表3 移植適応基準スコアリング

ポイント	0	1	2
発症から脳症までの日数	0〜5日	6〜10日	11日〜
%PT	20.1%〜	5.1〜20.0%	0〜5%
T-Bil	〜9.9	10〜14.9	15〜
D/T-Bil比	0.7〜	0.5〜0.69	0〜0.49
PLT	10.1万〜	5.1〜10.0万	5.0万以下
肝萎縮	なし	あり	—

脳症(肝性昏睡II度以上)発現時のデータをもって評価
〔持田 智：劇症肝炎：わが国における問題点．肝臓 50：497-506，2009〕

のため病因診断が最重要であり，的確な診断のためのプロセスを経ず，やみくもにグリチルリチン製剤やステロイドを投与したり，安易に新鮮凍結血漿を補給したりすることは避けるべきである．以下に，各種治療の意義を述べる．

1．ビタミンK

%PT値の低下に対しては，まずビタミンKの投与を行う．乳幼児でも1回に5 mg(年長小児以上では10 mg)を静注．%PT値の改善がみられない場合は，肝予備能の重篤な低下が示唆される．

2．ステロイド

血液疾患に基づく急性肝不全や自己免疫性肝炎など，成因によっては早期の適用(メチルプレドニゾロンパルス療法)が有効であるが，一般に成因不明の劇症化予防や治療に有効であるというエビデンスはなく，無作為化試験においてステロイド投与を行った患者の転帰悪化が示されている．易感染性の助長，創傷治癒遅延などのデメリットがあり，劇症化と判断し移植手術を控えた段階での投与は行うべきでない．

3．グルカゴン・インスリン療法

おもにわが国において肝再生促進により血液凝固能の改善に効果があるとされるが，欧米での評価は得られていない．末梢輸液では水分負荷が大きく，血糖・電解質(K)の注意深い管理が必要である．

4．新鮮凍結血漿

生検や中心静脈ルートの確保などの観血的処置を行う際に，必要と考えられる場合に凝固因子補充を目的として使用する．持続低用量の投与には，肝不全の予後を改善させる効果は全くない．

5．浸透圧利尿薬

脳症・脳浮腫の予防・治療にはマンニトール®(0.5〜1.0 g/kg/回)を用いる．グリセオール®は乳酸アシドーシスを招く場合があることと，シトリン欠損症などでは病態を増悪させうるため原則として使用しない．

6．抗ウイルス療法

A型肝炎では，対症療法として輸液やビタミン剤投与を行う．B型肝炎，単純ヘルペスでは，迅速かつ適切な抗ウイルス治療が行われれば，劇症化を阻止して治癒せしめうるので，診断の確定を急ぐべきである．B型キャリア例ではエンテカビルなどの核酸アナログ製剤を投与するが，その効果発現には数日を要するため，インターフェロンを併用した抗ウイルス療法を実施するのが望ましい．

7．抗凝固療法

血小板数が減少している症例では，肝類洞内の微小循環障害が広汎肝壊死の原因であると想定されることから，抗凝固療法を実施する．抗凝固療法にはアンチトロンビンIII製剤と合成蛋白分解酵素阻害薬を用い，ヘパリンは原則として併用しない．

8．高アンモニア対策

腸内細菌によるアンモニア産生を抑制する目的でカナマイシン，ラクツロースを投与し，必要時に浣腸を行って排便を促す．

9．肝不全用アミノ酸製剤

分枝鎖アミノ酸を高配合し，芳香族アミノ酸を除いた組成により，肝性脳症の治療とアンモニアの上昇抑制に有効であるが，高度の肝細胞機能障害においては窒素負荷を助長するため，重症肝不全における適用には考慮を要する．

10．血漿交換(plasma exchange：PE)，血液濾過透析(hemodiafiltration：HDF)

PEの適応は血液凝固能の低下に伴う出血傾向であり，脳症の予防・改善に効果は期待できない．血中有害物質の除去，脳症の進行阻止にはHDFを用いる．PEと持続HDF(continuous HDF：CHDF)の併用によって劇症化例を救命できる可能性があるが，しばしば肝移植までの橋渡しにし

かなり得ない．劇症化が危惧される症例では，顕性の出血傾向が出現する前に中心静脈ルート(ブラッドアクセス)を確保し，いつでも人工肝補助療法を施行できるよう備える．

11．その他

制酸剤・胃粘膜保護剤を投与し，カルニチン，亜鉛製剤，キレート剤(Wilson 病の診断兼治療)の使用を考慮する．グリチルリチン製剤には急性肝不全の予後を改善したり，劇症化を防ぐというエビデンスはない．

劇症化と肝移植について

小児，特に乳幼児の劇症化例では，肝性脳症の発現から脳浮腫や非可逆的中枢神経障害に至る経過が成人に比して急速である．実際に肝移植の対象となる可能性の高低を問わず，肝機能異常に血液凝固能低下や黄疸を伴う場合には，早期から治療手段の1つとして肝移植についても考慮した家族への説明(インフォームド・コンセント：IC)を行う必要がある．

わが国では脳死肝移植の施行率がきわめて低く，緊急的な肝移植に対応するためにはまずドナーの選定と検査が的確かつ迅速に行われなければならない．したがって，IC を行う際には，それに先立って緊急肝移植に対応可能な施設への連絡と十分な情報提供をしておくべきである．ドナー候補があり，患児の病状に対して人工肝補助療法を含めた有効な待機的治療が必要と考えられる場合は，可及的早期に(できればII度以上の肝性昏睡へ進行する前に)患児を移植施行施設へ搬送する．また，原則としてドナー検査は移植施設で進めるため，場合によっては患児の移送より先に家族に移植施設へ出向いてもらうことも必要である．

合併症

①腎機能障害：重症肝疾患では，黄疸の増悪や循環動態の異常などから続発性の腎機能障害(肝腎症候群)を呈することがある．

②消化管出血：Wilson 病など背景に肝硬変が存在する症例では，消化管粘膜のうっ血や静脈瘤があり吐・下血をきたしうる．治療過程のストレス性による急性胃粘膜病変などのリスクも大きい．

③低血糖：肝不全では実効的肝細胞量が減少する．特に年少児では体内グリコーゲン量が少なく低血糖に陥りやすいため，十分なブドウ糖(7.5～15%)の補給が必要である．ただし，シトリン欠損症における肝不全発症時には，高濃度のブドウ糖輸液はむしろ禁忌である．

④溶血性貧血：劇症肝炎型 Wilson 病では，赤血球膜に対する銅毒性により溶血発作をきたす．ハプトグロビン製剤の補充投与を行う．

⑤易感染性：重症化に伴って易感染性が問題となる．しかし，広域スペクトラム抗菌薬の予防投与には慎重であるべきで，初期から各種培養を提出して起因菌の同定に努める．

転帰・長期予後

小児急性肝不全のうち「非昏睡型」の予後は一般に良好である．一方，「昏睡型」(劇症肝不全)の予後は，わが国で肝移植が行われるようになる以前には救命率 30% 程度であったが，1995 年(肝移植導入)以降の救命率は約 70% となっている．ただし，時機を逸すれば，救命し得ても神経学的後遺症を遺すこともある．

また，劇症肝不全の移植では他の疾患に比して重篤な拒絶反応を生ずる率が高いとされるほか，原疾患の再発と考えられる経過をとる例も存在する．

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(虫明聡太郎)

Stool Color Card Screening for Early Detection of Biliary Atresia and Long-Term Native Liver Survival: A 19-Year Cohort Study in Japan

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Objective To evaluate the sensitivity and specificity of a stool color card used for a mass screening of biliary atresia conducted over 19 years. In addition, the age at Kasai procedure and the long-term probabilities of native liver survival were investigated.

Study design From 1994 to 2011, the stool color card was distributed to all pregnant women in Tochigi Prefecture, Japan. Before or during the postnatal 1-month health checkup, the mothers returned the completed stool color card to the attending pediatrician or obstetrician. All suspected cases of biliary atresia were referred for further examination. Diagnosis was confirmed by laparotomy or operative cholangiography for high-risk cases before the Kasai procedure. Patients with biliary atresia were followed from the date of their Kasai procedure until liver transplantation, death, or October 31, 2013, whichever comes sooner.

Results A total of 313 230 live born infants were screened; 34 patients with biliary atresia were diagnosed. The sensitivity and specificity of stool color card screening at the 1-month check-up was 76.5% (95% CI 62.2-90.7) and 99.9% (95% CI 99.9-100.0), respectively. Mean age at the time of Kasai procedure was 59.7 days. According to Kaplan-Meier analysis, the native liver survival probability at 5, 10, and 15 years was 87.6%, 76.9%, and 48.5%, respectively.

Conclusions The sensitivity and specificity of the stool color card have been demonstrated by our 19-year cohort study. We found that the timing of Kasai procedure and long-term native liver survival probabilities were improved, suggesting the beneficial effect of stool color card screening. *(J Pediatr 2015;166:897-902).*

Biliary atresia is the most frequent hepatic cause of death in early childhood, with an incidence of 0.7 in 10 000, 0.6 in 10 000, and 0.5 in 10 000 live births in the US, UK, and France, respectively.[1-3] In Japan, the incidence is greater, affecting approximately 1.0 in 10 000 live births.[4] Biliary atresia is characterized by a complete inability to excrete bile as a result of sclerosing inflammation of the extra, and possibly intra, hepatic bile ducts.[5] Patients with biliary atresia have 3 main clinical features: pale-pigmented stools, prolonged jaundice, and dark urine. Pale-pigmented stools appears within the first month after birth for most patients, and 2-5 months for others.[4,6] Although there is strong evidence that biliary atresia develops before birth and progresses after birth, its etiology remains unclear. The Kasai procedure[7] commonly is used as a first-line treatment for all types of biliary atresia.[8,9]

Prognosis for patients with biliary atresia is primarily related to the patient's age at the time of Kasai procedure and the anatomy of the bile duct remnant.[8-10] It is generally acknowledged that a Kasai procedure performed early, especially one that is performed before the patient reaches 60 days of age, can improve the long-term native liver survival and reduces likelihood of liver transplantations.[10,11] In Japan, 66.1% of living-donor liver transplantations performed for recipients younger than 18 years of age were attributable to biliary atresia.[12]

Serinet et al[10] highlighted the importance of screening for biliary atresia. The concept of a stool color card for mass screening was introduced for the first time to the local population in Tochigi Prefecture by Matsui and Dodoriki in early 1994, which resulted in early Kasai procedure (<60 days of age) in 2 of 3 patients with biliary atresia.[13] Since then, the stool color card had been distributed in the prefecture until March 2011. Subsequently, the concept of stool color card for mass screening was adopted and used in Taiwan in 2002 and resulted in earlier referral of patients with biliary atresia nationwide.[14]

In this present study, we aimed to determine the sensitivity and specificity of stool color card screening during the 19-year period, as well as its effect on the

JBAR Japanese Biliary Atresia Registry

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timing of Kasai procedure and long-term native liver survival in the Tochigi cohort.

