Supplementary Table S1 Information on identified mutations and the corresponding clinical phenotype of patients with intrahepatic cholestasis.

Patient ID	Age at onset	Sex	Sequencing type	Gene symbol	Genotype	Phenotype	**TBIL/DBIL** (μmol/L) **ALT/AST** (U/L) **GGT**(U/L) **TBA** (μmol/L)	Treatment	Outcomes
1	7d	M	WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) (Hom)	Cholestasis, hypoglycemia, chubby face	208.34/60.24 17/72 206 NA	Lactose-free formula and liposoluble vitamin supplements	Free of jaundice at 2.5-month-old; transaminase returned to normal at 9 months old
2	3d	M	WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) (Hom)	Cholestasis, hypoglycemia, raised prothrombin time, elevated plasma citrulline and arginine, food preference, chubby face	182.1/121.7 37/142 520 300	Lactose-free formula, liposoluble vitamin supplements and ursodeoxycholic acid (UDCA)	Free of jaundice at 4.5-month-old; transaminase returned to normal at 12 months old
3	15d	F	Trio-WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) c.615+5G>A (Comp het)	Cholestasis, elevated plasma citrulline and arginine, food preference	176.82/70.64 62/97 173 NA	Lactose-free formula and liposoluble vitamin supplements	Free of jaundice at 3-month-old; transaminase returned to normal at 4 months old
4	15d	M	Trio-WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251)	Cholestasis, hepatomegaly, elevated plasma citrulline and arginine, food	176.72/121.65 38/197 173	Lactose-free formula and liposoluble vitamin supplements	Free of jaundice at 5-month-old; transaminase returned

					(Hom)	preference, chubby face, failure to thrive	NA		to normal at 8 months old
5	10d	F	WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) (Hom)	Cholestasis, elevated plasma citrulline and arginine	76.2/34.2 34/86 306 NA	Lactose-free formula and liposoluble vitamin supplements	Free of jaundice at 5.5-month-old; transaminase returned to normal at 7 months old
6	4d	M	WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) c.1633_1644ins23; p.Ala555Glyfs*17 (NM_01160210) (Comp het)	Cholestasis, hypoglycemia, raised prothrombin time, raised triglyceride	196.1/139.2 32/78 283 243.8	Lactose-free formula, liposoluble vitamin supplements and UDCA	Free of jaundice at 4-month-old; transaminase returned to normal at 6 months old
7	1.5 m	F	WES	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) c.1177+1G>A (Comp het)	Cholestasis, acholic stools, hypoglycemia, elevated plasma citrulline and arginine, food preference for protein-rich foods and aversion to carbohydrates	147.67/95.83 62/124 186 204.6	Lactose-free formula, liposoluble vitamin supplements and UDCA	Free of jaundice at 3-month-old; transaminase returned to normal at 6 months old
8	10d	M	Panel	*SLC25A13*	c.1177+1G>A; IVS16ins3kb (Comp het)	Cholestasis, hypoglycemia, elevated plasma citrulline and arginine, chubby face	141.7/47.37 23/66 174 NA	Lactose-free formula, liposoluble vitamin supplements	Free of jaundice at 3-month-old; transaminase returned to normal at 6 months old
9	20d	M	Panel	*SLC25A13*	c.851_854del4;	Cholestasis, elevated plasma	48.9/37.1	Lactose-free formula,	Free of jaundice at 4-

					p.Met285Profs*2 (NM_014251) IVS16ins3kb (Comp het)	citrulline and arginine, food preference for protein-rich foods and aversion to carbohydrates	65/111 329 NA	liposoluble vitamin supplements	month-old; transaminase returned to normal at 5 months old
10	15d	M	Panel	*SLC25A13*	c.851_854del4; p.Met285Profs*2 c.443A>G;p.Try148Cys (NM_014251) (Comp het)	Cholestasis, elevated plasma citrulline and arginine, chubby face	66/56.8 51/75 178 NA	Lactose-free formula, liposoluble vitamin supplements	Free of jaundice at 3.5-month-old; transaminase returned to normal at 4 months old
11	15d	M	Panel	*SLC25A13*	c.1078C>T;p.Arg360* c.1399C>T;p.Arg467* (NM_014251) (Comp het)	Cholestasis, hypoglycemia, raised prothrombin time, elevated plasma citrulline and arginine	120.26/70.77 54/154 302 NA	Lactose-free formula, liposoluble vitamin supplements	Free of jaundice at 3.5-month-old; transaminase returned to normal at 5.5 months old
12	5d	M	Panel	*SLC25A13*	c.851_854del4; p.Met285Profs*2 c.1399C>T;p.Arg467* (NM_014251) (Comp het)	Cholestasis, raised prothrombin time, elevated plasma citrulline and arginine, chubby face	182.5/115.7 56/116 211 188.3	Lactose-free formula, liposoluble vitamin supplements	Free of jaundice at 3.5 month old; transaminase returned to normal at 5.5 months old
13	1m	M	Panel	*SLC25A13*	c.1095delT; p.Phe365Leufs*43 IVS16ins3kb (NM_014251) (Comp het)	Cholestasis, hypoglycemia, raised prothrombin time, elevated plasma citrulline and arginine, food preference, chubby face	163.4/108.7 58/116 187 196.2	Lactose-free formula, liposoluble vitamin supplements	Free of jaundice at 4.5-month-old; transaminase returned to normal at 6 months old
14	1.5 m	F	Panel	*SLC25A13*	c.851_854del4; p.Met285Profs*2	Cholestasis, acholic stools, hypoglycemia, elevated	117.15/72.89 35/105	Lactose-free formula, liposoluble vitamin	Free of jaundice at 4-month-old;

					(NM_014251) c.1177+1G>A; (Comp het)	plasma citrulline and arginine, raised triglyceride, liver hemangioendothelioma, food preference, chubby face, failure to thrive	140 191.3	supplements	transaminase returned to normal at 10 months old
15	4d	M	Panel	*SLC25A13*	IVS16ins3kb (Hom)	Cholestasis, hypoglycemia, raised triglyceride, raised prothrombin time, elevated plasma citrulline and arginine, Chubby face, failure to thrive	161.4/104.9 47/138 208 256.1	Lactose-free formula, liposoluble vitamin supplements and UDCA	Free of jaundice at 4-month-old; transaminase returned to normal at 5 months old
16	10d	F	Panel	*SLC25A13*	c.851_854del4; p.Met285Profs*2 (NM_014251) c.615+5G>A (Comp het)	Cholestasis, elevated plasma citrulline and arginine, chubby face	144.3/94.3 34/98 144.3 251.4	Lactose-free formula, liposoluble vitamin supplements and UDCA	Free of jaundice at 3.5-month-old; transaminase returned to normal at 5 months old
17	2m	M	WES	*JAG1*	c.439+1G>A (Het)	Cholestasis, pulmonary stenosis, atrial septal defect, butterfly vertebrae, dysmorphic facies	92/68 30.2/25.9 30.2 NA	Liposoluble vitamin supplements and UDCA	Free of jaundice at 7-month-old; transaminase slightly high at 3 years old
18	10d	M	Panel	*JAG1*	c.2783_2787del; p.His928fs*22 (NM_000214) (Het)	Cholestasis, pulmonary stenosis, dysmorphic facies	107.26/83.06 173/173 176 122.4	Liposoluble vitamin supplements and UDCA	Remain jaundice and high transaminase at 16 months old
19	3d	F	Panel	*JAG1*	c.2090G>A;p.Trp697* (NM_000214)	Cholestasis, acholic stools, pulmonary stenosis,	206.41/166.11 438/265	Liposoluble vitamin supplements and	Free of jaundice at 6-month-old;

