SUPPLEMENTAL MATERIAL FOR:

Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver

Evita van de Steeg, Viktor Stránecký, Hana Hartmannová, Lenka Nosková, Martin Hřebíček, Els Wagenaar, Anita van Esch, Dirk R. de Waart, Ronald P.J. Oude Elferink, Kathryn E. Kenworthy, Eva Sticová, Mohammad al-Edreesi, A.S. Knisely, Stanislav Kmoch, Milan Jirsa and Alfred H. Schinkel

Containing:

SUPPLEMENTAL RESULTS

Search for mutations associated with Rotor syndrome (RS) in the general population

To assess the general population frequency of RS-causing mutations, we searched the Database of Genomic Variants for genomic copy number variations (CNVs) within the *SLCO1B3* and *SLCO1B1* locus in healthy control samples. This yielded two deletions. Variation_49020, encompassing practically the whole *SLCO1B3* and *SLCO1B1* locus (chr12:20,901,315-21,296,099), was almost identical to the 405 kb deletion present in the R2 haplotype. It was found in 2 of 2026 investigated individuals, as was Variation_49025, which encompasses the first 2 exons of *SLCO1B1* (chr12:21,163,569-21,196,565), including the first coding exon (reference S1). A parallel search for CNVs in the raw data and genotypes generated on HapMap Phase III samples using Affymetrix SNP 6.0 arrays in 1115 individuals from 11 populations, as well as in another 200 DNA samples previously genotyped in our laboratory using the same technology, yielded 2 deletions and a single amplification: A deletion of chr12:20,909,063-20,921,469 encompassing exon 9 of *SLCO1B3*; a deletion of chr12:20,920,041-20,928,922 encompassing exons 9 and 10 of *SLCO1B3*; and an amplification of chr12:20,840,001-21,534,490 encompassing the whole *SLCO1B3* and *SLCO1B1* locus. In these data we also identified 12 individuals homozygous for extended stretches of the R1 haplotype block and 6 individuals homozygous for extended stretches of the R3 haplotype block. Genotyping of genomic DNA of these individuals obtained from Coriell Cell Repositories, did not find the chr12:g.(20,927,077)_(20,934,292)del(N_{205})ins mutation in the R1-haplotype samples, nor the c.1747+1G>A *SLCO1B3* mutation or the c.757C>T (p.R253X) *SLCO1B1* mutation in the R3-haplotype samples, suggesting that these mutations are relatively recent events.

We further genotyped 1,004 control samples of Central European ancestry to assess population frequencies of each of the RS deletions. One of the samples contained the heterozygous 405 kb deletion found in the R2 haplotype. Interestingly, the single identified individual having the R1-haplotype-associated c.1738C>T (p.R580X) mutation in *SLCO1B1* (in heterozygous state), was also homozygous for the R1 haplotype-associated deletion in *SLCO1B3*.

Expression of uptake and efflux transporters in *Slco1a/1b;Abcc2*$^{-/-}$, *Slco1a/1b;Abcc3*$^{-/-}$, and *Slco1a/1b;Abcc2;Abcc3*$^{-/-}$ mice

Quantitative RT-PCR analysis was performed for a range of functionally relevant uptake and efflux transporters in liver, kidney, and intestine of the single and combination knockout strains, as well as UDP-glycuronosyltransferase 1a1 (*Ugt1a1*) in liver (Supplemental Table 1). In the liver, expression of *Abcc2* was somewhat downregulated in *Slco1a/1b*$^{-/-}$ and *Slco1a/1b;Abcc3*$^{-/-}$ mice (1.6- and 1.8-fold, respectively) and expression of *Abcc3* was somewhat downregulated in *Slco1a/1b*$^{-/-}$ mice (1.8-fold). However, these changes in expression were not noticeable on Western blot (data not shown). Hepatic *Abcc3* mRNA was slightly (1.8-fold) upregulated in *Abcc2*$^{-/-}$ mice but not significantly different from wild-type in *Slco1a/1b;Abcc2*$^{-/-}$ mice. *Ugt1a1* expression was not significantly altered in any of the strains.

SUPPLEMENTAL METHODS

Mutation analysis. Long-range PCR encompassing the genomic regions of the deletion breakpoint boundaries was performed using an Expand Long Range dNTPack (Roche Applied Science). Resulting PCR products were gel-purified and sequenced using a primer walking approach. DNA sequencing of PCR products and genomic fragments covering 1 kb of the promoter regions and all of the exons, with their corresponding exon-intron boundaries, of *SLCO1B1*, *SLCO1B3*, and *SLCO1A2* was performed using a version 3.1 Dye Terminator cycle sequencing kit (Applied Biosystems) and electrophoresis on an ABI 3100 Avant Genetic Analyzer (Applied Biosystems). Data were analyzed using SeqScape software.

Histology and immunohistochemistry. Archival liver-biopsy specimens were available from 5 unrelated RS index subjects (probands, Families CE1, CE2, CE3, and P1; brother [A3 II.9] of proband from Family A3). Sections of paraffin-embedded material (formalin or Carnoy-solution fixative; 4-6 μm thick) were routinely stained with hematoxylin/eosin and periodic acid – Schiff techniques. For OATP1B1 and OATP1B3 immunostaining, similar sections, mounted on SuperFrost Plus slides (Dako) were routinely deparaffinized, rinsed in distilled water, and treated in 10 mM sodium citrate buffer, pH 6.0, for 30 min at 96°C. Endogenous peroxidase activity was blocked by 10 min incubation with 1% H_2O_2. After rinsing in distilled water the sections were incubated with primary mouse anti-OATP1B antibody (clone MDQ; recognizing the N-terminus of both OATP1B1 and OATP1B3), 1:100 dilution, overnight at 4°C (31). Bound antibody was visualized with horseradish peroxidase/diaminobenzidine (EnVision), with hematoxylin counterstaining. Adult human livers without cholestasis served as positive controls; for negative

controls, the primary antibody was replaced by buffer. Immunostaining for ABCC2, using primary mouse anti-ABCC2 antibody (clone M2III-6), was performed as described (14).

Mouse strains and conditions. All mice were of identical genetic background (>99% FVB) and between 9 and 14 weeks of age. Mice were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle. Mice received a standard diet (AM-II; Hope Farms) and acidified water *ad libitum*.