Methods

Participants were all infants born to mothers living in Tochigi Prefecture, situated about 100 km north of Tokyo (**Figure 1**; available at www.jpeds.com), from August 1994 to March 2011. Infants born in Tochigi Prefecture to mothers who lived outside of the prefecture before giving birth were not included. Under the Maternal and Child Health Law in Japan acted since 1965, all children in the country go through the same postnatal health management.

The stool color card (3rd edition; **Figure 2**) was placed within the Maternal and Child Health Handbook that was given to all pregnant women by their respective local government according to the Maternal and Child Health Law in Japan. Before or during the infant's 1-month health checkup, the mothers were asked to fill in the corresponding number of the image on the stool color card (**Figure 2**) that most resembled the color of her infant's stool. The card was then submitted to the attending pediatrician or obstetrician. A positive result was defined as a stool color determined by the guardian that matched either image 1, 2, or 3 before or during the infant's 1-month health checkup.

The Department of Pediatrics at the Jichi Medical University in Tochigi Prefecture (as the stool color card office), Japan was notified of all positive cases as soon as possible by telephone or fax. All stool color cards were collected and sent to the stool color card office at Jichi Medical University on a weekly basis. At the office, the cards were rechecked to confirm whether all corresponding numbers were properly recorded and that positive cases had been properly attended to. At the initial phase (first 3 years), all staff was trained on how to manage positive cases detected by the stool color card.

Verbal informed consent was obtained from all participants. The study protocol was reviewed and approved by the Ethics Board of the National Center for Child Health and Development.

Patients with Biliary Atresia and Long-Term Follow-Up

For patients with positive stool color card results, the possibility of other types of infantile cholestasis was eliminated by a pediatric specialist or pediatric hepatologist through clinical, biochemical, radiologic, histologic, and genetic investigations when necessary. A final diagnosis for high-risk cases was determined by laparotomy and/or by operative cholangiography prior to Kasai procedure by a pediatric hepatologist or surgeon. None of the false positive cases underwent any invasive procedures. All patients with biliary atresia received Kasai procedure at the soonest possibility performed in accordance with the Japanese Society of Pediatric Surgeons classification.[15]

Patients with biliary atresia in Tochigi Prefecture received Kasai procedure and were followed up regularly by their respective hospital (across 8 medical centers). Long-term follow-up was possible because all Japanese residents are covered by at least 1 health insurance plan that allows access to any necessary procedures post-Kasai procedure.[16] In addition, pediatric patients with any of the 514 intractable chronic diseases (including biliary atresia), defined by Ministry of Health, Labour and Welfare of Japan, are supported by a medical aid program.[16] Postsurgical procedures in Tochigi Prefecture are consistent with those in other areas of Japan. To ensure that no patient with biliary atresia in Tochigi Prefecture was overlooked, the patient list in our study was compared with that of the medical aid program covering the 514 intractable chronic diseases.

For the investigation of native liver survival probabilities, patients with biliary atresia in this study were observed from the date of Kasai procedure until liver transplantations, death, or October 31, 2013, whichever occurred sooner.

Statistical Analyses

Four reference data sets were used: nationwide data during stool color card screening between 1994 and 2011 from the Japanese Biliary Atresia Registry (JBAR), nationwide data before stool color card screening between 1989 and 1994 from JBAR,[17] Tochigi Prefecture data before stool color card screening between 1987 and 1992,[4] and Tochigi Prefecture data before stool color card screening between 1989 and 1991[18] (**Table I**). To quantify uncertainty, 95% CIs were used. The records of approximately 80%-90% of nationwide patients with biliary atresia diagnosed in hospitals that are part of the Japanese Society of Pediatric Surgeons were documented in JBAR. All patients with biliary atresia in our study were registered in JBAR. According to the Act on the Protection of Personal Information, only statistical data and not individual data can be used. Student t test or one-sample t test was performed to compare age at Kasai procedure. Kaplan-Meier analysis and the log-rank test were used to estimate the native liver survival probabilities with age (in months) as the time scale. IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York) was used for statistical analysis. $P < .05$ was considered statistically significant.

For analytical purposes, all 34 patients with biliary atresia were first considered as a whole (termed "all cases"), and then as 2 separate groups: patients identified using stool color chart and referred promptly (**Table I**).

Results

There were 313 230 live births in Tochigi Prefecture from August 1994 to March 2011 (**Figure 1**). We collected the stool color cards of 264 071 infants, yielding a return rate of 84.3% at the 1-month health check-up; 2014 showed a positive result, and 26 of them were diagnosed with biliary atresia. Finally, a total of 34 patients were diagnosed with biliary atresia in Tochigi prefecture during the study period. A patient with Alagille syndrome detected by the stool color card (stool color corresponding to image 2) at

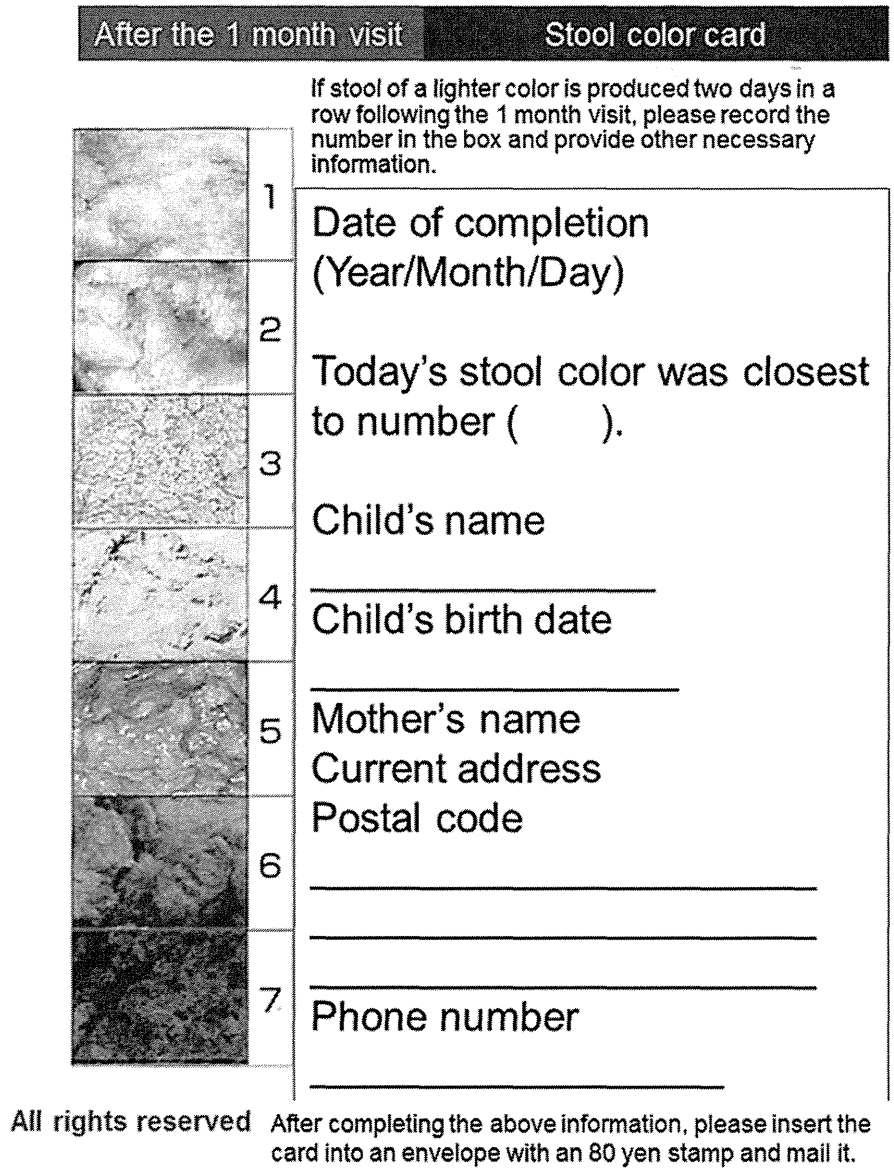

Figure 2. The 3rd edition stool color card used in Tochigi Prefecture from August 1994 to March 2011 consisted of 7 photographic images of stool color taken in both healthy infants and infants with biliary atresia. Images 1-3 denote abnormal stool color, whereas images 4-7 reflect normal stool color.

1-month health checkup was excluded. At the 1-month health checkup, the sensitivity, specificity, positive predictive value, and negative predictive value were 76.5% (26/34, 95% CI 62.2-90.7), 99.9% (313 018/313 196, 95% CI 99.9-100.0), 12.7% (26/204, 95% CI 8.2-17.3), and 99.9% (313 018/313 026, 95% CI 99.9-99.9), respectively. Incidence of biliary atresia was 1.1 in 10 000 infants (34/313 230, 95% CI: 0.7-1.5).