					(Het)	dysmorphic facies	622 129.2	UDCA	transaminase slightly high at 15 months old
20	1m	M	Panel	*JAG1*	c.1116dupT;p.Thr373fs*4 (NM_000214) (Het)	Cholestasis, acholic stools, hepatomegaly, dysmorphic facies	181.69/147.75 294/155 1155 117.4	Liposoluble vitamin supplements and UDCA	Free of jaundice at 10-month-old; transaminase slightly high at 12 months old
21	3d	F	WES	*JAG1*	c.810_811del; p.Cys271Argfs*5 (NM_000214) (Het)	Cholestasis, ventricular septal defect, dysmorphic facies	125.8/108.1 138/212 337 NA	Liposoluble vitamin supplements and UDCA	NA
22	9y	M	WES	*JAG1*	c.1797_1798del2; p.Cys599fs*17 (NM_000214) (Het)	Cholestasis, pruritus, cavernous transformation of the portal vein, aortic stenosis, atrioventricular block, renal cysts, dysmorphic facies	46.4/42.8 278/244 176 122.2	Liposoluble vitamin supplements and UDCA	Remain jaundice and pruritus at 10 years old
23	5d	M	Panel	*JAG1*	c.1899_1900delTG; p.Cys633* (NM_000214) (Het)	Cholestasis, biliary hypoplasia, ventricular septal defect, atrial septal defect, pulmonary stenosis, aortic stenosis, renal pelvis dilatation	165.9/146.7 211/234 671 131.6	Liposoluble vitamin supplements and UDCA	NA
24	1y	M	WES	*JAG1*	chr20:10018949-10654178 loss1 (Het)	Pruritus, atrial septal defect, aortic stenosis, persistent left superior vena cava, dysmorphic facies	39/32.7 38/35 316 41.6	Liposoluble vitamin supplements	Pruritus improved at 3 years old

25	7m	M	WES	*ATP8B1*	c.1543C>T;p.Gln515* (NM_005603) c.1932+3_1932+6del4 (Comp het)	Cholestasis, hepatomegaly	115.6/102.9 79/69 10 143	Liposoluble vitamin supplements	NA
26	9d	F	WES	*ABCB11*	c.390-2A>G c.76+29T>G (Comp het)	Cholestasis	174.6/150.9 283/320 37 136.9	Liposoluble vitamin supplements and UDCA	Remain jaundice at 6 months
27	7d	M	Panel	*ABCB11*	c.2344-8_2344-5del4 c.1456A>T;p.Ile486Phe (NM_003742) (Comp het)	Cholestasis, acholic stools, hepatomegaly	390.2/325.9 1405/1333 58 104.3	Liposoluble vitamin supplements and UDCA	Died at 3 month
28	7y	M	WES	*ABCB4*	c.2677G>A;p.Gly893Arg (NM_018849) c.202G>A;p.Gly68Arg (NM_000443) (Comp het)	Cholestasis, abdominal distention, ascites, hepatosplenomegaly, raised prothrombin time, epistaxis, diarrhea	77.5/59.9 234/518 197 NA	Liver transplantation at 7	Free of jaundice after liver transplantation
29	13y	M	WES	*ABCB4*	c.3598C>T;p.Pro1200Ser c.3250C>T;p.Arg1084Trp (NM_018849) (Comp het)	Cholestasis, poor appetite, splenomegaly, raised prothrombin time	136.96/106.96 327/498 305 119.4	Liver transplantation at 13.5	Free of jaundice after liver transplantation
30	10d	M	Panel	*TJP2*	c.3160A>G;p.Thr1054Ala c.3484G>A;p.Glu1162Lys	Cholestasis, acholic stools, low birth weight	136.3/97 75/207 110 NA	Liposoluble vitamin supplements and UDCA	Free of jaundice at 12-month-old; transaminase turned to normal at 18 months

					(NM_004817) (Comp het)				old
31	3.5 y	F	Panel	*HSD3B7*	c.45_46del;p.Gly17Leufs*26 c.319C>T;p.Gln107* (NM_025193) (Comp het)	Cholestasis, hepatosplenomegaly, fever, thrombocytopenia	32.7/18.6 24/38 8 12.3	Liposoluble vitamin supplements and UDCA	Free of jaundice at 4 years and 3 months old, remain thrombocytopenia
32	7d	M	Panel	*AKR1D1*	c.773T>C;p.Ile258Thr (NM_005989) c.580-13T>A (Comp het)	Cholestasis, hepatosplenomegaly, raised prothrombin time	360/267.3 518/844 47 NA	Liposoluble vitamin supplements and UDCA	died at 5 months
33	5d	M	Panel	*CYP27A1*	c.1263+1G>A c.1477-3C>G (Comp het)	Cholestasis, hepatomegaly	206.48/146.12 202/248 72 23	Liposoluble vitamin supplements and UDCA	Free of jaundice at 4-month-old; transaminase turned to normal at 5 months old
34	15d	M	Panel	*SLC10A1*	c.800C>T;p.Ser267Phe (NM_003049) (Hom)	Cholestasis	34.5/18.2 29/54 25 98.6	Liposoluble vitamin supplements	NA
35	3d	F	Panel	*SLC10A1*	c.800C>T;p.Ser267Phe (NM_003049) (Hom)	Cholestasis	38.3/16.47 9/22 14 154.7	Liposoluble vitamin supplements	Remain jaundice at 8.5y
36	1m	M	Panel	*SLC10A1*	c.800C>T;p.Ser267Phe (NM_003049) (Hom)	Cholestasis	65.6/61.6 245/182 74	Liposoluble vitamin supplements	Remain jaundice at 7 months

