**Supplementary methods**

**Immunohistochemistry and histopathology**

All liver specimens used for immunohistochemistry were 4 µm formalin-fixed paraffin-embedded (FFPE) sections. FGF19 immunohistochemistry was performed using Dako Target Retrieval Solution (S1699; Dako, Glostrup, Denmark) and Novolink Polymer Detection System kit (Leica, Newcastle, UK). Primary antibody (1:18000, MAB969-100; R&D Systems, Minneapolis, MN, USA) was incubated for one hour in room temperature (Supplementary Table 7). CK7 staining was performed at Diagnostic Center of Hospital District of Helsinki and Uusimaa in fully automated Ventana Benchmark Ultra IHC system (Ventana, Roche, Basel, Switzerland). Primary antibody (790-4462, Ventana, Roche) was incubated 16 minutes at 37°C. Collagen fibers were stained with Sirius red (ab150681; Abcam) following manufacturer’s instructions.

All scanned images were generated using 3DHISTECH Pannoramic 250 FLASH II digital slide scanner at Genome Biology Unit supported by HiLIFE and the Faculty of Medicine, University of Helsinki, and Biocenter Finland. Proportional CK7 and Sirius red stained areas of liver biopsies were quantified by measuring colour intensity and shade avoiding liver capsule using CaseViewer HistoQuant software (3DHISTECH, Budapest, Hungary).

CK7 stained proliferating bile ducts (0 = no, 1 = moderate, 2 = extensive) and periportal hepatocytes (0 = no staining, 1 = slight, 2 = multifocal periportal, 3 = extends to lobular areas, 4 = diffuse) were scored, and histological liver fibrosis was classified according to the Metavir staging. Two experienced pediatric pathologists, blinded to clinical data, performed scoring until a consensus was reached.

**In situ hybridization**

RNA in situ hybridization was performed on FFPE sections by using RNAscope Multiplex Fluorescent Reagent Kit v2 (323100; Advanced Cell Diagnostics, Newark, CA, USA) according to the manufacturer’s instruction. The target retrieval was performed for 15 min at 98°C and protease plus treatment for 15 min at 40°C. All the probes (Bio-Techne Ltd, Advanced Cell Diagnostics) were hybridized for two hours at 40°C. Localization of FGF19 (Hs-553981-C2) was detected with TSA Plus fluorophore Cyanine 3 (1:1000, NEL745001KT; Akoya Biosciences, Menlo Park, CA, USA), hepatocytes were identified with HPX (Hs-1083421-C1) and fluorescein (1:500, NEL741001KT; Akoya Biosciences), cholangiocytes with KRT19 (Hs- 426221-C3) and Cyanine 5 (1:3000, NEL745001KT; Akoya Biosciences), and activated stellate cells with ACTA2-O4 (Hs-1083431-C3) and Cyanine 5 (1:2000). Positive 3-plex control probe (Hs-320861) was run on all the samples. The sections were counterstained with DAPI.

**Supplementary Tables**

**Supplementary Table 1.** Clinical diagnoses and liver biochemistry presented separately for age-matched cholestatic disease controls with serum and/or liver RNA specimens.

|  |  |  |
| --- | --- | --- |
| Variable | Serum available | Liver RNA available |
| Number | 17 | 13 |
| Age, d | 46 (40-58) | 41 (32-63) |
| Diagnosis |  |  |
| Transient neonatal cholestasis | 8 (47%) | 3 (23%) |
| Bile duct hypoplasia | 2 (12%) | 4 (31%) |
| Alagille syndrome | 2 (12%) | 1 (8%) |
| Cytomegalovirus infection | 2 (12%) | 1 (8%) |
| Alpha-1 antitrypsin deficiency | 1 (6%) | 1 (8%) |
| Duodenal atresia | 1 (6%) | 0 (0%) |
| Progressive familial intrahepatic cholestasis type 2 | 1 (6%) | 0 (0%) |
| Choledochal malformation | 0 (0%) | 1 (8%) |
| Haemophagocytic lymphohistiocytosis and parenteral nutrition | 0 (0%) | 1 (8%) |
| Undetermined cholestasis | 0 (0%) | 1 (8%) |
| Liver biochemistry |  |  |
| Bilirubin, µmol/l | 114 (83-165) | 127 (92-165) |
| Conjugated Bilirubin, µmol/l | 81 (44-128) | 94 (66-123) |
| ALT, U/l | 50 (38-91) | 64 (50-137) |
| AST, U/l | 124 (86-164) | 154 (126-164) |
| GGT, U/l | 161 (68-195) | 192 (161-224) |
| APRI | 0.51 (0.27-0.81) | 0.75 (0.54-1.31) |