Among the 34 patients with biliary atresia, 8 were missed at the 1-month check-up (**Figure 1**). Of these patients, 2 (Patients 1 and 2) were in a neonatal intensive-care unit for more than a month after birth. Because their overall condition was poor, their guardians and the medical staff overlooked the presence of abnormal stool color. These 2 patients received Kasai procedure at 45 and 88 days of age, respectively. They did not undergo liver transplantations until October 2013. For 3 patients (Patients 3, 4, and 5), their guardians used the stool color card and reported pale-pigmented colored stool at the 1-month health checkup. However, no further examination was performed by their respective pediatricians because the infants did not present with visible jaundice. These 3 patients eventually underwent Kasai procedure at 62, 77, and 109 days after birth, respectively. Subsequently, Patients 3 and 5 underwent liver transplantations at 5 and 12 months of age, respectively. The guardian of 1 of the patients (Patient 6) failed to use the stool color card. The patient received Kasai procedure at 97 days of age and underwent liver transplantation at 72 months. One patient (Patient 7) did

Table I. Age at the time of the Kasai procedure in Tochigi cohort vs reference data, before and during stool color card screening

		Before stool color card screening		During stool color card screening	Tochigi stool color card cohort (August 1994-March 2011)		
	Year	Tochigi	JBAR	JBAR	All patients (N = 34)	Patients identified using stool color card and referred promptly (n = 30)	Patients with type III biliary atresia (n = 25)
Age at time of Kasai procedure, d							
Mean or mean ± SD	1987-1992[4]	70.3					
	1994-2011			67.7	59.7 ± 19.4*,†	56.2 ± 16.5‡,§	59.8 ± 19.1§,¶
	(1994-2002)			(67.8)			
	(2003-2011)			(67.6)			
Median (range)	1987-1992[4]	65.5					
	1994-2011			64.0	58.5 (18-109)	56.5 (18-88)	59.0 (18-109)
	(1994-2002)			(63.0)			
	(2003-2011)			(65.0)			
Number, % (95% CI)							
≤45	1989-1994[17]		18.9				
	1994-2011			20.4	8, 23.5 (9.3-37.8)	8, 26.6 (10.8-42.5)	6, 24.0 (7.3-40.7)
≤60	1989-1991[18]	34.0					
	1989-1994[17]		40.5				
	1994-2011			45.1	19, 55.9 (39.2-72.6)	19, 63.3 (46.1-80.6)**	14, 56.0 (36.5-75.5)
>80	1989-1994[17]		23.1				
	1994-2011			25.3	4, 11.8 (0.9-22.6)**,††	1, 3.4 (2.3-15.6)**,††	2, 8.0 (2.6-18.6)**,††
>90	1989-1991[18]	13.0					
	1989-1994[17]		15.1				
	1994-2011			16.2	2, 5.9 (2.0-13.8)**,††	0, 0.0**,††,‡‡	1, 4.0 (3.7-11.7)**,††,‡‡

*P = .023; ‡P = .001; ¶P = .000 vs JBAR data during screening (1994-2011), 1-sample t test.
†P = .003; §P = .000 vs Tochigi data before screening (1987-1992), 1-sample t test.
**P < .05 vs JBAR data during screening (1994-2011), ††P < .05 vs JBAR data before screening (1989-1994), and ‡‡P < .05 vs Tochigi data before screening (1987-1992).

not show abnormality at the 1-month health checkup. At 1.5 months of age, the patient's guardian noticed pale-pigmented stool and jaundice. The patient received Kasai procedure at 76 days of age and did not undergo liver transplantations. One patient (Patient 8) was not on our list but was later identified through the medical aid list. The patient received Kasai procedure at 66 days of age and did not undergo liver transplantations until October 2013. Therefore, with the exception of Patients 1, 2, 6, and 8, in which the usage of stool color card failed, 30 of the total 34 patients showed stool color changes around the time of the 1-month health checkup.

Demographic Data of Patients with Biliary Atresia

Among the 34 patients with biliary atresia, 11 (32.4%) were male and 23 (67.6%) were female. The numbers of patients who had type I, II, and III biliary atresia were 5 (14.7%), 1 (2.9%), and 25 (73.5%), respectively. The type of biliary atresia in 3 patients was unknown (8.8%). All patients with biliary atresia received Kasai procedure (1 patient with type I biliary atresia received hepaticojejunostomy, and all others received hepatoportoenterostomy).

Age at the Time of Kasai Procedure

The mean age at the time of Kasai procedure was 59.7 days in the 34 patients with biliary atresia (**Table I**). The percentage of Kasai procedure performed before 60 days of age was greater in patients with biliary atresia who were referred promptly after reporting of positive colors. The percentage of Kasai procedure performed after 80 days of age was significantly lower in the Tochigi cohort (**Table I**). The mean age ± SD of Kasai procedure for the 8 patients with biliary atresia who were missed at the 1-month checkup was significantly later compared with the other patients with biliary atresia (n = 26; 77.5 ± 20.4 days vs 54.3 ± 15.8 days; P = .002).

Long-Term Native Liver Survival Probabilities of Patients with Biliary Atresia

As of October 2013, 17 patients received liver transplants and 17 did not. One female patient died at 13 months without receiving a liver transplant. Kaplan-Meier survival analysis with the end point defined as liver transplant, death, or alive as of October 31, 2013, showed the native liver survival probability at 5, 10, and 15 years to be 87.6%, 76.9%, and 48.5%, respectively (**Table II**). The median survival estimated by Kaplan-Meier analysis is the earliest time at when the cumulative survival probability reached 50% or lower. In this study, the median native liver survival was 197.2 (95% CI 136.0-258.4), 207.9 (95% CI 184.6-231.3), and 212.5 (95% CI 146.7-278.4) months in all patients, patients who were referred promptly upon reporting of positive color, and patients with type III biliary atresia, respectively. There was no significant

Table II. Kaplan-Meier analysis of native liver survival at 5, 10, 15, and 20 years in the present study and previous reports

Countries	No. teams/ medical centers	Stool color card	Period	Study design	No. patients	Rate of native liver survival % (SE), y			
						5	10	15	20
Yokohama, Japan[19]	1	No	1970-1986	RCR	80	63.0	54.0	-	44.0
Sendai, Japan[20]	1	No	1975-1980	RCR	60	60.0	60.0	58.0	-
			1981-1986		50	68.0	60.0	51.0	-
US[21]	2	No	1972-1996	RCR	266	49.0	-	-	-
UK[22]	3	No	1999-2009	RCR	443	46.0 (95% CI 41-51)	40.0 (95% CI 34-46)	-	-
France[23]	45	No	1986-2009	RCR	1044	40.0 (1.6)	35.8 (1.6)	32.1 (1.7)	29.6 (2.0)
France[10]	27/45	No	1986-2002	RCR	695	37.9 (2.0)	32.4 (2.0)	28.5 (2.3)	-
Present study	8	Yes	1994-2011	Cohort study					
All patients with biliary atresia					34	87.6 (0.06)	76.9 (0.08)	48.5 (0.11)	-
Patients identified using the stool color card and referred promptly					30	89.6 (0.06)	77.6 (0.08)	55.5 (11.1)	-
Patients with type III biliary atresia					25	86.8 (0.07)	70.5 (0.10)	50.4 (0.12)	-

RCR, retrospective chart review in medical center(s).
In this study, the period of native liver survival was from the point of Kasai procedure until liver transplantation, death, or October 31, 2013, whichever occurred sooner.

difference across the 3 aforementioned groups mentioned on the basis of the log-rank test ($P > .05$).

Discussion

We have conducted a 19-year Japanese cohort study for screening of biliary atresia using the stool color card. The high stool color card sensitivity and specificity achieved are likely to have contributed to more patients with biliary atresia being diagnosed earlier, leading to a timely Kasai procedure. Accordingly, long-term native liver survival probabilities were improved. Serinet et al[10] reported that if every patient with biliary atresia were to undergo the Kasai procedure before 46 days of age, 5.7% of all liver transplantations performed annually in France in patients younger than 16 years could be spared.

In our cohort, the 5-, 10-, and 15-year native liver survival probabilities (**Table II**) were greater compared with studies conducted in US,[21] the UK,[22] and France,[10,23] where stool color card was not used. Notably, the 5- and 10-year native liver survival probabilities increased by more than 20% during 1994-2011 compared with studies conducted in the Japanese cities of Yokohama and Sendai where stool color card was not used (**Table II**).[19,20] The 15-year native liver survival probability estimated by Kaplan-Meier analysis in the Sendai patients was 51%-58% between 1975 and 1986[20] (**Table II**), which is greater than what we found in this study. It might be attributable to the data being collected from a single, highly specialized center, whereas our data were collected from 8 centers.

There are 2 other reports of long-term native liver survival rates in Japanese patients with biliary atresia. Notably, the method for the calculation of native liver survival and/or subjects selected in those studies was different from this study. In our case, we did not consider whether jaundice appeared or not after Kasai procedure. On the basis of JBAR data of 1989, Nio et al[24] reported the 5-year native liver survival rate was 62.0% in 735 patients who did not undergo liver transplantations, and 19 (2.6%) of patients were lost to follow-up. The 10-year native liver survival rate was 52.8% (57/108). The authors also found that when the Kasai procedure was performed at age of <60, 61-90, 91-120, and >120 days among patients with type III biliary atresia registered between 1953 and 2009 whose jaundice disappeared after Kasai procedure, the 10-year native liver survival rate was 74.4% (32/43), 74.5% (41/55), 100.0% (6/6), and 33.3% (1/3), respectively.[11]

Although the stool color card has been adopted and used in Taiwan,[14] Argentina,[25] and Switzerland,[26] the outcome was only reported in Taiwan, where the 5-year native liver survival rate (without jaundice) was 64.3% (18/28).[27]

According to JBAR data, the mean and median age at Kasai procedure was not significantly different between the periods of 1994-2002 and 2003-2011 (**Table I**), suggesting that the management of patients with biliary atresia did not change drastically over the years. Hence, the improvement of the probability of native liver survival revealed in this study is likely to be attributable to the younger age at Kasai procedure as a result of stool color card usage.

Although data of patients who did not use stool color card were available in JBAR, we could not access them because of the restriction imposed by the Act on the Protection of Personal Information. As such, the associations between stool color card usage/early Kasai procedure and the probability of long-term native liver survival cannot be statistically analyzed.

On the basis of our results in Tochigi Prefecture, the stool color card was gradually introduced to 16 other autonomous administrative divisions in Japan between 1999 and 2010. However, only patients from 2 of the regions were followed up. Nonetheless, the mean age of Kasai procedure after the introduction of stool color card was found to be significantly younger in those regions,[28,29] demonstrating excellent reproducibility and effectiveness of the stool color card.

In April 2012, a nationwide biliary atresia screening using an updated edition of the stool color card was initiated. In addition, a pilot study was launched in October 2013 in Beijing, China. The new edition of stool color card consists of digital photographic images to ensure quality control and

greater reproducibility. A modified screening protocol has also been devised. Instead of a single inspection point, the stool color card is now being inspected at 3 intervals; 2 weeks, 1 month, and 1-4 months after birth, allowing us to identify more patients with biliary atresia.