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37	2.5 m	M	Trio-WES	*LARS*	c.161A>G;p.Tyr54Cys c.478A>G;p.Ile160Val (NM_020117) (Comp het)	Cholestasis, diarrhea, pneumonia, perianal abscess	109.6/90 62/118 141 224	Fluid therapy, liposoluble vitamin supplements and UDCA	Jaundice and transaminase subsided at 5-month-old
38	6m	F	WES	*NBAS*	c.184del1G; p.Ala616Leufs*3 c.3596G>A;p.Cys1199Tyr (NM_015909) (Comp het)	Cholestasis, hepatomegaly, raised prothrombin time, fever, seizures, irritability	75.6/64.7 384.6/1118.9 75 NA	Non-biologic artificial liver replacement, liposoluble vitamin supplements and UDCA	Recurrent liver failure, died at 1 year
39	20d	F	WES	*TRMU*	c.96_97delAG;p.Arg361Gly c.1081C>G;p.Thr32Thrfs*12 (NM_018006) (Comp het)	Cholestasis, feeding intolerance, acholic stools, hepatomegaly, metabolic acidosis, hyperlactatemia	117.3/100.5 42/81 176 NA	L-cysteine, vitamin B, coenzyme Q10, moderate hydrolysis formula	Jaundice and transaminase resolved at 1 year old
40	10y	F	Panel	*ATP7B*	c.2621C>T;p.Ala87Val c.2333G>T;p.Arg778Leu (NM_000053) (Comp het)	Cholestasis, raised prothrombin time, K-F ring, low ceruloplasmin	72.4/49.1 43/139 280 30.6	Penicillamine, Zinc gluconate	Jaundice and transaminase resolved at 11-year-old
41	14d	M	Trio-WES	*NPC1*	c.112_113delAA; p.Lys38Glufs*19 c.2776G>A;p.Ala926Thr (NM_000271) (Comp het)	Cholestasis, hepatosplenomegaly, growth retardation	102.28/78.73 64/238 115 NA	Miglustat	Free of jaundice at 3-month-old; transaminase returned to normal at 16 months old

42	1y	F	WES	*VPS33B*	c.1081C>T;p.Gln361* c.244T>C;p.Cys82Arg (NM_018668) (Comp het)	Cholestasis, pruritus, hepatomegaly, failure to thrive, joint contractures, ichthyosis	355.73/285.19 19/23 16 173	Non-biological artificial liver support, UDCA and liposoluble vitamin supplements	Free of jaundice at 13 years old; mild pruritus
43	1.5 m	F	WES	*FAM111B*	c.1883G>A;p.Ser628Asn (NM_198947) (Het)	Cholestasis, poikiloderma, alopecia, heat intolerance, growth retardation	113.1/95.8 70/125 327 NA	Liposoluble vitamin supplements and UDCA	Free of jaundice at 4-month-old; transaminase slightly high at 2 years old
44	5.5 m	F	WES	*USP53*	c.91G>A;p.Gly31Ser c.394C>T;p.His132Tyr (NM_006446) (Comp het)	Cholestasis, raised prothrombin time, ecchymosis	128.2/96.3 70/123 56 NA	Coagulation factor, liposoluble vitamin supplements and UDCA	Jaundice and transaminase resolved at 7 months old
45	9m	F	Trio-WES	*ANK1*	c.971delT;p.Leu324Argfs*8 (NM_000037) (Het)	Cholestasis, hepatosplenomegaly, cholecystolithiasis, anaemia, raised triglyceride	321/210.7 221/71 204 NA	Splenectomy at 6y	Free of jaundice after splenectomy
46	7d	F	Trio-WES	*ABCC2*	c.4384delG; p.Glu1462Argfs*8 c.631_632+2del4; p.Ser211Hisfs*19 (NM_000392) (Comp het)	Cholestasis	172.49/101.57 36/50 94 NA	Liposoluble vitamin supplements	Persistent jaundice until 3 years old
47	4d	F	WES	*ABCC2*	c.3825C>G;p.Gly674Asp c.2021G>A;p.Tyr1275* (NM_000392)	Cholestasis	164.3/74.4 17/36 107	Liposoluble vitamin supplements	Free of jaundice at 16 months old

					(Comp het)		NA		
48	3d	M	Panel	*ABCC2*	c.1939G>T;p.Glu647* (NM_000392) c.2439+2T>C (Comp het)	Cholestasis	133.02/81.1 32/33 96 NA	Liposoluble vitamin supplements	Persistent jaundice until 3 years old
49	7d	M	Panel	*ABCC2*	c.1177C>T;p.Arg393Trp c.2026G>C;p.Gly676Arg (NM_000392) (Comp het)	Cholestasis	89.1/47.3 22/28 15 NA	Liposoluble vitamin supplements	Persistent jaundice until 10 years old
50	5d	F	Panel	*ABCC2*	c.1326G>A;p.Trp442* c.2366C>T;p.Ser789Phe (NM_000392) (Comp het)	Cholestasis	119.9/91.8 26/44 71 NA	Liposoluble vitamin supplements	Free of jaundice at 6 months old
51	4d	M	WES	*SLCO1B1* *SLCO1B3*	c.1738C>T;p.Arg580* (NM_006446) c.360_481del (Hom)	Cholestasis	102.1/57.5 20/26 12 NA	Liposoluble vitamin supplements	Persistent jaundice until 14 years old
52	1m	M	WES	*KIF12*	c.538C>T;p.Arg180Trp c.539G>A;p.Arg180Gln (NM_138424) (Comp het)	Cholestasis, gastrointestinal bleeding	218/156 23.9/34.6 315 NA	Liver transplantation at 6.5 years	Alive and well
53	15d	F	Trio-WES	*NCOA6*	c.1274A>G;p.Asn425Ser c.1047G>C;p.Leu349Phe (NM_014071) (Comp het)	Cholestasis, hepatosplenomegaly, developmental delay and growth delay	48.99/36.86 136/165 230 43	Liposoluble vitamin supplements and UDCA	Persistent jaundice until 9 months old
54	2d	M	Trio-	*CCDC88B*	c.3292C>T;p.Arg1098Tr	Cholestasis	114.89/89.09	Liposoluble vitamin	NA

			WES		p c.1307C>G;p.Ser436Cys (NM_032251) (Comp het)		197/264 85 NA	supplements and UDCA	
55	2m	M	Trio-WES	*USP24*	c.2282A>G;p.His761Arg (NM_015306) (Het)	Cholestasis	74.38/60.63 61/73 496 207	Liposoluble vitamin supplements and UDCA	Free of jaundice at 1 year old
56	1.5 y	M	Trio-WES	*ATP11C*	c.312_313insT;p.Thr105Tyrfs*8 (NM_001010986) (Hem)	Recurrent cholestasis, intellectual disability, developmental delay, hallux valgus deformity, agranulocytosis, recurrent acute liver failure	106.9/86.3 6081/2190 35 NA	Non-biological artificial liver support, UDCA and liposoluble vitamin supplements	Free of jaundice at 3.5 years old

Supplementary Table S2. List of the 210 genes included in the intrahepatic cholestasis panel.