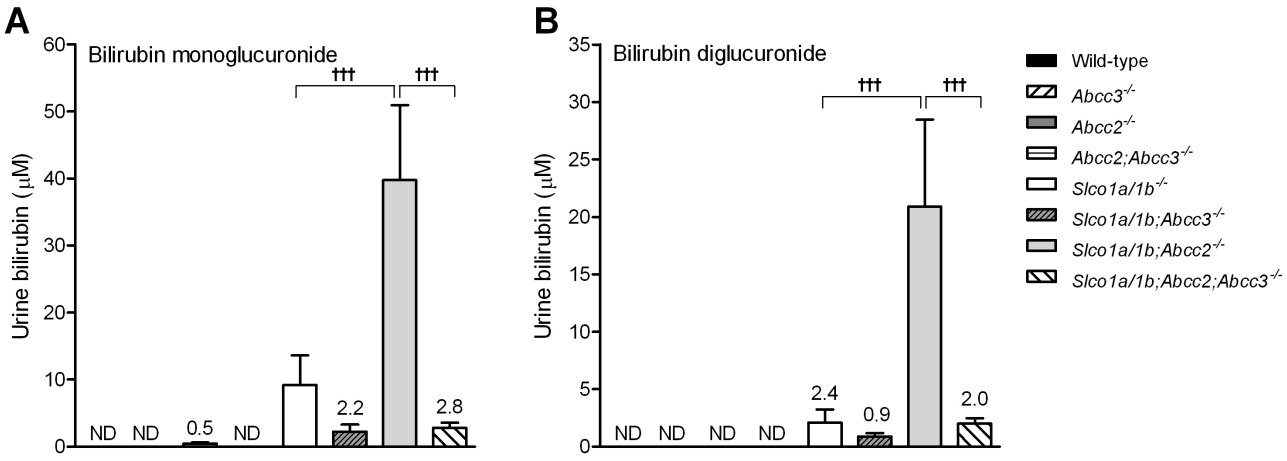
Clinical-chemical analysis of mouse plasma. Blood samples were isolated by cardiac puncture from isoflurane-anesthetized mice. Standard clinical-chemical analyses on EDTA plasma were performed on a Roche Hitachi 917 analyzer to determine levels of total and conjugated bilirubin, alkaline phosphatase, aspartate aminotransaminase, alanine aminotransaminase, γ-glutamyl transferase, lactate dehydrogenase, creatinine, urea, Na^+, K^+, Ca^{2+}, total protein, albumin, uric acid, cholesterol, and triglyceride.

Analysis of bilirubin in mouse plasma, bile and urine. Gallbladder cannulations and collection of bile in male wild-type, *Abcc2*$^{-/-}$, *Abcc3*$^{-/-}$, *Abcc2;Abcc3*$^{-/-}$, *Slco1a/1b*$^{-/-}$, *Slco1a/1b;Abcc2*$^{-/-}$, *Slco1a/1b;Abcc3*$^{-/-}$, and *Slco1a/1b;Abcc2;Abcc3*$^{-/-}$ mice (n = 4-7) were performed as described (25, 50, 51). Bile collected in the first 15 min after gall bladder cannulation was analyzed for bilirubin concentrations. We also collected urine (spot-collection beforehand) and heparin plasma (cardiac puncture afterwards) from these mice. For the detection of bilirubin in *Slco1a/1b*$^{-/-}$*;1B1*^{tg} and *Slco1a/1b*$^{-/-}$*;1B3*^{tg} mice we isolated heparin plasma by cardiac puncture and urine by spot-collection. Ascorbate (100 mg/ml) was added to all plasma (10 µl), urine (10

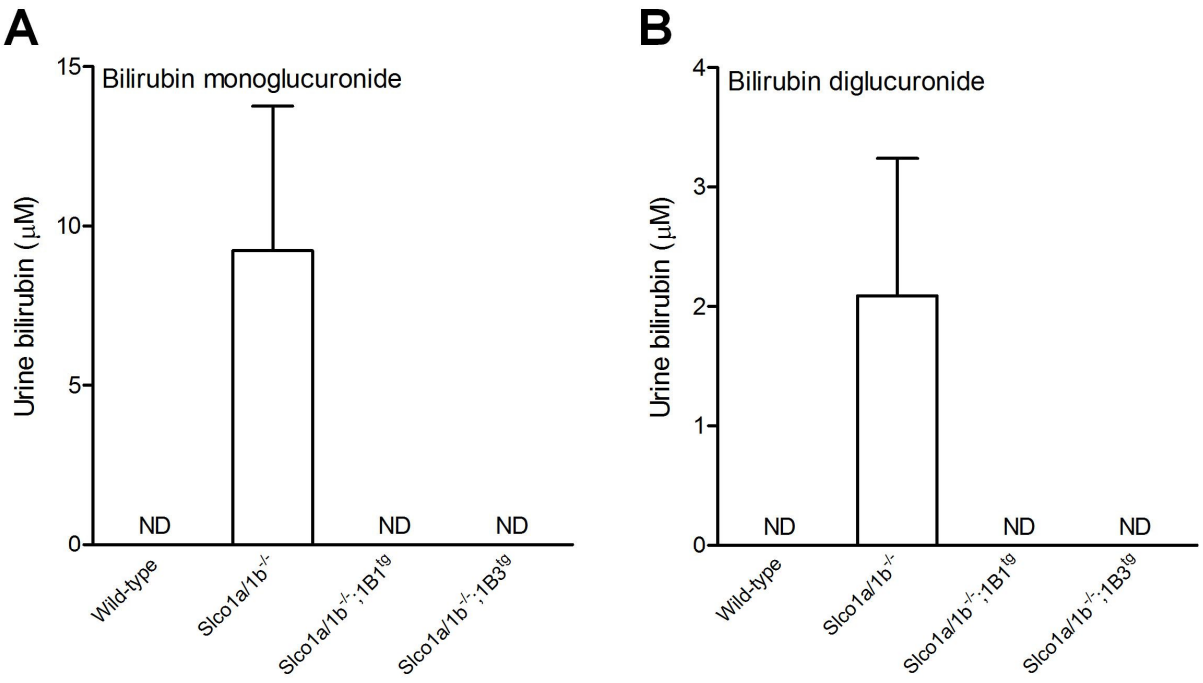
µl), and bile samples (2 µl) in order to prevent oxidation of bilirubin. All samples were immediately protected from the light, snap-frozen, and stored at -80°C until further analysis. Concentrations of bilirubin monoglucuronides (BMG), bilirubin diglucuronide (BDG), and unconjugated bilirubin (UCB) in plasma, bile, and urine were determined as described (51).