On the basis of our 19-year experience of stool color card usage in the Tochigi Prefecture cohort, the effectiveness of the stool color card, a non-invasive technique, was demonstrated. In particular, the stool color card was beneficial for patients with biliary atresia whose jaundice was not obvious. However, we are aware that the distribution of the stool color card in the community alone is not sufficient to achieve earlier detection of biliary atresia. Proper usage of the stool color card by guardians coupled with a sound knowledge of biliary atresia among healthcare personnel is essential. ■

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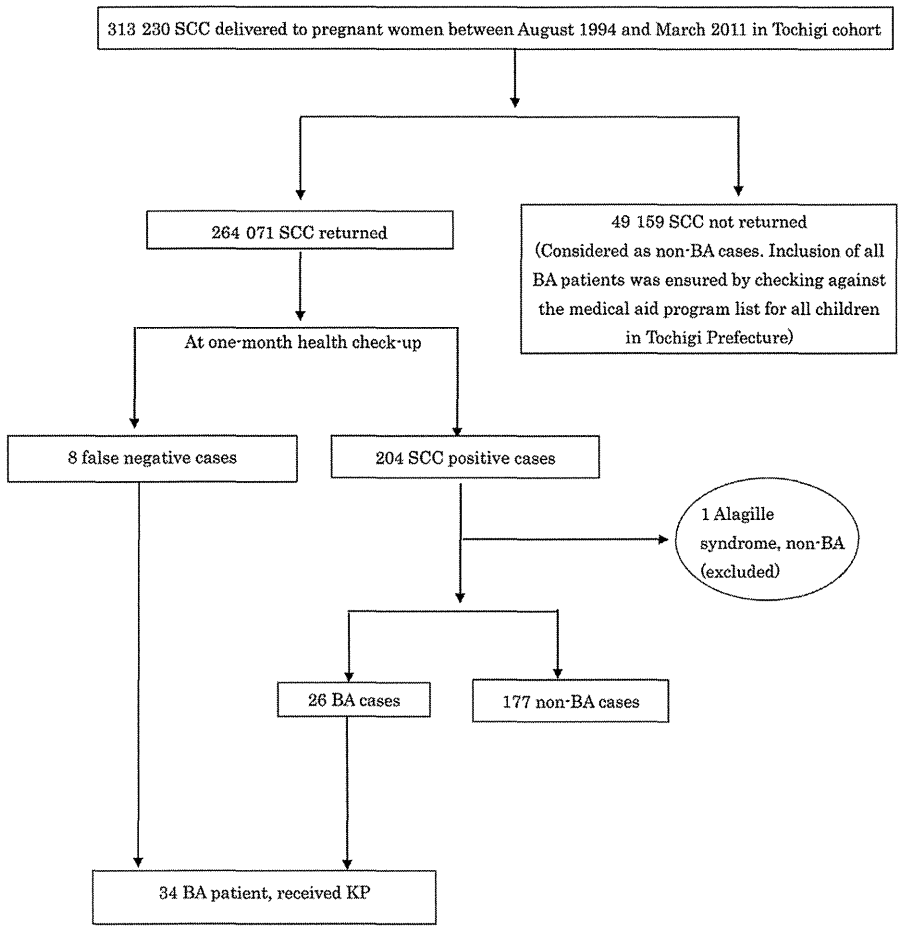

Figure 1. The flowchart for stool color chart screening. The numbers of participants, positive cases, false-negative cases, patients with biliary atresia in the Tochigi cohort from August 1994 to March 2011. *BA*, biliary atresia; *KP*, Kasai procedure; *SCC*, stool color card.

■ 特集 知っておきたい脾臓の話題

胆道閉鎖症術後遠隔期の諸問題

佐々木 英之* 田中 拡 渡邊 智彦 仁尾 正記

はじめに

胆道閉鎖症（BA）は1950年代に葛西手術が開発され，その後の術式ならびに術後管理の進歩により，その治療成績は向上してきた。それに伴い，長期の自己肝生存例も増加している。胆道閉鎖症全国登録（JBAR）では，全登録数2,792例中1,705例（61.1%）に葛西手術による黄疸消失を認めた。またJBARのKaplan-Meier生存率曲線では，20年自己肝生存率が48.5%である[1]。またJBARにおける自己肝生存例の追跡登録によると，10年目では食道静脈瘤が27.0%，脾機能亢進症が37.9%，20年目では食道静脈瘤が34.6%，脾機能亢進症が36.4%に認められていた[1]。また北米のグループスタディであるChildhood Liver Disease Research and Education Network（ChiLDREN）による検討でも，脾機能亢進症は1～25歳までの計163例中57例（35.0%）に認められると報告されている[2]。当科における20年以上自己肝生存を得ている92症例の検討でも，20例（21.7%）がなんらかの続発症を抱えていた[3]。その続発症のうち，主なものの一つは門脈圧亢進症である。

本稿ではBAの自己肝生存例における門脈圧亢進症，とくに脾腫・脾機能亢進症に焦点をあてて述べる。

I．門脈圧亢進症

門脈圧亢進症は肝硬変の進行に伴い発症するため，黄疸消失例では中長期的に注意を要する続発症である。症候としては，消化管の静脈瘤形成と脾機能亢進症，ならびに続発性肺血流異常がある。BAを含めた肝硬変に伴う脾腫の発生機序としては，肝内の抵抗増加に伴い門脈圧が亢進し，脾臓のうっ血により生ずると考えられてきた。組織学的には，静脈血のうっ滞の結果としての脾洞の拡大と増生が観察される一方で，近年のPETを用いた検討では脾臓の局所血流量は増加し，組織学的にも脾柱の増生，脾柱筆毛動脈の拡張，脾索毛細血管の脾洞との吻合など，脾動脈の血流増加を示す所見が認められる。つまり，肝硬変に伴う脾腫はうっ血のみではなく，肝硬変に伴うhyperdynamic circulationによる脾動脈血流増加も関与していることが考えられる[4]。

II．脾機能亢進症

1．消化管静脈瘤

門脈圧亢進により脾硬度が上昇することが知られており，脾硬度と門脈圧亢進症の症候との関連の報告がみられる。門脈圧亢進症で脾硬度が上昇する機序としては，脾血流の増加とうっ血による脾内圧上昇と，脾索の過形成や線維化が脾硬度上昇に影響していると推測されている。

種々の肝疾患において，脾硬度と食道静脈瘤の関連性の報告が認められる。BAと脾硬度とについては，論文としては発表されていないが，2014年の第41回日本胆道閉鎖症研究会において「胆道閉鎖症における肝・脾硬度測定による食道静脈瘤の予測診断能」という演題で発表が行われ，脾硬度と食道静脈瘤の関連について発表がなされた。今後さらに検討がなされるものと期待される。

2．続発性肺血流異常

肝硬変に伴う続発性肺血流異常の発症は重要な

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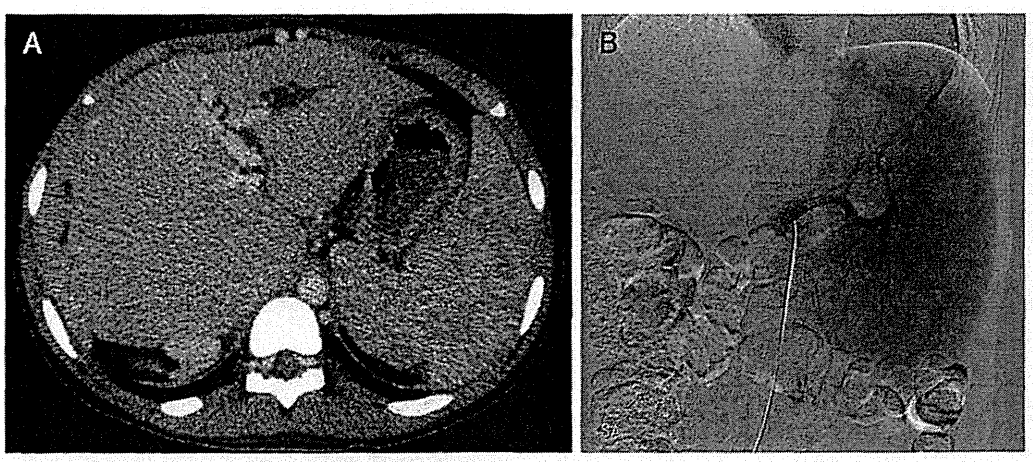

図 1 PSE 前の CT (A), PSE 前の血管造影 (B)

続発症の一つである。その発症には，エンドセリンや一酸化窒素などの因子の関与も示唆されている。門脈肺高血圧症の発症には，門脈圧亢進症に伴う hyperdynamic circulation による高心拍出状態に伴う肺循環における shear stress をきたし，肺血管のリモデリングをきたすという説や，エンドセリンなどの液性因子の関与などが考えられている。エンドセリンは血管収縮因子であり，ET-1, 2, 3 の 3 つのアイソフォームがある。肝硬変患者では末梢血の ET-1 濃度の上昇が認められる。また肝硬変において，脾臓はエンドセリンの産生に関与しているという報告も認められる[5]。さらに門脈肺高血圧症では，エンドセリン血中濃度が高かったという報告もある[6]。

また ChiLDREN での検討では，肝肺症候群を 163 例中 8 例 (4.9%) に認めていたが，8 例中 6 例では脾機能亢進症を伴っていた[2]。当科の検討でも，続発性肺血流異常を認めた 6 例中 3 例に部分的脾動脈塞栓術 (partial splenic embolization: PSE) を要する脾機能亢進症を認めていた[7]。その観点での脾機能亢進症の制御も，今後の研究のテーマとなりうると思われる。

Ⅲ. 脾機能亢進症に対する処置

一般的には肝硬変に伴う脾臓への処置を行う目的としては，脾機能亢進症に由来する血小板減少の改善，門脈圧亢進症 (胃食道静脈瘤，出血傾向) の改善，生体肝移植における small-for-size syndrome における門脈圧，門脈血流のコントロールがあげられる[8]。

脾機能亢進症においては脾臓での過剰な血流貯留，溶血により貧血が生じ，脾臓での血小板貯留の増加，半減期の減少，脾臓での血小板の破壊亢進により血小板減少が生ずる[8]。

脾機能亢進症に対しては，BA の小児例では脾摘後重症感染症の発症予防目的で PSE が選択される[9]。当科における PSE の適応としては，① 脾腫，② 血小板数が 10 万以下で減少傾向が進行する場合，③ 臨床的出血症状ありの 3 点が揃う場合としている (図 1)。

PSE は塞栓率 70～80% を目標に行い，塞栓物質はゼルフォームを用いている (図 2)。

PSE 後の疼痛，発熱は高頻度に認められるものの，解熱鎮痛剤で対応は可能である。また脾膿瘍合併のリスクも指摘されているが，当科では術後に十分な抗菌薬を投与することで，その発生をみていない。

脾臓への処置を行うと，門脈血流の血行動態に変化がみられる。一般的には，門脈血流は減少するとする報告が多い。しかし当科の経験において，PSE 後に食道静脈瘤の増悪を認めた症例もあり，注意を要する。当科では PSE 前後に上部消化管内視鏡検査を施行し，静脈瘤の変化に注意している。