AARS2	*ABCA1*	*ABCB11*	*ABCB4*	*ABCC2*	*ABCD3*	*ABCG8*	*ACOX2*
ADK	*AHCY*	*AIFM1*	*AKR1D1*	*ALAS2*	*ALDOB*	*ALG1*	*ALG11*
ALG12	*ALG13*	*ALG2*	*ALG3*	*ALG6*	*ALG8*	*ALG9*	*AMACR*
ANKS6	*AP1S1*	*APCS*	*APOA1*	*ASS1*	*ATP5F1A*	*ATP7B*	*ATP8B1*
B4GALT1	*BAAT*	*BCS1L*	*BLVRA*	*BMP2*	*C12orf65*	*C15ORF41*	*CC2D2A*
CCDC115	*CDAN1*	*CEP164*	*CEP83*	*CLDN1*	*COG1*	*COG2*	*COG4*
COG5	*COG6*	*COG7*	*COG8*	*CYC1*	*CYP7B1*	*DDOST*	*DGUOK*
DOLK	*DPAGT1*	*DPM1*	*DPM2*	*DPM3*	*DPYS*	*DYNC2H1*	*EARS2*
ELAC2	*EPHX1*	*FADD*	*FAH*	*FAN1*	*FARS2*	*FBP1*	*FBXL4*
FECH	*FGA*	*FTH1*	*GALT*	*GBA*	*GBE1*	*GFM1*	*GLIS2*
GLIS3	*GSN*	*GSTZ1*	*HAMP*	*HFE*	*HJV*	*HPD*	*HSD3B7*
IFT122	*IFT140*	*IFT172*	*IFT43*	*IFT80*	*IL31RA*	*INVS*	*JAG1*
LARS	*LBR*	*LIPA*	*LIPF*	*LYRM4*	*LYRM7*	*LYZ*	*MARS*
MAT1A	*MGAT2*	*MGME1*	*MOGS*	*MPDU1*	*MPI*	*MPV17*	*MRPL3*
MRPL44	*MRPS16*	*MRPS22*	*MTFMT*	*MTO1*	*MYO5B*	*NEK1*	*NEK8*
NGLY1	*NOTCH2*	*NPC1*	*NPC2*	*NPHP1*	*NPHP3*	*NPHP4*	*NR1H4*
OSMR	*PEX1*	*PEX10*	*PEX11B*	*PEX12*	*PEX13*	*PEX14*	*PEX16*
PEX19	*PEX2*	*PEX26*	*PEX3*	*PEX5*	*PEX6*	*PEX7*	*PGM1*
PKHD1	*PMM2*	*PNPT1*	*POLG*	*POMC*	*PSAP*	*RFT1*	*RMND1*
RPGRIP1L	*RRM2B*	*SC5D*	*SDHD*	*SERPINA1*	*SFXN4*	*SLC10A1*	*SLC10A2*
SLC25A13	*SLC25A4*	*SLC27A5*	*SLC35A1*	*SLC35A2*	*SLC35C1*	*SLC39A8*	*SLC40A1*
SLCO1B1	*SLCO1B3*	*SMPD1*	*SRD5A3*	*STT3A*	*STT3B*	*SUCLA2*	*SUCLG1*
SUMF1	*TARS2*	*TAT*	*TFAM*	*TFR2*	*TJP2*	*TK2*	*TMEM165*
TMEM199	*TMEM67*	*TRMT10C*	*TRMU*	*TSFM*	*TTC21B*	*TTR*	*TUFM*
TWNK	*UBR1*	*UGT1A1*	*UQCC2*	*UQCRB*	*UQCRC2*	*UQCRQ*	*UTP4*
VARS2	*VIL1*	*VIPAS39*	*VPS33B*	*WDR19*	*WDR34*	*WDR35*	*WDR60*
XPNPEP3	*ZNF423*						

Supplementary Methods

Construction of plasmid vector

cDNA of human NCOA6 were cloned into the pcDNA3.1-3×Flag vector using the Clon Express Entry One Step Cloning Kit (Vazyme, China). The corresponding mutations were introduced with the PCR-based DpnI-treatment method using the Mut Express II Fast Mutagenesis Kit V2 (Vazyme, China) and appropriate primers. The entire coding sequences of all the constructs were verified using sequencing. HEK293 cells were transiently transfected with wild-type or mutant constructs.

Cell culture and transfection

HEK293 cells were transiently transfected with wild type (WT) or mutant constructs. Briefly, HEK293 cells were seeded in 6-well plates with 2 mL of Dulbecco's Modified Eagle's Medium (DMEM) in each well at 37°C in an atmosphere of 5% CO_2 24 h prior to transfection. After the cells were 50%-70% confluent, HEK293 cells were transfected with purified constructs using PolyJet™ DNA In Vitro Transfection Reagent (SignaGen, American). Transfection was performed for 4-6 h with 2 μg of constructs.

Immunoblot analysis

HEK293 cells were rapidly washed with ice-cold PBS, and whole cell lysates were generated using lysis buffer containing protease inhibitors. The protein concentration was determined using a Micro BCA protein assay kit with bovine serum albumin as the standard (Pierce, Thermo). Total protein (20 μg) was separated using 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked in TBST (0.1% Tween 20 in TBS) containing 5% nonfat milk for 1 h at room temperature and incubated with primary antibodies against Flag (1: 3000, Sigma Aldrich) and GAPDH (1: 3000, ProTech) overnight at 4°C. The membranes were incubated with HRP-labeled secondary antibodies at room temperature for 1 h, and protein bands were visualized using a chemiluminescence reaction.

Transient transfections and luciferase assays

HEK293 cells were plated in 24-well plates 2 days earlier. They were transfected at day 0 with human BSEP promoter at 500 ng/well (in quadruplicate /per group) and also cotransfected with 50 ng FXR and 1ug WT and mutant NCOA6 plasmids. Luciferase activities were measured 24 h later using Promega kit (Promega, US).

Supplementary Data. Solved cases at the time of clinical testing

Metabolic liver disease

We identified homozygous or compound heterozygous variants in the *SLC25A13* gene in 16 patients with neonatal intrahepatic cholestatic caused by citrin deficiency (NICCD, OMIM#605814) (**Supplementary Table 1**). All 16 patients had typical clinical characteristics of NICCD. In particular, the NICCD patients in this study showed jaundice (16/16, 100%), raised plasma citrulline and arginine (14/16, 87.5%), hypoalbuminemia (11/16, 68.8%), chubby face (10/16, 62.5%), anemia (9/16, 56.3%), prolonged prothrombin time (6/16, 37.5%), hypoglycemia (5/16, 31.3%), raised triglyceride (3/16, 18.8%), failure to thrive (3/16, 18.8%). The latest follow-up showed that all the NICCD patients responded well with a dietary management of lactose-free formula. The median follow-up age was 31 months. Seven patients showed natural aversion to carbohydrates and favoring of protein. One patient had an 18-millimeter diameter hemangio-endotheliomas by abdominal ultrasound examination.

Niemann-Pick disease type C (OMIM#257220) due to variants in *NPC1* gene was another monogenetic metabolic liver disease identified in our cohort. We identified compound heterozygous variants: c.112_113del2 and c.2776G>A, in a 2-month boy with jaundice, hepatosplenomegaly and growth retardation. The patient started Miglustat (OGT 918, N-butyl-deoxynojirimycin) treatment at the age of 2-month. The symptoms of jaundice resolved when he was 3 months old. However, his transaminase levels remained elevated for 16 months. Until the last follow-up, the boy was 3.5 years old and had mild growth retardation (Height 97cm -2~-1SD, weight 14kg -2~-1SD).