SUPPLEMENTAL REFERENCE

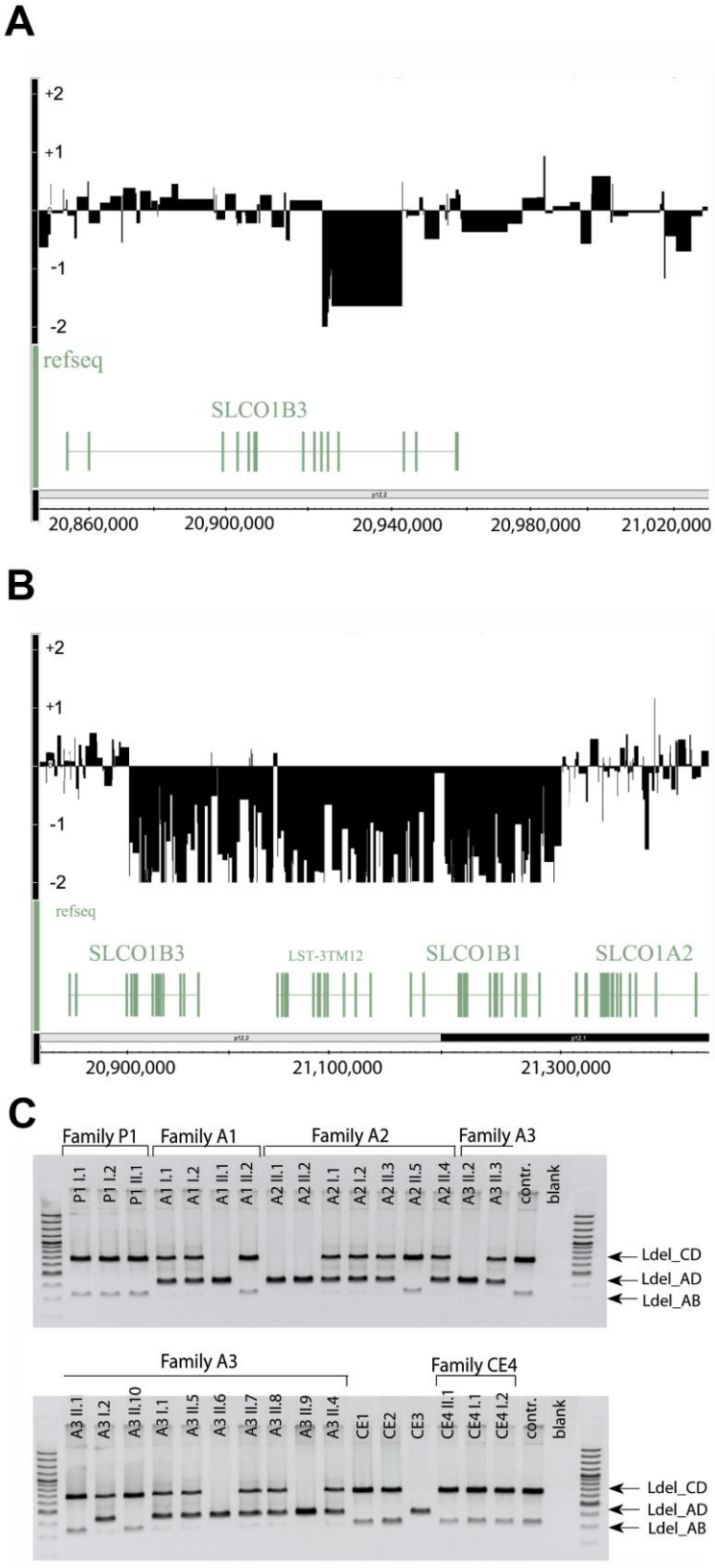
S1. Shaikh TH, et al. High-resolution mapping and analysis of copy number variations in the human genome: a data resource for clinical and research applications. *Genome Res.* 2009;19(9):1682-1690.



Supplemental Figure 1. Urinary bilirubin glucuronide levels in *Slco1a/1b*$^{-/-}$, *Slco1a/1b;Abcc3*$^{-/-}$, *Slco1a/1b;Abcc2*$^{-/-}$ and *Slco1a/1b;Abcc2;Abcc3*$^{-/-}$ mice. A, Bilirubin monoglucuronide and **B**, bilirubin diglucuronide concentrations in urine of male wild-type, *Abcc3*$^{-/-}$, *Abcc2*$^{-/-}$, *Abcc2;Abcc3*$^{-/-}$, *Slco1a/1b*$^{-/-}$, *Slco1a/1b;Abcc3*$^{-/-}$, *Slco1a/1b;Abcc2*$^{-/-}$, and *Slco1a/1b;Abcc2; Abcc3*$^{-/-}$ mice. Data are shown as means ± S.D. (n = 4-7). Urine was collected by spot-sampling. Unconjugated bilirubin concentrations were negligible. Bracketed comparisons: $^{\dagger\dagger\dagger}P < 0.001$. ND, not detectable; detection limit was 0.1 μM. For low bars measured values are presented above the bar.



Supplemental Figure 2. Urinary bilirubin glucuronide levels in *Slco1a/1b*$^{-/-}$, *Slco1a/1b*$^{-/-}$*;1B1*^{tg}, and *Slco1a/1b*$^{-/-}$*;1B3*^{tg} mice. **A**, Bilirubin monoglucuronide and **B**, bilirubin diglucuronide concentrations in urine of male wild-type, *Slco1a/1b*$^{-/-}$, *Slco1a/1b*$^{-/-}$*;1B1*^{tg}, and *Slco1a/1b*$^{-/-}$*;1B3*^{tg} mice. Data are shown as means ± S.D. (n = 4-7). Urine was collected by spot-sampling. Unconjugated bilirubin concentrations were negligible. ND, not detectable; detection limit was 0.1 μM. For low bars measured values are presented above the bar.