比較的早い時期に重症な脾機能亢進症がみられても，PSE により，その後の安定した経過が望める[9]。

成人の肝硬変症例に対して PSE を施行し，その

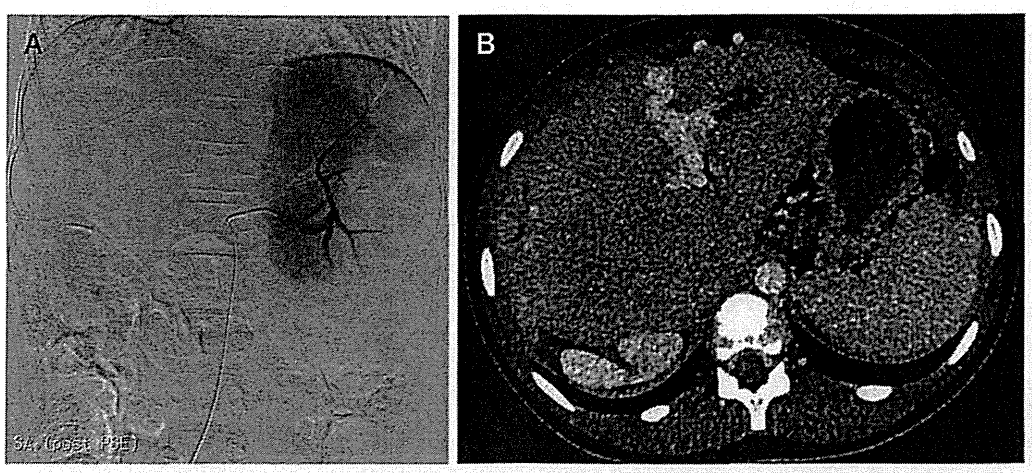

図 2 PSE後の血管造影（A），PSE施行後1年のCT（B）

後のアシアロシンチによる肝予備能評価で改善が認められたとする報告もある[10]。すなわち，肝臓と脾臓との臓器相関（肝脾相関）の観点から，脾腫が肝硬変の増悪に与える影響を考慮すると，脾機能亢進症への適切な介入はより意義が大きいと考えられる。年長児から成人では，脾摘後重症感染症について危険性が低下するため，PSEか脾摘かの選択は，肝病態や治療の見通し，肝移植を要する可能性などで決定される。PSEと脾摘とを比較した場合に，PSEでは脾機能亢進症が再発することが懸念される。当科の検討では，36例中11例（30.6%）でPSE後に再び血小板減少が認められた[9]。しかし，脾機能亢進症の再発の有無はその後の予後に影響を与えていなかった[9]。

背景の肝硬変が高度な場合は肝移植が必要となるが，正確な肝病態ならびに合併症管理が行われれば，消化管出血ならびに脾機能亢進症のみで肝移植となることはまれである。

おわりに

BAにおける脾腫・脾機能亢進症について述べた。前述のとおり，本症における脾機能亢進症は適切に管理を行えば，それのみで肝移植の適応となることはない。さらに，近年の脾臓の免疫や循環に対する機能の解明に伴い，脾機能亢進症の制御により本症の良好な自己肝生存を得る可能性を高めることも期待される。今後のこの方面からの研究の一助となれば幸いである。

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胆 道 29巻5号 870～873（2015）

＜報　告＞

先天性胆道拡張症の診断基準 2015

日本膵・胆管合流異常研究会
日本膵・胆管合流異常研究会診断基準検討委員会

膵・胆管合流異常の診療ガイドライン[1][2]が，日本膵・胆管合流異常研究会と日本胆道学会から2012年に作成され，さらに膵・胆管合流異常の診断基準2013[3][4]がその翌年に作成された．そのなかで，膵・胆管合流異常を有し，胆管に拡張を認める例を先天性胆道拡張症とした．そこで，先天性胆道拡張症の診断基準2015においては，いわゆる狭義の先天性胆道拡張症の診断基準を明らかにした．

定　義

先天性胆道拡張症（congenital biliary dilatation）とは，総胆管を含む肝外胆管が限局性に拡張する先天性の形成異常で，膵・胆管合流異常を合併するものをいう．ただし，肝内胆管の拡張を伴う例もある．

病　態

胆管拡張と膵・胆管合流異常により，胆汁と膵液の流出障害や相互逆流，胆道癌など肝，胆道および膵に様々な病態を引き起こす．

診断基準

先天性胆道拡張症の診断は，胆管拡張と膵・胆管合流異常の両者が画像または解剖学的に証明された場合になされる．ただし，結石，癌などによる胆道閉塞に起因する後天性，二次的な胆道拡張は除外する．

1．胆管拡張の診断

胆管拡張は，胆管径，拡張部位，拡張形態の特徴を参考に診断する．

1）胆管径

胆管径は，超音波検査，MRCP，CT（MD-CTのMPR像ほか）などの胆道に圧のかからない検査によって，総胆管の最も拡張した部位の内径を測定する．

胆管径は，年齢により変化するので，超音波検査による年齢別の胆管径の上限値（表1）を参考にする．

2）拡張部位

胆管拡張は，総胆管を含むものとする．また，総胆管を含む肝外胆管の拡張と同時に肝内胆管が拡張している例も，先天性胆道拡張症に含める．

3）拡張形態

拡張形態は，嚢胞型と円筒（紡錘）型の2つに分けられる．

狭義の先天性胆道拡張症は，戸谷分類（図1）のIa型，Ic型，IV-A型で表現される．

2．膵・胆管合流異常の診断

膵・胆管合流異常の診断は，先天性胆道拡張症の診断に必須であり，膵・胆管合流異常の診断基準2013に準拠してなされる．

解　説

1．定義

先天性胆道拡張症は，従来欧米ではcongenital choledochal cystと呼ばれてきた．Congenital choledochal cystは1959年にAlonso-Lejら[5]により3つのタイプに分類された．その後，Alonso-Lejの分類を基本として1977年にTodaniらは新たな分類[6]を提唱し，欧米で広く引用されるようになった．その後，先天性胆道拡張症は膵・胆管合流異常を高率に合併することが分か

日本膵・胆管合流異常研究会診断基準検討委員会
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表1　超音波検査による胆管拡張の年齢別参考値

年齢	基準値	上限値	拡張の診断
0歳	1.5mm	3.0mm	3.1mm 以上
1歳	1.7mm	3.2mm	3.3mm 以上
2歳	1.9mm	3.3mm	3.4mm 以上
3歳	2.1mm	3.5mm	3.6mm 以上
4歳	2.3mm	3.7mm	3.8mm 以上
5歳	2.4mm	3.9mm	4.0mm 以上
6歳	2.5mm	4.0mm	4.1mm 以上
7歳	2.7mm	4.2mm	4.3mm 以上
8歳	2.9mm	4.3mm	4.4mm 以上
9歳	3.1mm	4.4mm	4.5mm 以上
10歳	3.2mm	4.5mm	4.6mm 以上
11歳	3.3mm	4.6mm	4.7mm 以上
12歳	3.4mm	4.7mm	4.8mm 以上
13歳	3.5mm	4.8mm	4.9mm 以上
14歳	3.6mm	4.9mm	5.0mm 以上
15歳	3.7mm	5.0mm	5.1mm 以上
16歳	3.7mm	5.1mm	5.2mm 以上
17歳	3.7mm	5.2mm	5.3mm 以上
18歳	3.8mm	5.3mm	5.4mm 以上
19歳	3.8mm	5.4mm	5.5mm 以上
20歳代	3.9mm	5.9mm	6.0mm 以上
30歳代	3.9mm	6.3mm	6.4mm 以上
40歳代	4.3mm	6.7mm	6.8mm 以上
50歳代	4.6mm	7.2mm	7.3mm 以上
60歳代	4.9mm	7.7mm	7.8mm 以上
70歳代以上	5.3mm	8.5mm	8.6mm 以上

（文献11より引用）

り，戸谷は1995年に膵・胆管合流異常の概念を加えた分類[7]を発表した．その後国内外からの報告で，総胆管の限局性拡張を呈するI型と，I型に肝内胆管の拡張が加わったIV-A型の頻度が非常に高く，Ia型，Ic型およびIV-A型は，ほぼ全例に膵・胆管合流異常を合併するが，他のIb型，II型，III型，IV-B型，V型では膵・胆管合流異常の合併はほとんどみられないことが判明してきた．

そこで本診断基準では，総胆管を含む肝外胆管が限局性に拡張し，全例に膵・胆管合流異常を合併する戸谷Ia型，Ic型とIV-A型の先天性胆道拡張症を，狭義の先天性胆道拡張症と定義した．また，Caroli病，戸谷分類のIa型，Ic型，IV-A型以外で膵・胆管合流異常のない胆道拡張症などは狭義の先天性胆道拡張症に含めないことにした．

2. 病態

先天性胆道拡張症では，胆管拡張やしばしば合併する総胆管の十二指腸側の狭小部（narrow segment）によって胆汁の流出障害が起きる．また，合併する膵・胆管合流異常では，共通管が長く，乳頭部括約筋作用が膵胆管合流部に及ばないため，膵液と胆汁が相互に逆流する．膵液の胆道内への逆流（膵液胆道逆流現象）は高率に胆道癌を発生させ，胆汁の膵管内への逆流（胆汁膵管逆流現象）は膵炎を惹起させることがある．

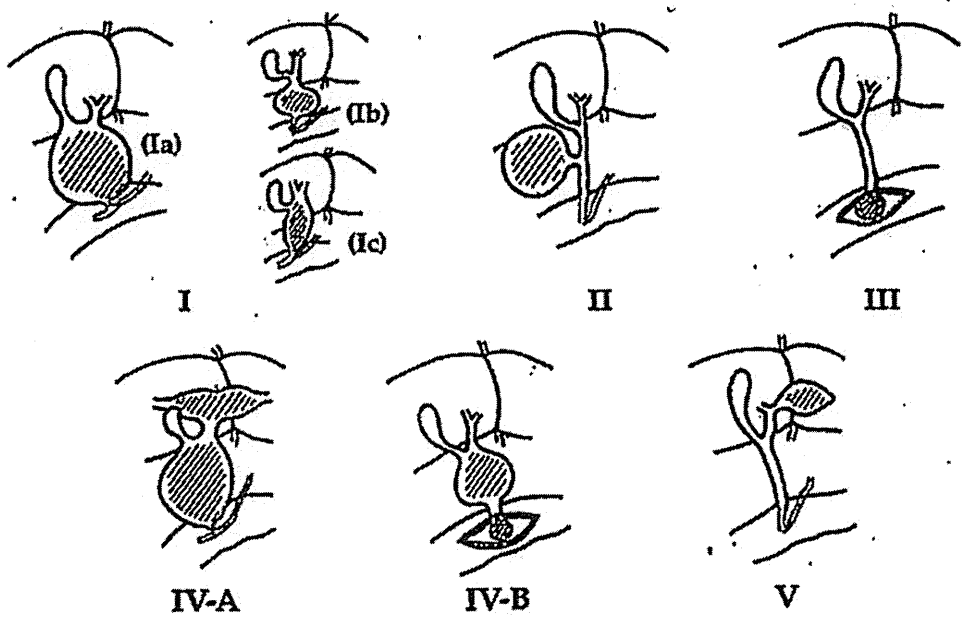

図1　戸谷分類（1995年改変）（文献7より引用）

3. 診断基準

1）胆管径

直接胆道造影（ERCP，経皮経肝胆道造影，術中胆道造影など）は，胆管内圧を上昇させて胆管が拡張する可能性がある検査なので，その計測値は参考にとどめ，胆管拡張の診断は慎重にすべきである．