Two variants were detected in the *ATB7B* gene in one patient: c.2621C>T;p.Ala87Val and c.2333G>T;p.Arg778Leu, all of which have been reported corresponded with Wilson disease. This patient was a ten years old boy, presenting with bilirubinemia, low ceruloplasmin, K-F rings, raised prothrombin time, hypoalbuminemia and cirrhosis. The symptoms of this patient improved slowly after treated with Penicillamine and Zinc gluconate. His bilirubin level and prothrombin time returned to normal range at the age of 11.

Syndromic cholestasis

Totally, nine patients presented with syndromic cholestasis for eight patients of Alagille syndrome (OMIM#601920) and one of arthrogryposis-renal dysfunction-cholestasis syndrome (ARC syndrome, OMIM#208085).

The eight Alagille syndrome patients in our cohort were found to harbor pathogenic or likely pathogenic variants in *JAG1*. The patients showed jaundice (7/8, 87.5%), dysmorphic facies (7/8, 87.5%), cardiovascular dysplasia (7/8, 87.5%), butterfly vertebrae (1/8, 12.5%). All the patients were treated with UDCA and liposoluble vitamin. Among the 8 patients, four got jaundice resolved, two had persistent jaundice, and two were lost to follow-up (Supplementary Table 1).

Novel compound heterozygous variants (c.1081C>T;p.Gln361stop and c.244T>C;p.Cys82Arg) in *VPS33B* gene were identified in a 12 years old girl, who presented with jaundice, pruritus, failure to thrive, irritability, joint contractures and ichthyosis. Biallelic variants in VPS33B are known to cause ARC syndrome. After treated

with non-biological artificial liver support system therapy for 15 days, UDCA and soluble vitamin supplements, the jaundice of this patient subsided at 13 years old, but ichthyosis and joint contractures persist. Her kidney function tests were normal.

Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) was the third cause accounted for 6/52 of our cohort at the time of clinical testing before reanalyze. Biallelic variants were encountered in *ATP8B1, ABCB11, ABCB4* and *TJP2* genes. At last follow-up, two patients with *ABCB4* variants underwent liver transplantation and recovered well. One patient with *ABCB11* variants died at the age of 3 months and the other one with *ABCB11* variants remain jaundice at 6 months old. The one with *ATP8B1* variants (c.1543C>T;p.Gln515* and c.1932+3_1932+6del4) diagnosed as PFIC-1 and was lost at the age of 12 months old. Biallelic loss of function variants in *TJP2* are responsible for PFIC4. Interestingly, we identified compound heterozygous missense variants in *TJP2* gene (c.3160A>G; p.Thr1054Ala and c.3484G>A; p.Glu1162Lys) in a 3-month-old patient with benign recurrent intrahepatic cholestasis. Addition to high-GGT cholestasis, this patient also suffered from low birth weight and anaemia. After treated with UDCA and liposoluble vitamin, DBIL of this patient subsided at 12-month-old and transaminase turned to normal at 18-months-old.

Bile acid synthesis defects

In this cohort, three patients were diagnosed as bile acid synthesis defects: one congenital bile acid synthesis defect type 1(CBAS1, OMIM#607765) with compound heterozygous variants in *HSD3B7* gene(c.45_46del;p.Gly17Leufs*26 and c.319C>T;p.Gln107*stop) , one congenital bile acid synthesis defect type 2(CBAS2, OMIM#235555) with compound heterozygous variants in *AKR1D1* gene (c.773T>C;p.Ile258Thr and c.580-13T>A), and one cerebrotendinous xanthomatosis(CTX, OMIM#213700) with biallelic variants in *CYP27A1* gene (c.1263+1G>A ;c.1477-3C>G). The clinical manifestations of the three patients were jaundice, hepatosplenomegaly and low bile acid levels ranged from 12.3 to 23 mmol/L. The CBAS2 patient also complicated with coagulation dysfunction and died at the age of 5 months. The CBAS1 patient had slight jaundice but complicated with thrombocytopenia. After treated with liposoluble vitamin and UDCA, the jaundice subsided at 4 years and 3 months old and thrombocytopenia remains. The jaundice of the CTX patient subsided at 4 months old and the transaminase turned to normal at 5 months old.

Infantile liver failure

Cholestasis also can be the symptom at onset of infantile liver failure. In our cohort, we diagnosed one infantile liver failure syndrome type 1(ILFS1, OMIM#615438), one infantile liver failure syndrome type 2(ILFS2, OMIM#616483) and one transient infantile liver failure (LFIT, OMIM#613070) - a kind of mitochondrial liver disease. Compound heterozygous mutations of *LARS* gene c.161A>G;p.Tyr54Cys/c.478A>G;p.Ile160Val were detected in the ILFS1 patient. The two variants were novel missense variants. This patient had an onset at 2.5 months old and presented as jaundice, diarrhea, anaemia, pneumonia and perianal abscess. After treated with fluid therapy, liposoluble vitamin and UDCA, the jaundice of this patient resolved at 5 months old.

Compound heterozygous mutations of novel *NBAS* gene c.184delG; p.Ala616Leufs*3/c.3596G>A;p.Cys1199Tyr were detected in the ILFS2 patient. The variant c.184delG is a frameshift variant and c.3596G>A is a missense variant. This patient had an onset at 6 months old and presented as jaundice, hepatomegaly, raised prothrombin time, fever, seizures, vomiting and irritability. This patient was treated with non-biologic artificial liver replacement, fluid therapy, liposoluble vitamin and UDCA. The symptoms had improved for 2 months. At the age of 1 year, the patient died of recurrent liver failure trigged by respiratory infection.

Novel compound heterozygous mutations of *TRMU* gene c.96_97delAG; p.Arg361Gly/c.1081C>G;p.Thr32Thrfs*12 were detected in one LFIT patient. This patient had an onset at 20 days and presented as Jaundice, feeding intolerance, acholic stools, hepatomegaly, metabolic acidosis and hyperlactatemia. After treated with L-cysteine, vitamin B, coenzyme Q10 and moderate hydrolysis formula, the symptoms resolved at 1 year old.

Detecting disease causing variants in a Phenocopy Gene in 19% of patients with intrahepatic cholestasis

Bilirubin transport syndromes include Dubbin-Johnson syndrome (DJS, OMIM#237500) and Rotor syndrome (OMIM#237450) are common phenocopy of intrahepatic cholestasis present as jaundice, but normal bile acid and transaminase level. In this cohort, we found 8 variants of *ABCC2* gene in 4 patients with DJS, including 3 missense mutations (c.3825C>G, c.1177C>T, c.2026G>C), 2 frameshift mutations (c.4384delG, c.631_632+2delAGGT), 2 nonsense mutation (c.2021G>A, c.1939G>T), and 1 splicing mutation(c.2439+2T>C). Five of these were novel. *SLCO1B1* gene c.1738C>T homozygous mutation and *SLCO1B3* gene c.360_481del homozygous mutation were found in a Rotor syndrome patient. These five patients had similar manifestations recurrent jaundice and sclera. Four of them had persistent jaundice and one of them recovered at 3 years old.