Supplemental Figure 3. Mapping of deletions in the *SLCO1B* locus (A and B) and PCR-based genotyping of the R2 haplotype-linked deletion in individuals from RS families (C). Log_2 of fluorescence intensity ratios for microarray probes distributed along the RS candidate locus in approximately 10 kb intervals for haplotypes R1 (**A**), and R2 (**B**), respectively. The value 0 indicates the presence of two copies of the genomic sequence complementary to the probe sequence. Regions with a large rectangular drop (**A**) or with multiple irregular rectangular drops (**B**) to the baseline noise value -2 indicate loss of two copies due to homozygous deletions in probands CE1 (R1 haplotype) and CE3 (R2 haplotype), respectively. The discrepancy between the results of the microarray-based low resolution mapping and sequencing-based exact mapping of the deletion breakpoints, especially for *SLCO1B3* in proband CE1 (Figure 4), is explained by long physical distances between the variations genotyped with the microarray probes. **C**, PCR-based genotyping documents segregation of the R2 haplotype-linked deletion in 4 of 8 investigated RS families. Primer pair Ldel_AD amplifies across the R2 deletion, whereas primer pairs Ldel_AB and Ldel_CD amplify from within the R2 deletion area to just outside the R2 deletion area, each covering one of the two deletion junctions. For primer positions see Supplemental Table 4.

Supplemental Table 1. RT-PCR analysis (ΔCt values) of expression of endogenous uptake and efflux transporters in liver, kidney and small intestine of male wild-type and knockout mice.

A. Liver RT-PCR

	Wild-type	*Abcc2*$^{-/-}$	*Abcc3*$^{-/-}$	*Abcc2/3*$^{-/-}$	*Slco1a/1b*$^{-/-}$	*Slco1a/1b; Abcc2*$^{-/-}$	*Slco1a/1b; Abcc3*$^{-/-}$	*Slco1a/1b; Abcc2/3*$^{-/-}$
Abcc2	-3.30 ± 0.23	-	-3.04 ± 0.40	-	-2.64 ± 0.05*	-	-2.43 ± 0.28*	-
Abcc3	1.82 ± 0.14	0.98 ± 0.20*	-	-	2.68 ± 0.06*	2.21 ± 0.63	-	-
Abcc4	8.67 ± 0.35	6.42 ± 0.78***	8.17 ± 0.46	6.47 ± 0.45***	8.78 ± 0.40	9.28 ± 0.90	8.21 ± 0.49	7.98 ± 0.13
Abcg2	0.31 ± 0.26	0.31 ± 0.28	0.22 ± 0.41	0.18 ± 0.28	0.35 ± 0.05	0.68 ± 0.26	0.14 ± 0.39	0.13 ± 0.45
Abcb1a	5.19 ± 0.33	6.51 ± 0.72	6.63 ± 0.90	7.21 ± 0.34*	6.10 ± 0.86	7.25 ± 0.30*	6.96 ± 1.28	7.21 ± 1.28*
Abcb1b	7.39 ± 0.51	7.81 ± 0.77	6.98 ± 1.05	7.50 ± 0.65	7.95 ± 0.05	8.53 ± 0.43	7.74 ± 0.69	8.11 ± 0.43
Abcb11	-2.53 ± 0.90	-2.95 ± 0.21	-2.84 ± 0.16	-2.82 ± 0.12	-2.76 ± 0.30	-2.53 ± 0.39	-2.57 ± 0.03	-3.20 ± 0.16
Slco1a1	-2.10 ± 0.15	-1.66 ± 0.24	-1.66 ± 0.49	-1.55 ± 0.29	-	-	-	-
Slco1a4	-0.30 ± 0.39	1.13 ± 0.32*	0.90 ± 0.65*	2.36 ± 0.45**	-	-	-	-
Slco1b2	-4.76 ± 0.16	-4.33 ± 0.04	-4.38 ± 0.34	-4.30 ± 0.11	-	-	-	-
Slco2b1	-1.08 ± 0.18	-0.84 ± 0.73	-1.04 ± 0.49	-0.33 ± 0.32	-0.85 ± 0.05	-0.57 ± 0.30	-0.60 ± 0.49	-1.00 ± 0.17
Slc10a1	-4.36 ± 0.10	-3.90 ± 0.12	-3.99 ± 0.28	-3.86 ± 0.07	-4.36 ± 0.13	-3.91 ± 0.57	-4.07 ± 0.37	-4.54 ± 0.21
Ugt1a1	2.28 ± 0.16	2.07 ± 0.14	1.81 ± 0.45	2.34 ± 0.23	2.06 ± 1.18	1.74 ± 0.24	1.87 ± 0.22	2.39 ± 0.04

B. Kidney RT-PCR

	Wild-type	*Abcc2*$^{-/-}$	*Abcc3*$^{-/-}$	*Abcc2/3*$^{-/-}$	*Slco1a/1b*$^{-/-}$	*Slco1a/1b; Abcc2*$^{-/-}$	*Slco1a/1b; Abcc3*$^{-/-}$	*Slco1a/1b; Abcc2/3*$^{-/-}$
Abcc2	-1.74 ± 0.33	-	-1.70 ± 0.14	-	-1.48 ± 0.31	-	-1.58 ± 0.06	-
Abcc3	10.1 ± 1.43	10.7 ± 1.55	-	-	10.2 ± 2.69	8.63 ± 0.43	-	-
Abcc4	3.14 ± 0.13	2.18 ± 0.10**	4.13 ± 0.17**	3.12 ± 0.40	3.65 ± 0.21	2.52 ± 0.25*	4.26 ± 0.09***	2.59 ± 0.41
Abcg2	-1.37 ± 0.12	-0.91 ± 0.15	-0.93 ± 0.16	-1.26 ± 0.55	-1.07 ± 0.28	-0.98 ± 0.14	-0.83 ± 0.16	-1.18 ± 0.12
Abcb1a	4.69 ± 0.20	4.93 ± 0.09	5.95 ± 0.37**	5.51 ± 0.52	5.63 ± 0.69	4.83 ± 0.18	5.64 ± 0.36	4.78 ± 0.44
Abcb1b	4.76 ± 0.09	4.83 ± 0.36	4.41 ± 0.18	4.60 ± 0.47	4.20 ± 0.17	4.89 ± 0.45	4.76 ± 0.74	4.33 ± 0.41
Slco1a1	-1.04 ± 0.15	-0.71 ± 0.16	-0.75 ± 0.14	-1.35 ± 0.14	-	-	-	-
Slco1a6	-3.04 ± 0.21	-2.86 ± 0.14	-2.87 ± 0.34	-3.09 ± 0.43	-	-	-	-
Slco2b1	2.68 ± 0.08	2.91 ± 0.46	2.42 ± 0.13	2.55 ± 0.67	2.64 ± 0.11	2.99 ± 0.05	2.82 ± 0.18	2.54 ± 0.29