Data are presented as median with interquartile range or frequencies. ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase; APRI, aspartate transaminase to platelet ratio index.

**Supplementary Table 2.** List of antibodies and staining colours used in the study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target | Catalog number | Manufacturer | Positive control | Negative control |
| FGF19 (IHC) | MAB969-100 | R&D Systems | - | Normal liver, ovary |
| CK7 (IHC) | 790-4462 | Ventana | Normal liver (cholangiocytes) | Normal liver (parenchyme) |
| Sirius red (staining) | ab150681 | Abcam | Normal liver (capsule), portal plates | Normal liver (parenchyme) |
| FGF19 (ISH) | Hs-553981-C2 | Bio-Techne Ltd | - | Normal liver, ovary |
| HPX (ISH) | Hs-1083421-C1 | Bio-Techne Ltd | Normal liver (hepatocytes) | Normal liver (cholangiocytes), ovary |
| KRT19 (ISH) | Hs- 426221-C3 | Bio-Techne Ltd | Normal liver (cholangiocytes) | Normal liver (parenchyme), ovary |
| ACTA2 (ISH) | Hs-1083431-C3 | Bio-Techne Ltd | - | Normal liver (cholangiocytes), ovary |

ACTA2, actin alpha 2; CK7, cytokeratin 7; FGF19, fibroblast growth factor 19; HPX, hemopexin; IHC, immunohistochemistry; ISH, in situ hybridization; KRT19, keratin 19

**Supplementary Table 3.** Clinical characteristics presented separately for BA patients with serum and/or liver RNA specimens.

|  |  |  |
| --- | --- | --- |
| Variable | Serum available | Liver RNA available |
| Number of patients | 74 | 69 |
| Male, n (%) | 32 (43%) | 28 (41%) |
| Place of care, n (%) |  |  |
| Helsinki, Finland | 15 (20%) | 17 (25%) |
| London, UK | 59 (80%) | 52 (75%) |
| Type 3 BA, n (%) | 74 (100%) | 68 (99%) |
| Cystic disease, n (%) | 11 (15%) | 12 (17%) |
| Splenic malformation, n (%) | 8 (11%) | 11 (16%) |
| Associated anomalies, n (%) | 15 (20%) | 15 (22%) |
| Age at KPE, d | 56 (41-75) | 58 (44 - 78) |
| Clearance of jaundice\*, n (%) | 43 (58%) | 37 (54%) |
| Outcome, n (%) |  |  |
| Native liver | 30 (41%) | 24 (35%) |
| Died with native liver | 3 (4%) | 4 (6%) |
| LT | 41 (55%) | 40 (58%) |
| Died after LT | 0 (0%) | 1 (1%) |
| Native liver survival, y | 2.4 (0.9 - 7.5) | 2.2 (0.9 - 6.5) |
| Age at LT, y | 1.1 (0.70 - 2.3) | 1.2 (0.8 - 2.3) |
| Liver biochemistry at KPE |  |  |
| Bilirubin, µmol/l | 145 (124 - 181) | 155 (133 - 184) |
| Conjugated Bilirubin, µmol/l | 114 (96 - 137) | 118 (100 - 144) |
| ALT, U/l | 124 (65 - 213) | 126 (87 - 271) |
| AST, U/l | 199 (147 - 277) | 205 (160 - 285) |
| GGT, U/l | 585 (251 - 875) | 491 (214 - 858) |
| APRI | 0.88 (0.56 - 1.28) | 0.94 (0.59 - 1.45) |

Data are presented as median with interquartile range or frequencies. BA, biliary atresia; KPE, Kasai portoenterostomy; LT, liver transplantation; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase; APRI, aspartate transaminase to platelet ratio index. \*Decrease of serum bilirubin concentration < 20 µmol/l after KPE.