胆管径は，年齢により変化する[8)~10)]ので，超音波検査による年齢別の胆管径の上限値（表1）[11)]を参考にして拡張の有無について診断する．

2）胆管の拡張形態

先天性胆道拡張症には以下のような胆管の形態的特徴[12)~14)]を示す例が多いので，これらを参考にして診断する．

(1) 拡張した総胆管の十二指腸側に狭小部（narrow segment）がみられる．

(2) 拡張が総胆管から三管合流部を越えて肝臓側に及ぶ場合は，胆嚢管合流部の起始部が限局性に拡張している．

(3) 肝内胆管が限局性に拡張している場合は，肝門部に相対的狭窄がみられる．

(4) 肝内胆管の拡張部とそれより上流の胆管とは著明な口径差がある．

3）膵・胆管合流異常

膵・胆管合流異常とは，解剖学的に膵管と胆管が十二指腸壁外で合流する先天性の形成異常である．膵・胆管合流異常の診断には，画像または解剖学的検索によって，膵管と胆管が異常に長い共通管をもって合流するか異常な形で合流すること，または膵管と胆管が十二指腸壁外で合流することを確認する必要がある．画像診断には，直接胆道造影（ERCP，経皮経肝胆道造影，術中胆道造影など）や，EUSまたはMD-CTのMPR像などを用いる．また，高アミラーゼ胆汁は，膵・胆管合流異常の存在を強く示唆しており有力な補助診断となる．

4. 参考

1）つぎのような所見は，先天性胆道拡張症の存在を疑わせるので診断の参考となる[1)]．

(1) 出生前超音波検査による肝下面の嚢胞性病変

(2) 新生児期の直接型優位の間歇性黄疸

(3) 小児期から繰り返す腹痛発作

(4) 小児の腹痛時の高アミラーゼ血・尿症

(5) 小児の胆道穿孔による胆汁性腹膜炎

2）以下の類義語が使われているが，先天性胆道拡張症（congenital biliary dilatation）を推奨する．

先天性胆管拡張症 congenital bile duct dilatation（戸谷 1995）[7)]

先天性総胆管嚢胞 congenital choledochal cyst（Alonso-Lej 1959）[5)]

総胆管嚢胞 choledochal cyst

本論文に関連し，開示すべき利益相反はなし

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11) 濵田吉則，濵田　洋，高橋良彰，ほか．胆管径か

らみた胆管拡張の定義．胆と膵　2014；35：943—945
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Diagnostic criteria for congenital biliary dilatation 2015

The Japanese Study Group on Pancreaticobiliary Maljunction (JSPBM)
The Committee of JSPBM for Diagnostic Criteria for Pancreaticobiliary Maljunction

Congenital biliary dilatation (CBD) is a congenital malformation involving both extrahepatic bile duct dilatation and pancreaticobiliary maljunction (PBM). Although pathogenesis of bile duct dilatation is unknown, PBM causes reciprocal reflux between the pancreatic juice and bile and results in various biliary and pancreatic pathologies.

For a diagnosis of CBD, both abnormal dilatation of the bile duct and PBM must be evident. Bile duct dilatation should be diagnosed based on age-related limits on the maximum diameter of the common bile duct using diagnostic imaging (e.g., ultrasonography, magnetic resonance cholangiopancreatography, and multiplanar reconstruction imaging by multidetector row computed tomography). Endoscopic retrograde cholangiopancreatography, percutaneous transhepatic cholangiopancreatography, and operative cholangiography would be avoided as bile duct measurement tools. Typical concomitant anatomical characteristics of extra- and intra-hepatic bile ducts should be also considered when diagnosing CBD. Diagnosis of PBM, an abnormally long common channel, and/or an abnormal union between the pancreatic and bile ducts must be established by various radiological imaging.

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Prediction of interindividual differences in hepatic functions and drug sensitivity by using human iPS-derived hepatocytes

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Interindividual differences in hepatic metabolism, which are mainly due to genetic polymorphism in its gene, have a large influence on individual drug efficacy and adverse reaction. Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem (iPS) cells have the potential to predict interindividual differences in drug metabolism capacity and drug response. However, it remains uncertain whether human iPSC-derived HLCs can reproduce the interindividual difference in hepatic metabolism and drug response. We found that cytochrome P450 (CYP) metabolism capacity and drug responsiveness of the primary human hepatocytes (PHH)-iPS-HLCs were highly correlated with those of PHHs, suggesting that the PHH-iPS-HLCs retained donor-specific CYP metabolism capacity and drug responsiveness. We also demonstrated that the interindividual differences, which are due to the diversity of individual SNPs in the CYP gene, could also be reproduced in PHH-iPS-HLCs. We succeeded in establishing, to our knowledge, the first PHH-iPS-HLC panel that reflects the interindividual differences of hepatic drug-metabolizing capacity and drug responsiveness.

human iPS cells | hepatocyte | CYP2D6 | personalized drug therapy | SNP

Drug-induced liver injury (DILI) is a leading cause of the withdrawal of drugs from the market. Human induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (HLCs) are expected to be useful for the prediction of DILI in the early phase of drug development. Many groups, including our own, have reported that the human iPS-HLCs have the ability to metabolize drugs, and thus these cells could be used to detect the cytotoxicity of drugs that are known to cause DILI (1, 2). However, to accurately predict DILI, it will be necessary to establish a panel of human iPS-HLCs that better represents the genetic variation of the human population because there are large interindividual differences in the drug metabolism capacity and drug responsiveness of hepatocytes (3). However, it remains unclear whether the drug metabolism capacity and drug responsiveness of human iPS-HLCs could reflect those of donor parental primary human hepatocytes (PHHs). To address this issue, we generated the HLCs differentiated from human iPSCs which had been established from PHHs (PHH-iPS-HLCs). Then, we compared the drug metabolism capacity and drug responsiveness of PHH-iPS-HLCs with those of their parental PHHs, which are genetically identical to the PHH-iPS-HLCs.

Interindividual differences of cytochrome P450 (CYP) metabolism capacity are closely related to genetic polymorphisms, especially single nucleotide polymorphisms (SNPs), in CYP genes (4). Among the various CYPs expressed in the liver, CYP2D6 is responsible for the metabolism of approximately a quarter of commercially used drugs and has the largest phenotypic variability, largely due to SNPs (5). It is known that certain alleles result in the poor metabolizer phenotype due to a decrease of CYP2D6 metabolism. Therefore, the appropriate dosage for drugs that are metabolized by CYP2D6, such as tamoxifen, varies widely among individuals (6). Indeed, in the 1980s, polymorphism in CYP2D6 appears to have contributed to the withdrawal of CYP2D6-metabolized drugs such as perhexiline from the market in many countries (7). If we could establish a panel of HLCs that better represents the diversity of genetic polymorphisms in the human population, it might be possible to determine the appropriate dosage of a drug for a particular individual. However, it is not known whether the drug metabolism capacity and drug responsiveness of HLCs reflect the genetic diversity, including SNPs, in CYP genes. Therefore, in this study we generated HLCs from several PHHs that have various SNPs on CYP2D6 and then compared the CYP2D6 metabolism capacity and responses to CYP2D6-metabolized drugs between the PHH-iPS-HLCs and parental PHHs.

Significance

We found that individual cytochrome P450 (CYP) metabolism capacity and drug sensitivity could be predicted by examining them in the primary human hepatocytes–human induced pluripotent stem cells–hepatocyte-like cells (PHH-iPS-HLCs). We also confirmed that interindividual differences of CYP metabolism capacity and drug responsiveness that are due to the diversity of individual single nucleotide polymorphisms in the CYP gene could also be reproduced in the PHH-iPS-HLCs. These findings suggest that interindividual differences in drug metabolism capacity and drug response could be predicted by using HLCs differentiated from human iPS cells. We believe that iPS-HLCs would be a powerful technology not only for accurate and efficient drug development, but also for personalized drug therapy.

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Data deposition: The DNA microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE61287).

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To this end, PHHs were reprogrammed into human iPSCs and then differentiated into the HLCs. To examine whether the HLCs could reproduce the characteristics of donor PHHs, we first compared the CYP metabolism capacity and response to a hepatotoxic drug between PHHs and genetically identical PHH-iPS-HLCs (12 donors were used in this study). Next, analyses of hepatic functions, including comparisons of the gene expression of liver-specific genes and CYPs, were performed to examine whether the hepatic characteristics of PHHs were reproduced in the HLCs. To the best of our knowledge, this is the first study to compare the functions between iPSC-derived cells from various donors and their parental cells with identical genetic backgrounds. Finally, we examined whether the PHH-iPS-HLCs exhibited a capacity for drug metabolism and drug responsiveness that reflect the genetic diversity such as SNPs on CYP genes.

