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Loss of Organic Anion Transporting Polypeptide 1B3 Function Causes Marked Delay in Indocyanine Green Clearance Without any Clinical Symptoms

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The indocyanine green (ICG) retention test is widely used for preoperative evaluation of liver function. Individuals who have markedly poor ICG clearance without severe liver diseases are defined as patients with a "constitutional ICG excretory defect."[(1)] However, the underlying molecular mechanisms remain unknown. We hypothesized that the ICG clearance defect involved impaired function of hepatic uptake transporters such as organic anion transporting polypeptide 1B1 (OATP1B1, gene symbol *SLCO1B1*), OATP1B3 (*SLCO1B3*), and sodium-taurocholate cotransporting polypeptide (NTCP, *SLC10A1*). Homozygous inactivation of both *SLCO1B1* and *SLCO1B3* causes Rotor syndrome, for which three disease-causing haplotypes linked to *SLCO1B1* and *SLCO1B3* are documented.[(2)] We also reported a Japanese population-specific haplotype: a homozygous c.1738C>T (p.R580X) nonsense mutation in *SLCO1B1*, and a homozygous insertion of a 6.5-kbp long interspersed element (LINE-1, L1) retrotransposon in intron 5 of *SLOC1B3* that induced exon skipping and a premature stop codon that

Abbreviations: ICG, indocyanine green; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; P1-P4, patients 1-4.

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TABLE 1. Patient Characteristics and Genotyping of *SLCO1B1*, *SLCO1B3*, and *SLC10A1*

Subject	Age (years)	Gender	Liver Disease	Platelets ($\times 10^4/\mu L$)	Albumin (g/dL)	ALT (IU/L)	ALP (IU/L)	T. Bil (mg/dL)	D. Bil (mg/dL)	Total bile acid (μmol/L)	PT-INR	ICG-R15 (%)	ICG-k
A. Patient Characteristics*													
P1	44	F	PBC (stage I)	12.6	4.3	63	417	0.7	0.2	3.3	0.88	90.7	NA
P2	58	F	PBC (stage I)	23.6	4.1	32	443	0.8	0.3	2.5	0.95	79.3	NA
P3	62	M	Fatty liver	23.4	4.4	38	313	0.8	0.3	1.0	0.97	86	0.024
P4	53	F	HCC	27.1	4.6	27	201	1.4	0.1	5.2	1.26	70.8	0.023

	SLCO1B1							*SLCO1B3*		*SLC10A1*		
Subject	c.388A>G p.N130D rs2306283	c.571T>C p.L191L rs4149057	c.757C>T p.R253X rs183501729	c.1738C>T p.R580X rs71581941	chr12: 21239315T>C 3′UTR	chr12: 21239628T>G 3′UTR rs4149087	chr12: 21239652A>G 3′UTR rs4149088	c.1747+1 G>A	L1 insertion	c.225G>A p.T75T rs4646285	c.263T>C p.I88T rs148467625	c.755G>A p.R252H rs147226818
B. Genotyping of *SLCO1B1*, *SLCO1B3*, and *SLC10A1*†												
P1	A/G	T/C	**C/C**	**C/C**	T/C	T/T	A/A	**G/G**	**L1/L1**	G/A	T/C	**G/G**
P2	G/G	T/T	**C/C**	**C/C**	T/T	T/G	A/G	**G/G**	**L1/L1**	G/G	T/T	**G/G**
P3	G/G	T/T	**C/C**	**C/C**	T/T	T/G	A/G	**G/G**	**L1/L1**	G/G	T/T	**G/G**
P4	G/G	T/T	**C/C**	**C/C**	T/T	T/G	A/G	**G/G**	**L1/L1**	G/G	T/T	**G/G**

*Normal reference values of laboratory data: platelets, 13.0-36.9 $\times$ $10^4/\mu L$; albumin, 3.8-5.2 g/dL; ALT, 5-40 IU/L; ALP, 115-359 IU/L; total bilirubin, 0.3-1.2 mg/dL; direct bilirubin, <0.4 mg/dL; total bile acid, <10 μmol/L; PT-INR, 0.85-1.15; ICG-R15, <10%; ICG-k, 0.179-0.199.

†c.757C>T and c.1738C>T in *SLCO1B1* and c.1747+1G>A and L1 insertion in *SLCO1B3* are variants associated with Rotor syndrome, and 755G>A in *SLC10A1* is a variant associated with hypercholanemia (shown in bold). The following variants have not been reported to be pathogenic: c.388A>G, c.571T>C, chr12:21239315T>C, chr12:21239628T>G and chr12:21239652A>G in *SLCO1B1*, and c.225G>A and c.263T>C in *SLC10A1*.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; D. Bil, direct bilirubin; HCC, hepatocellular carcinoma; ICG-R15, indocyanine green retention rate at 15 minutes; ICG-k, indocyanine green elimination rate constant; NA, not available; PBC, primary biliary cholangitis; PT-INR, international normalized ratio of prothrombin time; T. Bil, total bilirubin.