**Supplementary Table 4.** Biochemical and clinical predictors for native liver survival assessed with univariable Cox proportional hazards regression model.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Coefficient | HR (95% CI) | p-value |
| FGF19 | 0.351 | 1.42 (1.14 - 1.78) | 0.002 |
| Conjugated Bilirubin, µmol/l | 0.002 | 1.00 (1.00 - 1.00) | 0.1 |
| GGT, U/l | 0.000 | 1.00 (1.00 - 1.00) | 0.9 |
| APRI | 0.265 | 1.30 (1.09 - 1.55) | 0.003 |
| Total bile acids, µmol/l | 0.002 | 1.00 (1.00 - 1.01) | 0.6 |
| Primary bile acids, µmol/l | -0.001 | 1.00 (0.99 - 1.01) | 0.7 |
| CA, µmol/l | -0.005 | 1.00 (0.98 - 1.01) | 0.6 |
| CDCA, µmol/l | -0.0009 | 1.00 (0.99 - 1.01) | 0.9 |
| Age at KPE, d | 0.013 | 1.01 (1.00 - 1.03) | 0.02 |
| Splenic malformation | 0.441 | 1.56 (0.69 - 3.50) | 0.3 |
| Congenital disorders | -0.144 | 0.87 (0.40 - 1.87) | 0.7 |
| Cystic disease | -0.765 | 0.47 (0.17 - 1.31) | 0.1 |

Biochemical factors measured at time of KPE. Logarithmically transformed serum FGF19 levels were used in Cox proportional hazards regression model. KPE, Kasai portoenterostomy; HR, hazard ratio; CI, confidence interval; GGT, gamma-glutamyltransferase; APRI, aspartate transaminase to platelet ratio index; CA, cholic acids; CDCA, chenodeoxycholic acids.

**Supplementary Table 5.** Internal validation of Cox proportional hazards regression models for serum FGF19 predicting native liver survival in all BA patients and different BA sub-cohorts using Bootstrapping method.

|  |  |  |
| --- | --- | --- |
| Patient cohort | Coefficient (95% CI) | HR (95% CI) |
| All BA patients | 0.364 (0.127 - 0.641) | 1.45 (1.14 - 1.90) |
| Without associated anomalies | 0.434 (0.150 - 0.761) | 1.56 (1.16 - 2.14) |
| KPE <60 days | 0.512 (0.175 - 0.927) | 1.70 (1.19 - 2.53) |
| Patients with COJ\* | 0.583 (0.240 - 1.126) | 1.84 (1.27 - 3.083) |
| Patients without COJ | 0.009 (-0.352 - 0.327) | 1.02 (0.70 - 1.37) |

Coefficients analyzed from 1000 regression model repetitions of resampling and replacements of the data. Logarithmically transformed serum FGF19 levels were used in Cox proportional hazards regression models. \*Decrease of serum bilirubin concentration < 20 µmol/l after KPE. BA, biliary atresia; KPE, Kasai portoenterostomy; HR, hazard ratio; CI, confidence interval; COJ, clearance of jaundice.