C. Small intestine RT-PCR

	Wild-type	***Abcc2*$^{-/-}$**	***Abcc3*$^{-/-}$**	***Abcc2/3*$^{-/-}$**	***Slco1a/1b*$^{-/-}$**	***Slco1a/1b; Abcc2*$^{-/-}$**	***Slco1a/1b; Abcc3*$^{-/-}$**	***Slco1a/1b; Abcc2/3*$^{-/-}$**
Abcc2	0.21 ± 0.32	-	0.17 ± 0.75	-	0.55 ± 0.53	-	0.62 ± 0.45	-
Abcc3	4.85 ± 0.88	4.44 ± 0.93	-	-	5.24 ± 0.65	6.14 ± 0.41	-	-
Abcc4	10.2 ± 0.33	10.2 ± 0.51	9.87 ± 0.32	10.2 ± 0.02	10.6 ± 0.62	10.2 ± 1.06	9.64 ± 0.87	9.65 ± 0.80
Abcg2	3.36 ± 1.02	2.52 ± 1.34	3.24 ± 0.67	3.57 ± 0.30	3.11 ± 0.68	4.28 ± 0.82	3.06 ± 0.65	3.25 ± 0.30
Abcb1a	1.04 ± 0.26	2.37 ± 0.57*	1.90 ± 0.94	0.64 ± 0.35	2.32 ± 0.22*	2.29 ± 0.11*	2.09 ± 0.87	1.36 ± 0.06
Abcb1b	9.71 ± 0.60	9.15 ± 1.26	10.7 ± 0.84	9.66 ± 0.31	10.5 ± 0.72	10.7 ± 6.20	9.68 ± 1.69	10.1 ± 0.13
Slco1a4	5.18 ± 1.40	3.90 ± 1.43	3.96 ± 0.76	4.72 ± 0.91	-	-	-	-
Slco2b1	3.82 ± 0.45	4.37 ± 1.19	4.13 ± 0.20	3.99 ± 0.17	4.37 ± 0.19	4.55 ± 0.60	3.64 ± 0.38	3.60 ± 0.32
Slc10a2	11.3 ± 0.80	10.7 ± 0.61	10.3 ± 0.36	11.1 ± 0.22	12.5 ± 1.65	11.8 ± 1.60	11.0 ± 0.90	10.2 ± 0.59
Ostα	1.52 ± 0.29	1.26 ± 0.66	1.59 ± 0.23	1.57 ± 0.58	1.30 ± 0.23	1.53 ± 0.18	1.06 ± 0.55	1 61 ± 0.63
Ostβ	0.82 ± 0.18	0.63 ± 0.40	1.10 ± 0.46	0.75 ± 0.24	0.79 ± 0.22	0.66 ± 0.42	0.36 ± 0.77	0.14 ± 1.01

Analysis of the results was done by comparative Ct method. Quantification of the target cDNAs in all samples was normalized against the endogenous control β-actin ($Ct_{target} - Ct_{\beta\text{-actin}} = \Delta Ct$). Accordingly, the lower the value, the higher the expression level. Data are presented as means ± S.D. (n = 3); each sample was assayed in duplicate. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared with wild-type mice. One-way ANOVA followed by Tukey's multiple comparison test was used to determine statistical significance.

Supplemental Table 2. Laboratory findings in RS index subjects.

RS index subject	Total bilirubin (μM)	Direct bilirubin (%)	Urinary porphyrin output	Coproporphyrin I (% of total coproporphyrin)	Liver uptake of anionic tracers*	Liver on light microscopy	Liver on electron microscopy
Reference	<17 μM	<20%	<200 μg/24 h	<40%	high	normal	normal
CE1 (m)	170	70	80 – 500	57	low	normal***	n.d.
CE2 (m)	41 - 121	53 - 72	n.d.	n.d.	low	normal***	n.d.
CE3 (f)	60 - 90	33	n.d.	n.d.	n.d.	normal***	n.d.
CE4 II.1 (f)	45 - 60	50 - 60	124	n.d.	low	n.d.	n.d.
P1 II.1 (m)	97	77	n.d.	62	low	normal***	normal
A1 II.1 (f)	102	57	206	195 μg/24 h**	low	n.d.	n.d.
A2 II.1 (m)	53	68	n.d.	n.d.	n.d.	n.d.	n.d.
A2 II.2 (m)	68	75	<25	21 μg/24 h**	low	n.d.	n.d.
A3 II.2 (m)	49	66	n.d.	n.d.	low	n.d.	n.d.
A3 II.6 (m)	34	60	n.d.	n.d.	n.d.	n.d.	n.d.
A3 II.9 (m)	53	68	n.d.	n.d.	low	normal***	normal

Boldfaced – probands. m, male; f, female; n.d., not done.

*) Bromosulfophthalein (used only in RS subject CE2) or radiotracer.

**) Total coproporphyrin output, isomers were not fractionated.

***) Immunostaining for ABCC2 was unremarkable in all 5 tested RS subjects. Moreover, no predictably pathogenic ABCC2 mutations have been found in any of the 8 probands.

Supplemental Table 3. Single nucleotide variations in exons and splice sites of *SLCO1B* genes detected in RS index subjects, their parents and selected siblings.