Sodium-taurocholate co-transporting polypeptide (NTCP) deficiency is a new hereditary bile acid metabolic disease due to biallelic mutations of the *SLC10A1* gene. We found homozygous variant c.800C>T in 3 patients which is unknown variant in NTCP deficiency. These patients had jaundice and marked and persistent high bile acid ranged from 98.6 to 237 mmol/L. Except the one lost, the other two still had jaundice at the last follow-up.

In our cohort, we found 1 novel frameshift variant c.971delT in *ANK1* gene which causes hereditary spherocytosis (HS, OMIM#182900). This was a 5.5 years old boy had jaundice, hepatosplenomegaly, cholecystolithiasis, anaemia and raised triglyceride onset at 9 months old. Then he received splenectomy at 6 years old and the jaundice subsided after the surgery.

Diagnostic Yield and Novel Candidate Genes by Exome Sequencing in 166 children with intrahepatic cholestasis

- Supplementary Figure

- Figure S1. Reanalysis WES data in newly reported genes identified compound heterozygous variants of *USP53* in a patient with low GGT cholestasis.
- Figure S2. Reanalysis of phenotype and WES data identified a de novo variant in *FAM111B* gene from a patient with high GGT cholestasis.
- Figure S3. Candidate genes *ATP11C*,*CCDC88B* and *USP24* shows high expression level in human glandular cells. Data from https://www.proteinatlas.org/
- Figure S4. Trio-based WES discovered compound heterozygous variants in *CCDC88B* and heterozygous variant in *USP24* from 2 patients with cholestasis independently.

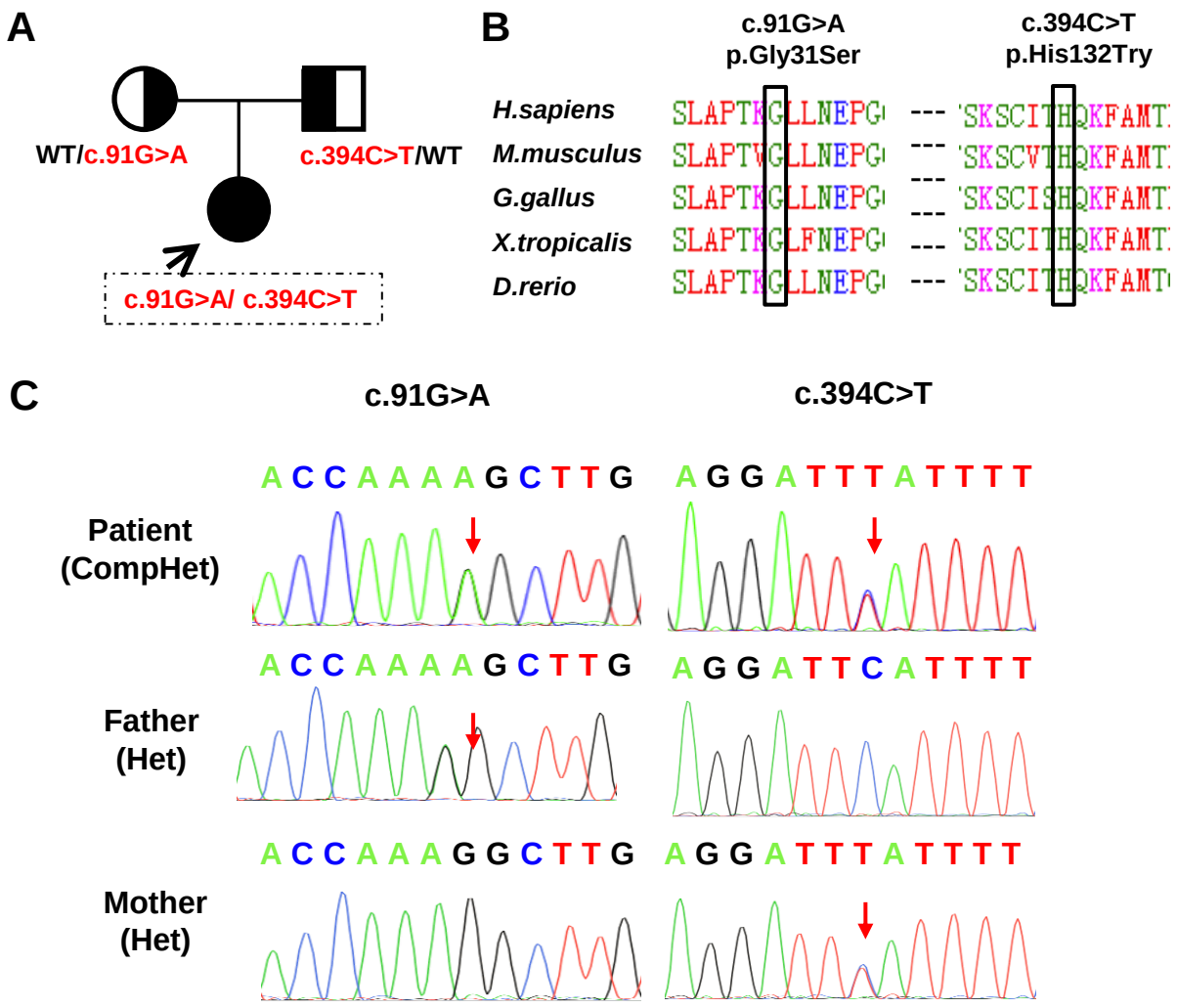

Figure S1. Reanalysis WES data in newly reported genes identified compound heterozygous variants of *USP53* in a patient with low GGT cholestasis.

A. Pedigrees of the family with *USP53*-related low GGT cholestasis.

B. The Gly131 and the His132 residues of USP53 are well conserved from *Homo sapiens* to *Danio rerio*.

C. Sequencing chromatograms showing the compound heterozygous variants c.91G>A and c.394C>T in the *USP53* gene of the *USP53*-related family.