Results

Reprogramming of PHHs to Human iPSCs. To examine whether the HLCs could reproduce interindividual differences in liver functions, we first tried to generate human iPSCs from the PHHs of 12 donors. PHHs were transduced with a Yamanaka 4 factor-expressing SeV (SeVdp-iPS) vector (*SI Appendix*, Fig. S1*A*) in the presence of SB431542, PD0325901, and a rock inhibitor, which could promote the somatic reprogramming (8). The reprogramming procedure is shown in *SI Appendix*, Fig. S1*B*. The human iPSCs generated from PHHs (PHH-iPSCs) were positive for alkaline phosphatase (*SI Appendix*, Fig. S1*B*, *Right*), NANOG, OCT4, SSEA4, SOX2, Tra1-81, and KLF4 (Fig. 1*A*). The gene expression levels of the pluripotent markers (*OCT3/4*, *SOX2*, and *NANOG*) in the PHH-iPSCs were approximately equal to those in human embryonic stem cells (ESCs) (*SI Appendix*, Fig. S1*C*, *Left*). The gene expression levels of the hepatic markers [*albumin (ALB)*, *CYP3A4*, and *αAT*] in the PHH-iPSCs were significantly lower than those in the parental PHHs (*SI Appendix*, Fig. S1*C*, *Right*). We also confirmed that the PHH-iPSCs have the ability to differentiate into the three embryonic germ layers in vitro by embryoid body formation and in vivo by teratoma formation (*SI Appendix*, Fig. S2 *A* and *B*, respectively). To verify that the PHH-iPSCs originated from PHHs, short tandem repeat analysis was performed in the PHH-iPSCs and parental PHHs (*SI Appendix*, Fig. S2*C*). The results showed that the PHH-iPSCs were indeed originated from PHHs. Taken together, these results indicated that the generation of human iPSCs from PHHs was successfully performed. It is known that a transient epigenetic memory of the original cells is retained in early-passage iPSCs, but not in late-passage iPSCs (9). To examine whether the hepatic differentiation capacity of PHH-iPSCs depends on their passage number, PHH-iPSCs having various passage numbers were differentiated into the hepatic lineage (Fig. 1*B*). The *tyrosine aminotransferase* (*TAT*) expression levels and albumin (ALB) secretion levels in early passage PHH-iPS-HLCs (fewer than 10 passages) were higher than those of late passage PHH-iPS-HLCs (more than 14 passages). These results suggest that the hepatic differentiation tendency is maintained in early passage PHH-iPSCs, but not in late passage PHH-iPSCs. In addition, the hepatic functions of late passage PHH-iPS-HLCs were similar to those in the HLCs derived from late passage non–PHH-derived iPS cells (such as dermal cells, blood cells, and Human Umbilical Vein Endothelial Cells (HUVEC)-derived iPS cells) (*SI Appendix*, Fig. S3). Therefore, PHH-iPSCs, which were passaged more than 20 times, were used in our study to avoid any potential effect of transient epigenetic memory retained in parental PHHs on hepatic functions.

HLCs Were Differentiated from PHH-iPSCs Independent of Their Differentiation Tendency. To compare the hepatic characteristics among the PHH-iPS-HLCs that were generated from PHHs of

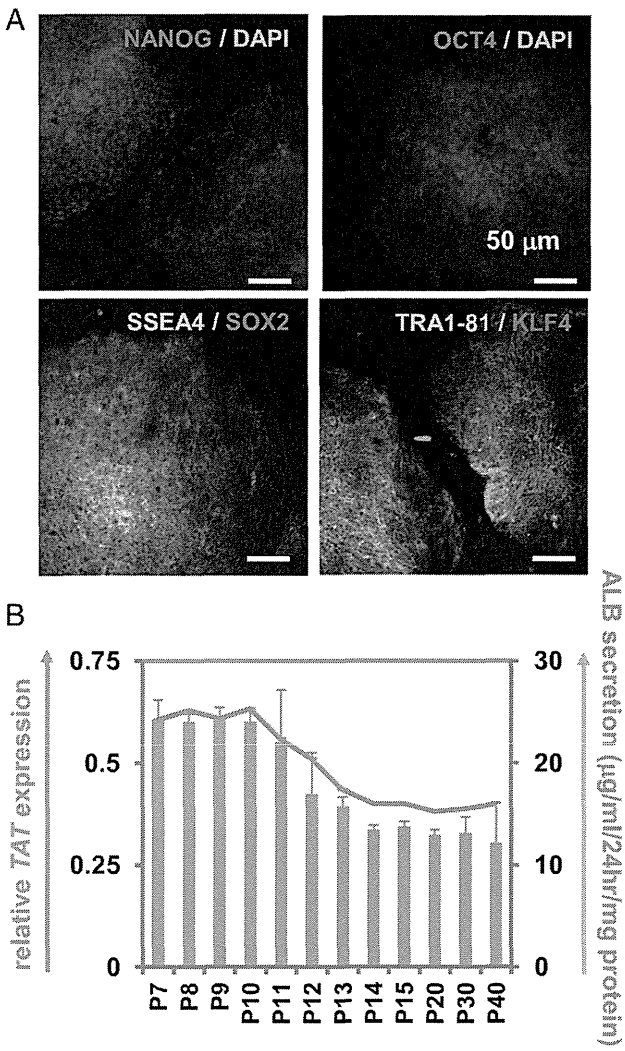

Fig. 1. Establishment and characterization of human iPSCs generated from PHHs. (*A*) The PHH-iPSCs were subjected to immunostaining with anti-NANOG (red), OCT4 (red), SSEA4 (green), SOX2 (red), TRA1-81 (green), and KLF4 (red) antibodies. Nuclei were counterstained with DAPI (blue) (*Upper*). (*B*) The *TAT* expression and ALB secretion levels in the PHH-iPS-HLCs (P7–P40) were examined. On the *y* axis, the gene expression level of *TAT* in PHHs was taken as 1.0.

the 12 donors, all of the PHH-iPSCs were differentiated into the HLCs as described in Fig. 2*A*. However, the differences in hepatic function among PHH-iPS-HLCs could not be properly compared because there were large inter-PHH-iPSC line differences in the hepatic differentiation efficiency based on ALB or asialoglycoprotein receptor 1 (ASGR1) expression analysis (Fig. 2*B*). In addition, there were also large inter-PHH-iPS-HLC line differences in ALB or urea secretion capacities (Fig. 2*C*). These results suggest that it is impossible to compare the hepatic characteristics among PHH-iPS-HLCs without compensating for the differences in the hepatic differentiation efficiency. Recently, we developed a method to maintain and proliferate the hepatoblast-like cells (HBCs) generated from human ESCs/iPSCs by using human laminin 111 (LN111) (10). To examine whether the hepatic differentiation efficiency could be made uniform by generating the HLCs following purification and proliferation of the HBCs, the PHH-iPS-HBCs were cultured on LN111 as

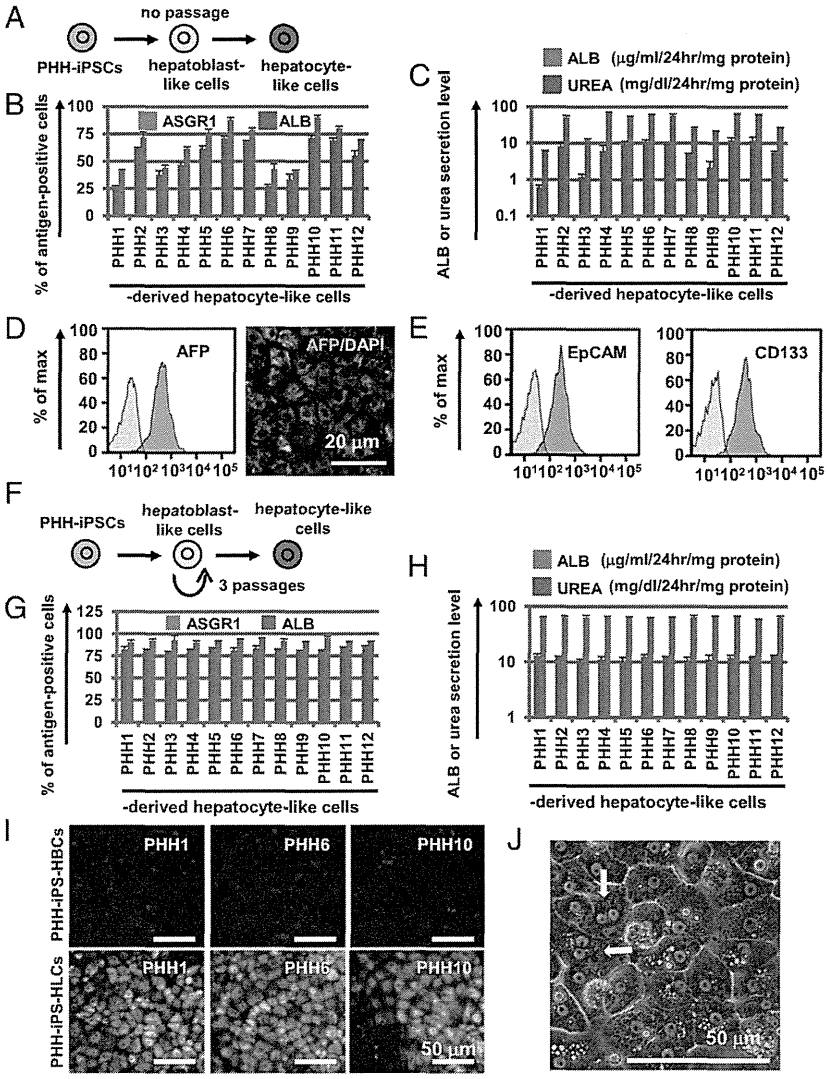

Fig. 2. Highly efficient hepatocyte differentiation from PHH-iPSCs independent of their differentiation tendency. (*A*) PHH-iPSCs were differentiated into the HLCs via the HBCs. (*B*) On day 25 of differentiation, the efficiency of hepatocyte differentiation was measured by estimating the percentage of ASGR1- or ALB-positive cells using FACS analysis. (*C*) The amount of ALB or urea secretion was examined in PHH-iPS-HLCs. (*D*) The percentage of AFP-positive cells in PHH-iPS-HBCs was examined by using FACS analysis (*Left*). The PHH-iPS-HBCs were subjected to immunostaining with anti-AFP (green) antibodies. Nuclei were counterstained with DAPI (blue) (*Right*). (*E*) The percentage of EpCAM- and CD133-positive cells in PHH-iPS-HBCs was examined by using FACS analysis (*Left*). (*F*) PHH-iPSCs were differentiated into the hepatic lineage, and then PHH-iPS-HBCs were purified and maintained for three passages on human LN111. Thereafter, expanded PHH-iPS-HBCs were differentiated into the HLCs. (*G*) The efficiency of hepatic differentiation from PHH-iPS-HBCs was measured by estimating the percentage of ASGR1- or ALB-positive cells using FACS analysis. (*H*) The amount of ALB or urea secretion in PHH-iPS-HLCs was examined. Data represent the mean ± SD from three independent differentiations. (*I*) The PHH1-, 6-, or 10-iPS-HBCs and -HLCs were subjected to immunostaining with anti-αAT (green) antibodies. Nuclei were counterstained with DAPI (blue). (*J*) A phase-contrast micrograph of PHH-iPS-HLCs.