Subject (family identifier, generation number, subject number, family status)		***SLCO1B1***								
		rs2306283 c.388A>G (p.N130D) Exon 5	rs11045818 c.411G>A (p.S137S) Exon 5	rs11045819 c.463C>A (p.P155T) Exon 5	**c.481+1G>T** Exon 5	rs4149056 c.521T>C (p.A174V) Exon 6	rs4149057 c.571T>C (p.L191L) Exon 6	rs2291075 c.597C>T (p.F199F) Exon 6	**c.757C>T (p.R253X)** Exon 8	**rs71581941 c.1738C>T (p.R580X)** Exon 13
CE1	**Proband**	GG	GG	CC	GG	CC	TT	TT	CC	**TT**
CE2	**Proband**	GG	GG	CC	GG	CC	TT	TT	CC	**TT**
CE3	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
CE4 I.1	Father	AG	GG	CC	GG	TC	TC	CT	CC	C**T**
CE4 I.2	Mother	AG	GG	CC	GG	TC	TC	CT	CC	C**T**
CE4 II.1	**Proband**	GG	GG	CC	GG	CC	TT	TT	CC	**TT**
P1 I.1	Father	GG	GG	CC	GG	TT	TT	CT	C**T**	CC
P1 I.2	Mother	GG	GG	CC	GG	TT	TT	CT	C**T**	CC
P1 II.1	**Proband**	GG	GG	CC	GG	TT	TT	TT	**TT**	CC
A1 I.1	Father	A-	G-	C-	G-	C-	C-	C-	C-	C-
A1 I.2	Mother	A-	G-	C-	G-	C-	C-	C-	C-	C-
A1 II.1	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A1 II.2	Brother	AA	GG	CC	GG	CC	CC	CC	CC	CC
A2 I.1	Father	A-	G-	C-	G-	T-	C-	C-	C-	C-
A2 I.2	Mother	A-	G-	C-	**T**-	T-	T-	C-	C-	C-
A2 II.1	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A2 II.2	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A2 II.3	Sister	A-	G-	C-	**T**-	T-	T-	C-	C-	C-
A2 II.4	Sister	A-	G-	C-	G-	T-	C-	C-	C-	C-
A2 II.5	Brother	AA	GG	CC	G**T**	TT	TC	CC	CC	CC
A3 I.1	Father	G-	A-	A-	G-	T-	C-	T-	C-	C-
A3 I.2	Mother	G-	G-	C-	G-	T-	T-	T-	C-	C-
A3 II.2	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.6	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.9	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.10	Brother	GG	GA	CA	GG	TT	TC	TT	CC	CC

Subject (family identifier, generation number, subject number, family status)		***SLCO1B3***						
		rs4149117 c.334T>G (p.S112A) Exon 4	rs3764009 c.360-3C>T Exon 5	rs7311358 c.699G>A (p.M233I) Exon 7	rs60140950 c.767G>C (p.G256A) Exon 8	rs2053098 c.1557A>G (p.A519A) Exon 12	**c.1747+1G>A** Exon 13	rs3764006 c.1833A>G (p.G611G) Exon 14
CE1	**Proband**	GG	TT	AA	GG	**na**	GG	AA
CE2	**Proband**	GG	TT	AA	GG	**na**	GG	AA
CE3	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
CE4 I.1	Father	GG	TT	AA	GG	G-	GG	AA
CE4 I.2	Mother	GG	TT	AA	GG	G-	GG	AA
CE4 II.1	**Proband**	GG	TT	AA	GG	**na**	GG	AA
P1 I.1	Father	TG	CT	GA	GG	AG	G**A**	AA
P1 I.2	Mother	TG	CC	GA	GG	AG	G**A**	AA
P1 II.1	**Proband**	TT	CC	GG	GG	AA	**AA**	AA
A1 I.1	Father	G-	T-	A-	C-	G-	G-	AA
A1 I.2	Mother	G-	T-	A-	G-	G-	G-	AA
A1 II.1	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A1 II.2	Brother	GG	TT	AA	GC	GG	GG	AA
A2 I.1	Father	G-	T-	A-	G-	G-	G-	A-
A2 I.2	Mother	T-	C-	G-	G-	A-	G-	A-
A2 II.1	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A2 II.2	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A2 II.3	Sister	T-	C-	G-	G-	A-	G-	A-
A2 II.4	Sister	G-	T-	A-	G-	G-	G-	A-
A2 II.5	Brother	TG	CT	GA	GG	GA	GG	AA
A3 I.1	Father	G-	T-	A-	G-	G-	G-	A-
A3 I.2	Mother	G-	T-	A-	G-	G-	G-	G-
A3 II.2	**Proband**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.6	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.9	**Brother**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
A3 II.10	Brother	GG	TT	AA	GC	GG	GG	AG

Predictably pathogenic mutations and RS index subjects are in **bold**, **na** – no amplification.

Supplemental Table 4. Primers used for PCR amplification, sequencing, copy number analysis and mapping of the found deletions.