**Supplementary Table 6.** Predictors for native liver survival assessed with multivariable Cox proportional hazards regression models including serum FGF19 with previously established prognosticators KPE age and APRI.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Coefficient | HR (95% CI) | p-value |
| All patients |  |  |  |
| FGF19 | 0.309 | 1.36 (1.09 - 1.69) | 0.006 |
| APRI | 0.250 | 1.28 (1.06 - 1.55) | 0.01 |
| Age at KPE, d | 0.010 | 1.01 (1.00 - 1.02) | 0.10 |
| Patients with isolated BA |  |  |  |
| FGF19 | 0.424 | 1.53 (1.18 - 1.99) | 0.002 |
| APRI | 0.288 | 1.33 (1.10 - 1.62) | 0.004 |
| Age at KPE, d | 0.009 | 1.01 (0.99 - 1.03) | 0.23 |
| Patients <60 d of age at KPE |  |  |  |
| FGF19 | 0.389 | 1.48 (1.03 - 2.10) | 0.03 |
| APRI | 0.180 | 1.20 (0.86 – 1.66) | 0.28 |
| Age at KPE, d | 0.025 | 1.03 (0.98 - 1.07) | 0.25 |
| Patients with COJ\* |  |  |  |
| FGF19 | 0.522 | 1.68 (1.12 – 2.54) | 0.01 |
| APRI | 0.352 | 1.42 (0.91 - 2.21) | 0.12 |
| Age at KPE, d | 0.003 | 1.00 (0.98 - 1.03) | 0.78 |
| Patients without COJ |  |  |  |
| FGF19 | 0.033 | 1.03 (0.76 - 1.40) | 0.83 |
| APRI | 0.359 | 1.43 (1.11 - 1.85) | 0.006 |
| Age at KPE, d | 0.0001 | 1.00 (0.98 - 1.02) | 0.99 |

Serum FGF19 and APRI measured at KPE. Logarithmically transformed serum FGF19 levels were used in multivariable Cox proportional hazards regression models. \*Decrease of serum bilirubin concentration < 20 µmol/l after KPE. KPE, Kasai portoenterostomy; HR, hazard ratio; CI, confidence interval; APRI, aspartate transaminase to platelet ratio index; COJ, clearance of jaundice.

**Supplementary Table 7.** Serum bile acid concentration in BA patients at KPE and in normal controls.

|  |  |  |  |
| --- | --- | --- | --- |
|  | BA patients | Normal controls | p-values |
| Number of patients | 50 | 4 |  |
| Total bile acids, µmol/l | 122.4 (80.1 – 158.1) | 3.4 (3.2 - 3.6) | 0.001 |
| Primary bile acids, µmol/l | 89.9 (64.4 – 133.7) | 2.6 (2.2 - 2.7) | 0.001 |
| CDCA, µmol/l | 46.1 (33.1 – 72.7) | 2.2 (1.9 - 2.3) | 0.001 |
| CA, µmol/l | 38.3 (31.3 – 52.0) | 0.40 (0.33 - 0.41) | 0.001 |
| Secondary bile acids, µmol/l | 4.88 (3.55 - 10.25) | 0.86 (0.76 - 1.14) | 0.001 |
| DCA, µmol/l | 1.34 (1.08 - 1.91) | 0.42 (0.41 - 0.66) | 0.02 |
| LCA, µmol/l | 1.88 (1.31 - 3.40) | 0.28 (0.25 - 0.28) | 0.001 |
| UDCA, µmol/l | 0.79 (0.52 - 3.22) | 0.02 (0.07 - 0.18) | 0.002 |

Data is presented as median with interquartile range and p-values are calculated using Mann-Whitney U test. Concentrations are for combined conjugated and unconjugated bile acids. BA, biliary atresia; CDCA, chenodeoxycholic acids; CA, cholic acids; DCA, deoxycholic acids; LCA, lithocholic acids; UDCA, ursodeoxycholic acids.