previously described (10), and then differentiated into the HLCs. Almost all of the cells were positive for the hepatoblast marker [alpha-fetoprotein (AFP)] (Fig. 2*D*). In addition, the PHH-iPS-HBCs were positive for two other hepatoblast markers, EpCAM and CD133 (Fig. 2*E*). To examine the hepatic differentiation efficiency of the PHH-iPS-HBCs maintained on LN111-coated dishes for three passages (Fig. 2*F*), the HBCs were differentiated into the HLCs, and then the percentage of ALB- and ASGR1-positive cells was measured by FACS analysis (Fig. 2*G*). All 12 PHH-iPS-HBCs could efficiently differentiate into the HLCs, yielding more than 75% or 85% ASGR1- or ALB-positive cells, respectively. In addition, there was little difference between the PHH-iPSC lines in ALB or urea secretion capacities (Fig. 2*H*). Although there were large differences in the hepatic differentiation capacity among the PHH1/6/10 (Fig. 2*B*), PHH1/6/10-iPS-HBCs could efficiently differentiate into the HLCs that homogeneously expressed αAT (Fig. 2*I*). After the hepatic differentiation of the PHH-iPS-HBCs, the morphology of the HLCs was similar to that of the PHHs: polygonal with distinct round binuclei (Fig. 2*J*). These results indicated that the hepatic differentiation efficiency of the 12 PHH-iPSC lines could be rendered uniform by inducing hepatic maturation after the establishment of self-renewing HBCs. Therefore, we expected that differences in the hepatic characteristics among the HLCs generated from the 12 individual donor PHH-iPS-HBCs could be properly compared. In addition, the hepatic differentiation efficiency could be rendered uniform not only in the PHH-iPSC lines but also in non–PHH-iPSC lines and human ESCs by performing hepatic maturation after the establishment of self-renewing HBCs (*SI Appendix*, Fig. S4). In Figs. 3 and 4, the HLCs were differentiated after the HBC proliferation step to normalize the hepatic differentiation efficiency.

PHH-iPS-HLCs Retained Donor-Specific Drug Metabolism Capacity and Drug Responsiveness. To examine whether the hepatic functions of individual PHH-iPS-HLCs reflect those of individual PHHs, the CYP metabolism capacity and drug responsiveness of PHH-iPS-HLCs were compared with those of PHHs. PHHs are often used as a positive control to assess the hepatic functions of the HLCs, although in all of the previous reports, the donor of PHHs has been different from that of human iPSCs. Because it is generally considered that CYP activity differs widely among individuals, the hepatic functions of the HLCs should be compared with those of genetically identical PHHs to accurately evaluate the hepatic functions of the HLCs. The CYP1A2, -2C9, and -3A4 activity levels in the PHH-iPS-HLCs were ~60% of

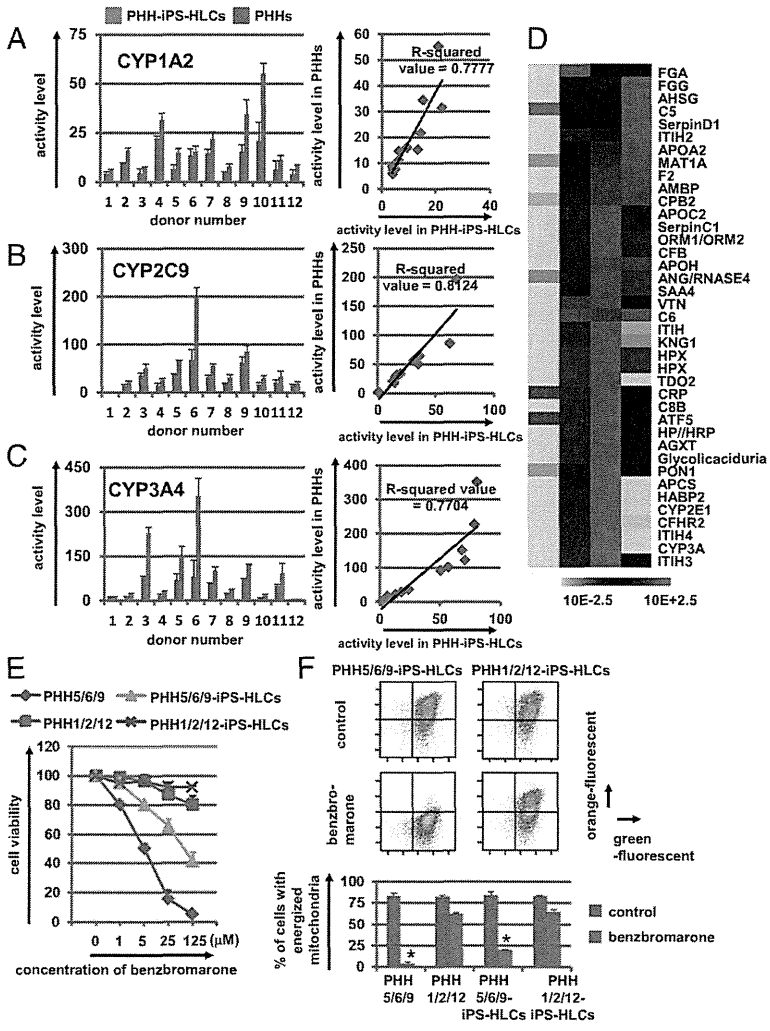

Fig. 3. The drug metabolism capacity and drug responsiveness of PHH-iPS-HLCs were highly correlated with those of their parental PHHs. (*A–C*) CYP1A2 (*A*), -2C9 (*B*), and -3A4 (*C*) activity levels in PHH-iPS-HLCs and PHHs were measured by LC-MS/MS analysis. The R-squared values are indicated in each figure. (*D*) The global gene expression analysis was performed in PHH9-iPSCs, PHH9-iPS-HLCs, PHH9s, and HepG2 (PHH-iPSCs, PHH-iPS-HLCs, and PHHs are genetically identical). Heat-map analyses of liver-specific genes are shown. (*E*) The cell viability of PHH5/6/9, PHH1/2/12, PHH5/6/9-iPS-HLCs, and PHH1/2/12-iPS-HLCs was examined after 24 h exposure to different concentrations of benzbromarone. The cell viability was expressed as a percentage of that in the cells treated only with solvent. (*F*) The percentage of cells with energized mitochondria in the DMSO-treated (control, *Upper*) or benzbromarone-treated (*Lower*) cells based on FACS analysis. Double-positive cells (green+/orange+) represent energized cells, whereas single-positive cells (green+/orange−) represent apoptotic and necrotic cells. Data represent the mean ± SD from three independent experiments (*Lower Graph*). Student *t* test indicated that the percentages in the "control" were significantly higher than those in the "benzbromarone" group ($P < 0.01$). The "PHH5/6/9" represents the average value of cell viability (*E*) or mitochondrial membrane potential (*F*) in PHH5, PHH6, and PHH9. The "PHH1/2/12" represents the average value of cell viability or mitochondrial membrane potential in PHH1, PHH2, and PHH12. PHH5, PHH6, and PHH9 were the top three with respect to CYP2C9 activity levels, whereas PHH1, PHH2, and PHH12 had the lowest CYP2C9 activity levels.

those in the PHHs (Fig. 3 *A–C* and *SI Appendix*, Fig. S5). Interestingly, the CYP1A2, -2C9, and -3A4 activity levels in the PHH-iPS-HLCs were highly correlated with those in the PHHs (the R-squared values were more than 0.77) (Fig. 3 *A*, *B*, and *C*, respectively). These results suggest that it would be possible to predict the individual CYP activity levels through analysis of the CYP activity levels of the PHH-iPS-HLCs. Because the average and variance of CYP3A4 activity levels in PHH-iPS-HLCs, non–PHH-iPS-HLCs, and human ES-HLCs were similar to each other (*SI Appendix*, Fig. S6), the drug metabolism capacity of PHH-iPS-HLCs might be similar to that of nonliver tissue-derived iPS-HLCs and human ES-HLCs. Therefore, it might be possible to predict the diversity of drug metabolism capacity among donors by using nonliver tissue-derived iPS-HLCs and human ES-HLCs as well as PHH-iPS-HLCs. On the other hand, the CYP induction capacities of PHH-iPS-HLCs were weakly correlated with those of PHHs (*SI Appendix*, Fig. S7 *A–C*). To further investigate the characteristics of the HLCs, DNA microarray analyses were performed in genetically identical undifferentiated iPSCs, PHH-iPS-HLCs, and PHHs. The gene expression patterns of liver-specific genes, CYPs, and transporters in the PHH-iPS-HLCs were similar to those in PHHs (Fig. 3*D* and *SI Appendix*, Fig. S7 *D* and *E*, respectively). Next, the hepatotoxic drug responsiveness of PHH-iPS-HLCs was compared with that of PHHs. Benzbromarone, which is known to cause hepatotoxicity by CYP2C9 metabolism (11), was treated to PHH5/6/9 and PHH5/6/9-iPS-HLCs, which have high CYP2C9 activity, or PHH1/2/12 and PHH1/2/12-iPS-HLCs which have low CYP2C9 activity (Fig. 3*E*). The susceptibility of the PHH5/6/9 and PHH5/6/9-iPS-HLCs to benzbromarone was higher than that of PHH1/2/12 and PHH1/2/12-iPS-HLCs, respectively. These results were attributed to the higher CYP2C9 activity levels in PHH5/6/9 and PHH5/6/9-iPS-HLCs compared with those in PHH1/2/12 and PHH1/2/12-iPS-HLCs. Because it is also known that benzbromarone causes mitochondrial toxicity (12), an assay of mitochondrial membrane potential was performed in benzbromarone-treated PHHs and PHH-iPS-HLCs (Fig. 3*F*). The mitochondrial toxicity observed in PHH5/6/9 and PHH5/6/9-iPS-HLCs was more severe than that in PHH1/2/12 and PHH1/2/12-iPS-HLCs, respectively. Taken together, these results suggest that the hepatic functions of the individual PHH-iPS-HLCs were highly correlated with those of individual PHHs.

Interindividual Differences in CYP2D6-Mediated Metabolism and Drug Toxicity, Which Are Caused by SNPs in CYP2D6, Are Reproduced in the PHH-iPS-HLCs. Because certain SNPs are known to have a large impact on CYP activity, the genetic variability of CYP plays an important role in interindividual differences in drug response. CYP2D6 shows the large phenotypic variability due to genetic polymorphism (13). We next examined whether the PHHs used