrosectomy and laparoscopic pancreatic necrosectomy have recently been reported as less invasive treatments in adults and a few children[90-92]. Pancreatic abscesses generally require percutaneous, endoscopic, or surgical drainage.

Pancreatic pseudocysts are cysts that develop due to injury of the pancreatic duct and extravasation of fluid. These occur 4 wk or later after the onset of pancreatitis. Treatment is indicated for pseudocysts if their size does not decrease, if they are accompanied by abdominal pain, or if there are complications of infection or hemorrhage. Endoscopic ultrasound-guided transgastric puncture and drainage can safely be performed in these cases[93,94].

## CONCLUSION

Currently, our approach to acute pancreatitis in children mainly depends on physician experience and knowledge gained from acute pancreatitis in adults. Acute pancreatitis in children tends to be considered a difficult disease, even by pediatric gastroenterologists. However, with recent advances in diagnostic techniques and treatment methods, unfamiliar and difficult diseases are becoming controllable diseases once they are better understood. In order to improve treatment outcomes in patients with childhood acute pancreatitis, future studies focusing on developing a scoring system for predicting the severity of acute pancreatitis and identifying the potential effective treatment modalities for children should be conducted.

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