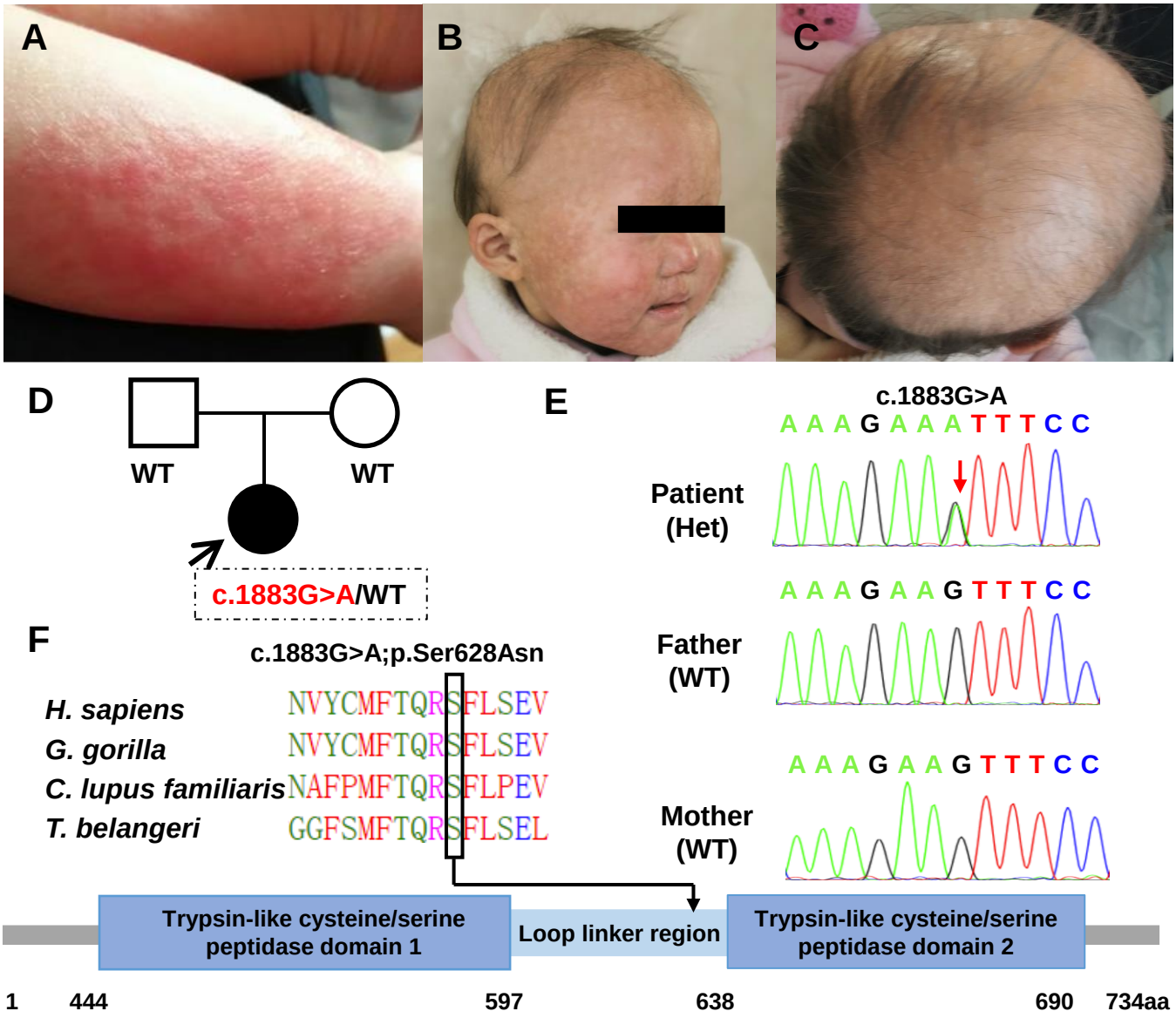
A
B
C
D
WT
WT
c.1883G>A/WT
E
c.1883G>A
A A A G A A A T T T C C
Patient
(Het)
A A A G A A G T T T C C
Father
(WT)
A A A G A A G T T T C C
Mother
(WT)
F
c.1883G>A;p.Ser628Asn
H. sapiens NVYCMFTQRSFLSEV
G. gorilla NVYCMFTQRSFLSEV
C. lupus familiaris NAFPMFTQRSFLPEV
T. belangeri GGFSMFTQRSFLSEL
Trypsin-like cysteine/serine peptidase domain 1
Loop linker region
Trypsin-like cysteine/serine peptidase domain 2
1
444
597
638
690
734aa

Figure S2. Reanalysis of phenotype and WES data identified a de novo variant in *FAM111B* gene from a patient with hereditary fibrosing poikiloderma with tendon contracture, myopathy, and pulmonary fibrosis (POIKTMP) presenting as high GGT cholestasis.

(A-C) The skin lesions of the proband.

A. Eczema-like dermatosis of limbs at the age of 6 months old.

B. Poikiloderma on the face and absence of eyebrows at the age of 2 years old.

C. Alopecia at he age of 2 years old.

D. Pedigrees of the family with *FAM111B*-related high GGT cholestasis.

E. Sequencing chromatograms showing the de novo heterozygous variants c.1883G>A in the *FAM111B* gene of the POIKTMP family.

F. The Ser628 residue of FAM111B is located at the loop link region between the two Trypsin-like cysteine/serine peptidase domain of the FAM111B protein and well conserved from *Homo sapiens* to *Tupaia belangeri*.