Name	Primer sequence 5´- 3´	Genomic position (NCBI36/hg18)	Amplified region
***SLCO1A2*: PCR & sequencing from genomic DNA**			
SLCO1A2_A1	TCAACACCTGGGAGTGGGTGGT	12:21438995-21439016	Exon 1
SLCO1A2_S1	CAGCTGCGTGCTGGGAGACC	12:21439850-21439869	
SLCO1A2_A2	ACTTTCAGTAGGTAGCATGTATGTCAGG	12:21418296-21418323	Exon 2
SLCO1A2_S2	ACAGTCAGTTCTGAGAAGAACACCC	12:21418796-21418820	
SLCO1A2_A3	TCCTGTGTAGACACACCCTCAGT	12:21378607-21378629	Exon 3
SLCO1A2_S3	TGAAAAGCTTTCTTTTAACCATGTGACCA	12:21379178-21379206	
SLCO1A2_A4	CGGTCAGATTAAATGACCTAAAACAGCA	12:21362876-21362903	Exon 4
SLCO1A2_S4	AGGCATTTTGTCAACAGACGGCA	12:21363178-21363200	
SLCO1A2_A5	AGTGAGGCAGCCAAGAGCACCA	12:21358579-21358600	Exon 5
SLCO1A2_S5	TGCAACCCTAGCACAAATCCAAGT	12:21358994-21359017	
SLCO1A2_A6	GCACCCGGCCCTTTGACTCATT	12:21350818-21350839	Exon 6
SLCO1A2_S6	TGCACATTGCCCACATTGTCCTCA	12:21351232-21351255	
SLCO1A2_A7	AGGGTCACCTCCAGGGGCACTA	12:21348381-21348402	Exon 7
SLCO1A2_S7	TGCACTAGGGGTGCCCTGAGAA	12:21348917-21348938	
SLCO1A2_A8	CAAATTGAAGGTCAAGTAAGGCCATGA	12:21345274-21345300	Exon 8
SLCO1A2_S8	ACCTGTGACCCAGCATGAAAGGGA	12:21345696-21345719	
SLCO1A2_A9	TCTTGACTGGTGACTGTTGATGACA	12:21344418-21344442	Exon 9
SLCO1A2_S9	GCCAATTTTATAGTTGGTTGGGACCCG	12:21344854-21344880	
SLCO1A2_A10	ACACCCGCCATCACACTGTTCG	12:21341539-21341560	Exon 10
SLCO1A2_S10	AGCCAGTCACAAAATGCAAAGCCA	12:21341806-21341829	
SLCO1A2_A11	TGATCTGATCGTGCATTGCCATTGT	12:21339713-21339737	Exon 11
SLCO1A2_S11	CCCTGTGGGTAATGTGTAACTAAGTGGGG	12:21340130-21340158	
SLCO1A2_A12	AGGCCCAGTTCATAGTTGGGAAGT	12:21338079-21338102	Exon 12
SLCO1A2_S12	TGGCTATGTGGTCATCAAAGCAGGT	12:21338479-21338503	
SLCO1A2_A13	AGTCCTGAGTGACTTTCTTAGAACAAGC	12:21336285-21336312	Exon 13
SLCO1A2_S13	TCCATCTTGATCCAACTGGCTGTG	12:21336604-21336627	
SLCO1A2_A14	TGCAGCACACCAACATGGCACA	12:21319419-21319440	Exon 14
SLCO1A2_S14	GGGCTCCTGTGTAGGCTGCCA	12:21319783-21319803	
SLCO1A2_A15	TGGTGCTGCGTTATGCACAGTCT	12:21318608-21318630	Exon 15
SLCO1A2_S15	ACTTGAAAAGGCCCTGTCTGACCT	12:21319148-21319171	
SLCO1A2_A16	GGGGGCTGTTATTGATGTCCCTCC	12:21313537-21313560	Exon 16
SLCO1A2_S16	AGATCAGCATGCAAAACCATCAGC	12:21314067-21314090	
***SLCO1B1*: PCR & sequencing from genomic DNA**			
SLCO1B1P1A1	AGCGTGTGGAAGACACAGAGCA	12:21175537-21175558	Exon 1
SLCO1B1P1S1	AGGTGGTTAATCATCACTGGACTTGT	12:21175339-21175364	
SLCO1B1P2A1	TTGCTTTTCTATACATTAAAGTTCC	12:21185902-21185926	Exon 2
SLCO1B1P2S1	ATTGACCTAGCAGAGTGGTAACG	12:21185567-21185589	
SLCO1B1P3A1	TAAATTTACCCCAGTTGATAACC	12:21217061-21217083	Exon 3
SLCO1B1P3S1	CATGTGCCTATTGACATTATATAGTCC	12:21216782-21216808	

SLCO1B1P4A1	ACAAGGTACTGATAGTGGCACAGAG	12:21218935-21218959	Exon 4
SLCO1B1P4S1	ACGCATGAAGGAGCACCTTACCCT	12:21218456-21218479	
SLCO1B1P5A1	TGCAGTTGGCCCTGTTCAATCCA	12:21221369-21221391	Exon 5
SLCO1B1P5S1	TAACCCACTTAGCCTGGGGTGT	12:21220748-21220769	
SLCO1B1P6A1	ACAGAGATCCCAGGGTAAAGCCA	12:21223160-21223182	Exon 6
SLCO1B1P6S1	CAGCATAAGAATGGACTAATACACC	12:21222600-21222624	
SLCO1B1P7A1	TGCTTAGAAAGACGTTATCATGG	12:21223245-21223267	Exon 7
SLCO1B1P7S1	CATGGTGAATAAGAACCATGC	12:21223062-21223082	
SLCO1B1P8A1	AGTGCAAAAGAAAGCCAACTCCA	12:21241485-21241507	Exon 8
SLCO1B1P8S1	ACTTTCTTCATACCATTATTTCCCTGAACC	12:21241066-21241095	
SLCO1B1P9A1	CAAAATCACTTTCACAATAAAATACC	12:21244938-21244963	Exon 9
SLCO1B1P9S1	GCCTGTGGTATTGCAGGCTATTCTC	12:21244563-21244587	
SLCO1B1P10A1	TGATCCATCCAAGATTACAGTGGTGGT	12:21247266-21247292	Exon 10
SLCO1B1P10S1	ACCGGGGACTGTTGAGGGGT	12:21246396-21246415	
SLCO1B1P11A1	TGTGCTTTTGAATAAGGAGAGG	12:21250256-21250277	Exon 11
SLCO1B1P11S1	TCTCTGCTTTCACTTTACTTCTTCC	12:21249974-21249998	
SLCO1B1P12A1	TCATTAGGTGTGTTTATAGTCTCATGC	12:21261536-21261562	Exon 12
SLCO1B1P12S1	AATGTATTTGCAGCACTGTTAGG	12:21261254-21261276	
SLCO1B1P13A1	TTAACAATCGAATTCTCCTTTAGG	12:21266789-21266812	Exon 13
SLCO1B1P13S1	GGAGAAGGTTTAATGTTGTTTCG	12:21266427-21266449	
SLCO1B1P14A1	GAGATACGAGATTGCTTGATACC	12:21269173-21269195	Exon 14
SLCO1B1P14S1	CATGCAGTTACATTTAAAATATGTTCC	12:21268864-21268890	
SLCO1B1P15A1	CAAAGTCAATTTTTCCTAATACATTACC	12:21284053-21284080	Exon 15
SLCO1B1P15S1	TTTTTCTTTAGGATCTGGATACTGG	12:21283087-21283111	
***SLCO1B3*: PCR & sequencing from genomic DNA**			
SLCO1B3P1A1	AGGGCTCAGAACAAAAGTGTGGAGA	12:20855086-20855110	Exon 1
SLCO1B3P1S1	GGCTTCTGGGGTGAACTCCTAGAATTA	12:20854770-20854796	
SLCO1B3P2A1	TGTTCTTTTTGACAGTTAGTGGCCTTTCT	12:20860081-20860109	Exon 2
SLCO1B3P2S1	CCTGTGGTCAGGAAATAGCAGGCCC	12:20859738-20859762	
SLCO1B3P3A1	TGTTTTTCAACTTATGCAAGTATGG	12:20899398-20899422	Exon 3
SLCO1B3P3S1	AAACTGTTTTAGTTCCATGTACC	12:20899146-20899168	
SLCO1B3P4A1	GCAGCAGGTGAAGTTGTGAAGCC	12:20903107-20903129	Exon 4
SLCO1B3P4S1	AGGGAAGGTACAATGTCTTGGGCA	12:20902532-20902555	
SLCO1B3P5A1	TGTGTTGTTTAAGAATCGACTGC	12:20905493-20905515	Exon 5
SLCO1B3P5S1	TCTGGTAATTTGGAGAAGACAGC	12:20905100-20905122	
SLCO1B3P6A1	TGACATTATTATTTCAAGGGTAGATCC	12:20906801-20906827	Exon 6
SLCO1B3P6S1	TGAATATGAATCACTTGTAATTAGG	12:20906482-20906506	
SLCO1B3P7A1	TTCTTTGGAAGAATGGTGTCC	12:20907103-20907123	Exon 7
SLCO1B3P7S1	TGATTACATTCCCTGGATCTACC	12:20906787-20906809	
SLCO1B3P8A1	AGCAGAAACCTAATCCTCTTCCCCT	12:20919862-20919886	Exon 8
SLCO1B3P8S1	GGTTTACTTTCTTCATCTATGGAGGACTGC	12:20919328-20919357	
SLCO1B3P9A1	CAGCAGTGTTTCATTTATCAAGC	12:20922162-20922184	Exon 9
SLCO1B3P9S1	CAATTTGGTTAATTCACATGTTCC	12:20921805-20921828	
SLCO1B3P10A1	TGCACATAATCTTTTAATTTGATGG	12:20923872-20923896	Exon 10
SLCO1B3P10S1	GAAATAAGAATGGGTGAATTTGG	12:20923526-20923548	
SLCO1B3P11A1	TCTGTGATTTTGATTAAGGAGAGG	12:20925247-20925270	Exon 11
SLCO1B3P11S1	TCTCCTTATCCCCTTGTCTCC	12:20924970-20924990	