**Supplementary figure legends**

**Supplementary Figure 1. Serum FGF19 concentration distribution, ROC curve for native liver survival and native liver survival in sub-cohorts of BA.** (A)SerumFGF19 levels in all BA patients on a linear scale and (B) according to sex. (C) ROC curve of serum FGF19 concentration for native liver survival after KPE in BA. The optimal cutoff 109 pg/ml was identified based on maximum sum of specificity and sensitivity. Kaplan-Meier native liver survival curves according to serum FGF19 above or below 109 pg/ml at the time of KPE for (D) patients without COJ (P = 0.98), (E) patients with associated anomalies (P = 0.37), and (F) patients >60 days of age at the time of KPE (P = 0.09). The shaded areas represent 95% CI. BA, biliary atresia; KPE, Kasai portoenterostomy; AUC, area under curve; ROC, receiving operator system; COJ, clearance of jaundice; CI, confidence interval.

**Supplementary Figure 2. Serum concentration and liver mRNA expression of FGF19 by a year of sample acquisition.** (A) No diminution of serum concentrations (R = -0.24, P = 0.03) or (B) liver expression (R = -0.13, P = 0.30) occurred as function of storage time.

**Supplementary Figure 3. Internal validation of regression analyses with Bootstrapping method.** Predictive value of FGF19 for native liver survival in validation cohorts of (A) all patients, (B) patients with isolated BA, (C) patients <60 days of age at the time of KPE, (D) COJ patients, and (E) patients without COJ. Density plots display distribution and mean of hazard ratios analyzed from 1000 regression model repetitions of resampling and replacements of the data. Logarithmically transformed serum FGF19 levels were used in Cox proportional hazards regression models. KPE, Kasai portoenterostomy; COJ, clearance of jaundice.

**Supplementary Figure 4. Expression of liver *FGF19* and other bile acid metabolism genes normalized to cholangiocyte and hepatocyte control genes.** (A) Liver mRNA expression of *FGF19* normalized to *KRT19* for cholangiocytes and (B) HMGCR for hepatocytes in BA at the time of KPE. (C) Liver mRNA expression of FXR (*NR1H4*), SHP (*NR0B2*), *FGFR4,* *KLB*, *CYP7A1*, *CYP8B1*, and *CYP27A1* normalized to *KRT19* (cholangiocytes) and (D) *HMGCR* (hepatocytes). Violin plots display median, interquartile range and individual data points. Expression levels are presented as fold-changes relative to normal controls and P-values were corrected for multiple comparisons with Benjamini- Hochberg method. \*P < 0.05, \*\*P < 0.01. BA, biliary atresia; DC, disease control; FGF19, fibroblast growth factor 19; NC, normal control.

**Supplementary Figure 5. Liver mRNA expression of bile acid transporter genes.** Liver mRNA expression of (A) MRP2 (*ABCC2*), MRP3 (*ABCC3*), and MRP4 (*ABCC4*) and MDR3 (*ABCB4*), and (B) NTCP (*SLC10A1*), BSEP (*ABCB11*), OSTα (*SLC51A*) and OSTβ (*SLC51B*) in BA at the time of KPE, age-matched cholestatic disease controls (DC) and normal controls (NC). Violin plots display median, interquartile range and individual data points. Expression levels were normalised to arithmetic mean of four housekeeping genes (*GAPDH, ACTB, B2M,* and *HPRT1*) and presented as fold-changes relative to normal controls. P-values were corrected for multiple comparisons with Benjamini- Hochberg method. \*P < 0.05, \*\*P < 0.01. BA, biliary atresia; DC, disease control; NC, normal control.

**Supplementary Figure 6. Liver FGF19 expression localized mainly in hepatocytes**. Liver *In situ* hybridization at the time of KPE in BA. (A) *FGF19*, *HPX* (hepatocytes), *KRT19* (cholangiocytes), and merge. (B) *FGF19*, *HPX*, *ACTA2* (activated myofibroblasts), and merge. *FGF19* expressed in hepatocytes with *HPX* and in *ACTA2* negative parenchymal cells. (C) Immunostaining of FGF19 was observed in periportal hepatocytes, bile ductular cholangiocytes and occasional spindle-like parenchymal cells. (D) No immunostaining was seen in healthy liver. (For details, see Methods). BA, biliary atresia; FGF19, fibroblast growth factor 19; NC, normal control.