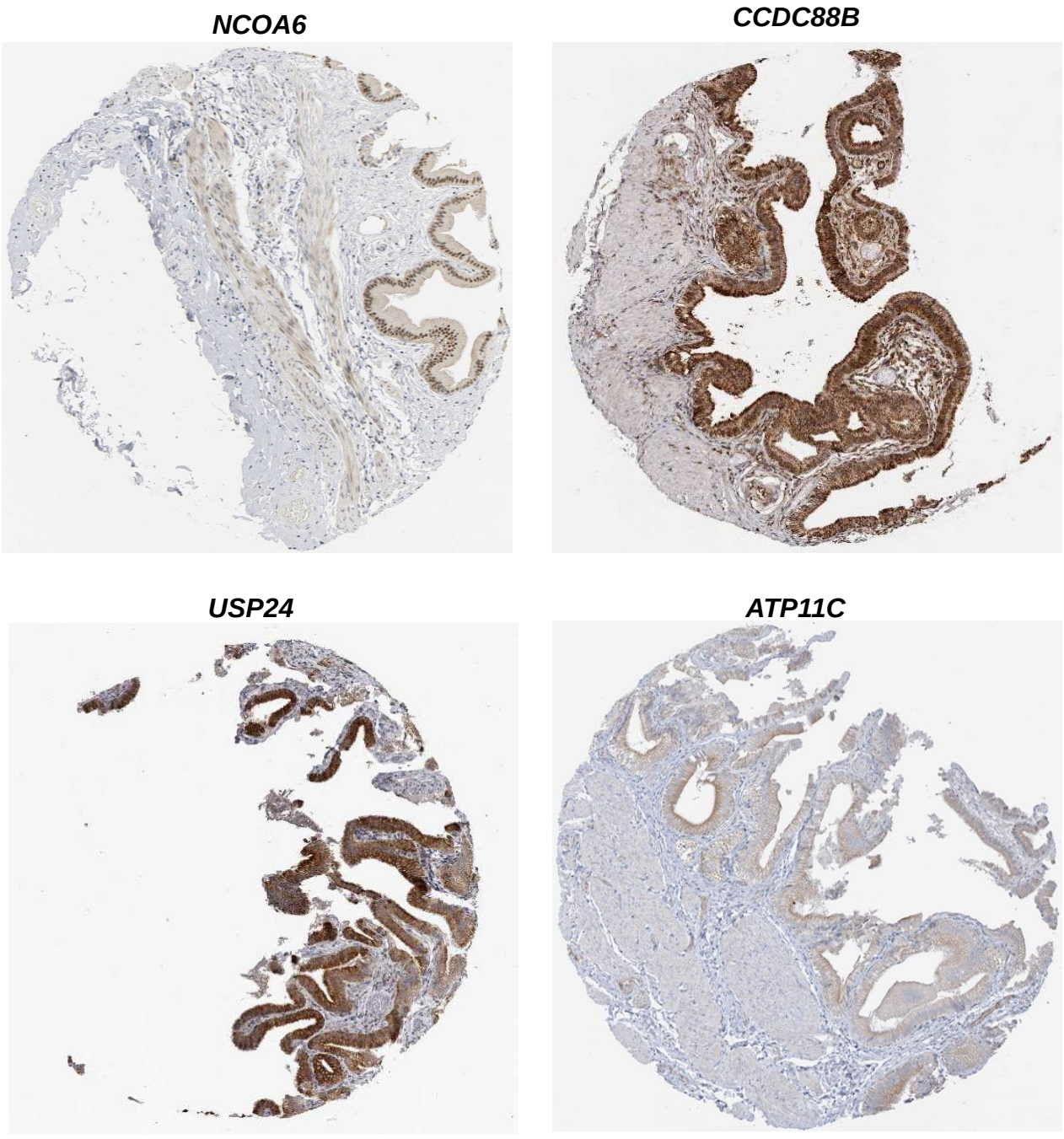

Figure S3. Candidate genes NCOA6, *CCDC88B*, *USP24* and *ATP11C* shows high expression level in human glandular cells.
Data from https://www.proteinatlas.org/

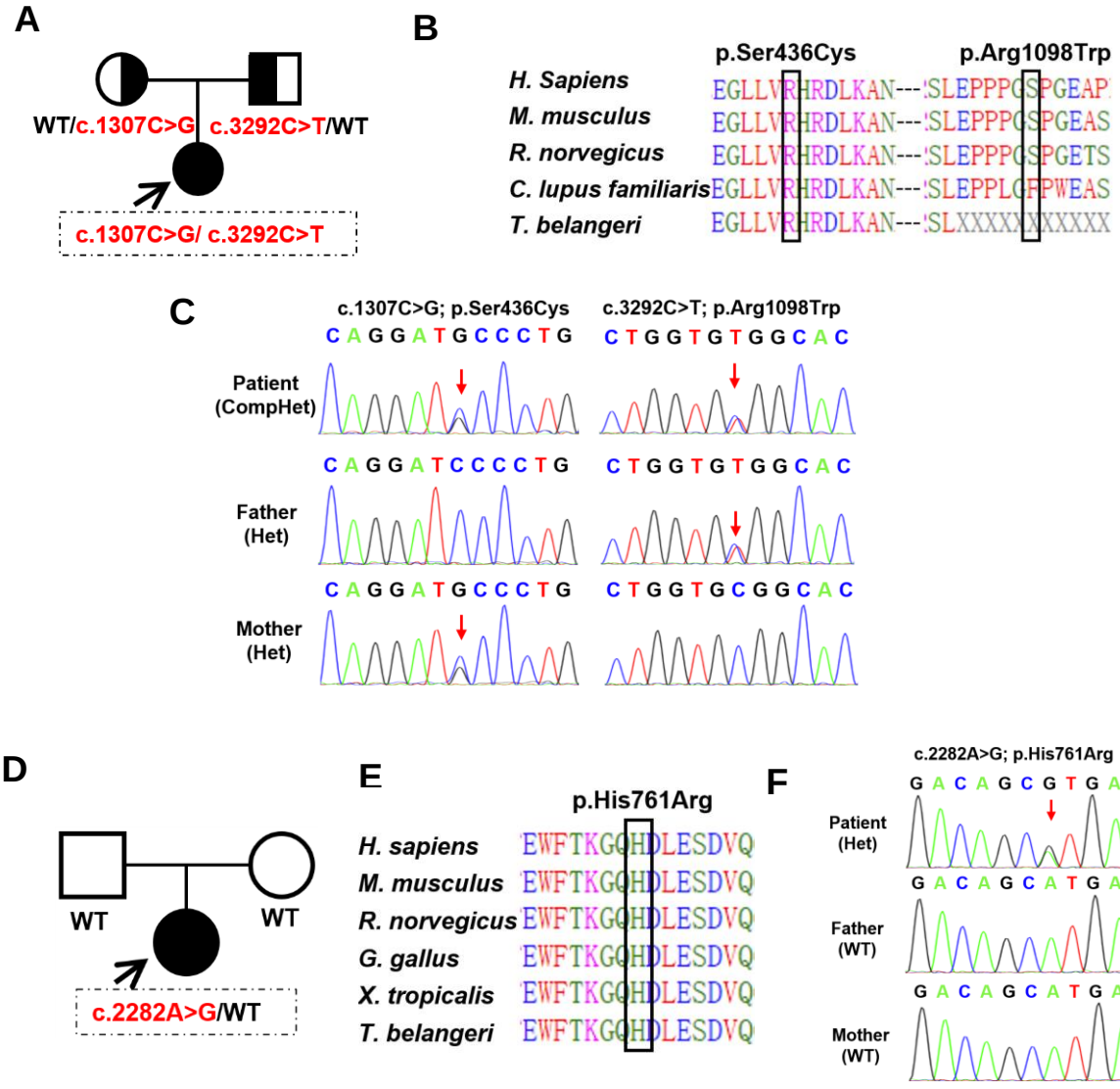
A
WT/c.1307C>G
c.3292C>T/WT
c.1307C>G/ c.3292C>T
B
p.Ser436Cys
p.Arg1098Trp
H. Sapiens
M. musculus
R. norvegicus
C. lupus familiaris
T. belangeri
C
c.1307C>G; p.Ser436Cys
c.3292C>T; p.Arg1098Trp
Patient (CompHet)
Father (Het)
Mother (Het)
D
WT
WT
c.2282A>G/WT
E
p.His761Arg
H. sapiens
M. musculus
R. norvegicus
G. gallus
X. tropicalis
T. belangeri
F
c.2282A>G; p.His761Arg
Patient (Het)
Father (WT)
Mother (WT)

Figure S4. Trio-based WES discovered compound heterozygous variants in *CCDC88B* and heterozygous variant in *USP24* from 2 patients with cholestasis independently.

A. Pedigrees of the family with *CCDC88B*-related low GGT cholestasis.

B. The Ser436 residue of CCDC88B is well conserved from *Homo sapiens* to *Tupaia belangeri* and the Arg1098 residue is conserved from *Homo sapiens* to *Rattus norvegicus*.

C. Sequencing chromatograms showing the compound heterozygous variants c.1307C>G and c.3292C>T in the *CDCC88B* gene of the *CDCC88B*-related family.

D. Pedigrees of the family with USP24-related high GGT cholestasis.

E. The His761 residue of USP24 is well conserved from *Homo sapiens* to *Tupaia belangeri*.

F. Sequencing chromatograms showing the heterozygous variant c.2282A>G in the *USP24* gene of the *USP24*-related family.