SLCO1B3P12A1	TGAGCTCAAAATACAGAAAAATATGC	12:20927864-20927889	Exon 12
SLCO1B3P12S1	ATTCATAGCCCTGTTGTATTTGG	12:20927559-20927581	
SLCO1B3P13A1	TCACAAAATAGAAATGATTCTTACC	12:20942809-20942833	Exon 13
SLCO1B3P13S1	GTATTCATTCTACCAGGGAGAGG	12:20942530-20942552	
SLCO1B3P14A1	CAAAGTAATTGTTCACTAAATGGTAGC	12:20945767-20945793	Exon 14
SLCO1B3P14S1	GATTCCTGGGTGGATGTAAGC	12:20945403-20945423	
SLCO1B3P15A1	ATGGGAGGTTGGAAGAAGATCC	12:20960951-20960972	Exon 15
SLCO1B3P15S1	TTCTTTCTTTTAAGATATGCATACTGG	12:20960109-20960135	Exon 15
Copy number analysis by quantitative PCR			
SLCO1B3_1_L	TTAAAGACCCACATAAATGGAAAAA	12:20894106-20894130	*SLCO1B3* intron 1
SLCO1B3_1_R	GCAGAGAAATCTGGGTAGCACT	12:20894176-20894197	
SLCO1B3_2_L	AGTTAGGCATAGTGGTGCACAG	12:20896533-20896554	*SLCO1B3* intron 1
SLCO1B3_2_R	CTCTTGGGCTCTATCAATCCTC	12:20896588-20896609	
SLCO1B3_3_L	AAAAGCAAAATTTCTTATATCCCTGT	12:20930418-20930443	*SLCO1B3* intron 11
SLCO1B3_3_R	TTCTACATATGGCATTGTTGGTAGA	12:20930467-20930491	
SLCO1B3_4_L	TCCTAGGAACAACAGGCACTC	12:20930615-20930635	*SLCO1B3* intron 11
SLCO1B3_4_R	TCATGGGACCTCCTCAGTTT	12:20930677-20930696	
SLCO1B3_5_L	TGTAATTTGGACATGCAAGACA	12:20960410-20960431	*SLCO1B3* exon 14
SLCO1B3_5_R	CATCTTAATGAATCAATGCAATGTTAG	12:20960445-20960471	
SLCO1B1_1_L	TCCATCATTCATATAGAACGGAGAT	12:21216917-21216941	*SLCO1B1* exon 2
SLCO1B1_1_R	CAATTTCAAAGCTTCCGTCAA	12:21216972-21216992	
SLCO1B1_2_L	AGAGACGAGGTAGAGGCAAAAA	12:21221733-21221754	*SLCO1B1* intron 4
SLCO1B1_2_R	GAATCTCCAGAAAGATTTACAAACG	12:21221791-21221815	
SLCO1B1_3_L	TGGATGAAGCAAACTTAGAATCC	12:21283308-21283330	*SLCO1B1* exon 14
SLCO1B1_3_R	TCCCCTTAACAATGTGTTTCACT	12:21283373-21283395	
SLCO1B1_4_L	TTTGCAATCAATGAAAATAAGAAGA	12:21296103-21296127	Intergenic between *SLCO1B3*&*SLCO1B1*
SLCO1B1_4_R	GAGAGAGACTCGGTTAGTGAGACTG	12:21296222-21296246	
SLCO1B1_5_L	CTTCCCCTGTGCCTATGTCT	12:21302281-21302300	Intergenic between *SLCO1B3*&*SLCO1B1*
SLCO1B1_5_R	CCAAAACCATAGAAACCCTCAA	12:21302325-21302346	
SLCO1B1_6_L	TTGTGCAACTGTTCATAGTACTCTCTT	12:21304533-21304559	Intergenic – between *SLCO1B3*&*SLCO1B1*
SLCO1B1_6_R	AGCAGACTCAGATTGCTAAAATCA	12:21304618-21304641	
Fine resolution mapping of the deletions			
LDEL_A_U	TTGCCTCCACAAAGTTCTATT	12:20898799-20898819	Regions surrounding the large R2 deletion
LDEL_D_L	TAGTGCTGAAAGTTTTAGCCA	12:21303755-21303775	
LDEL_B_L	ATTTTTCCCTATACAAGTTGA	12:20899086-20899106	Regions in the deleted sequence
LDEL_C_U	TGTCACCTGCAAGCGAAGATT	12:21303145-21303165	
ROTOR_934.4_L	AATAGCCTGTTCCTGAACAAT	12:20934470-20934490	Regions surrounding the deletion in *SLCO1B3* linked with the R1 haplotype
ROTOR_926.8_U	ACCACGCCTGGCCAATTCTTT	12:20926892-20926912	