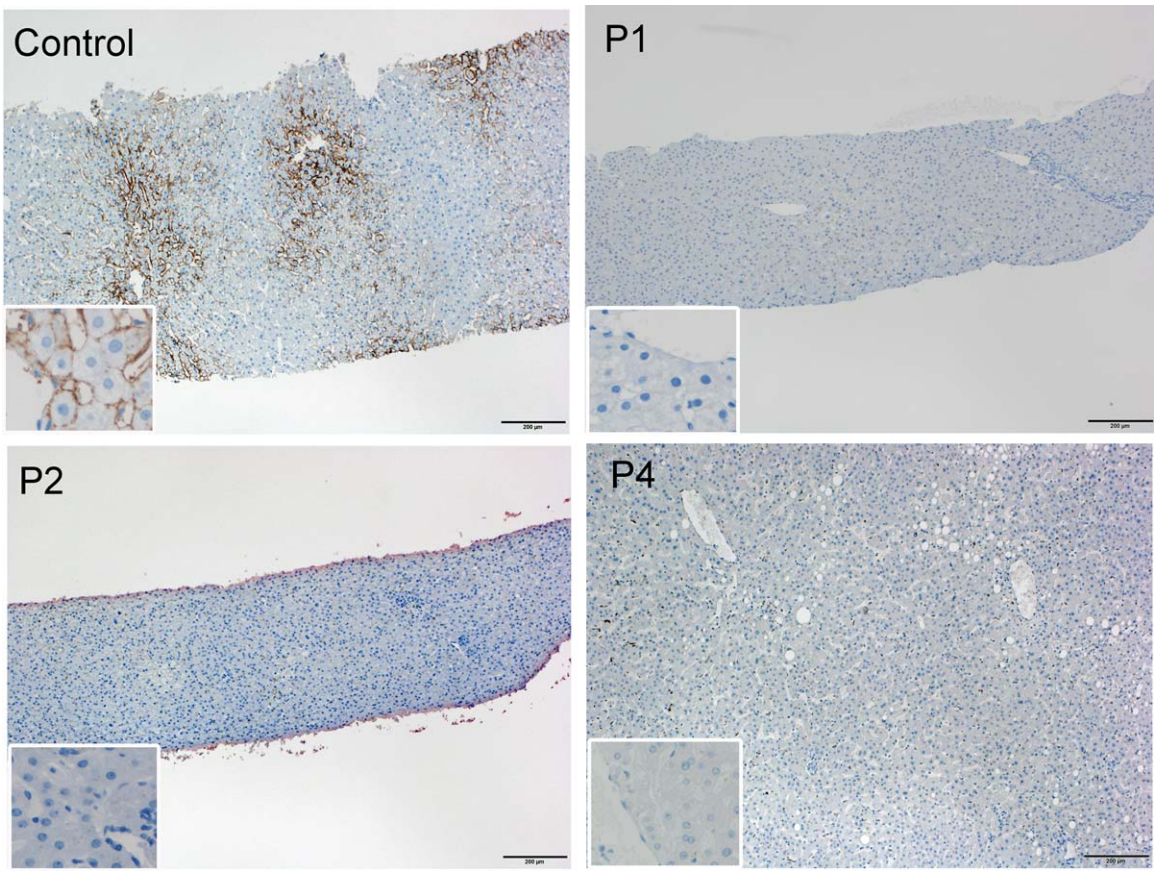

FIG. 1. Hepatic OATP1B3 protein expression in patients P1, P2, and P4 and a control. Positive staining for OATP1B3 is observed along the basolateral membrane of hepatocytes in centrilobular areas in control liver tissue (antibody HPA004943; Sigma-Aldrich, St. Louis, MO). In contrast, OATP1B3 expression is not detected in any of the patients. Scale bar, 200 μm. Inset, high magnification.

generated truncated proteins.[3] A recent case report showed that homozygous c.755G>A (p.R252H) missense mutation in *SLC10A1* led to the functional loss of NTCP resulting in hypercholanemia.[4] Here, by genotyping these transporters, we report cases of an ICG clearance defect due to a deficiency of OATP1B3.

Cases

Four patients (P1-P4) underwent an ICG retention test—popular in Japan—for assessing liver function. All four patients exhibited extremely high ICG retention at 15 minutes, ranging from 70.8% to 90.7% (Table 1A). ICG elimination rate constants for P3 and P4 were markedly low. Background liver diseases included primary biliary cholangitis (Ludwig stage I), fatty liver, and hepatocellular carcinoma. P4 was taking metformin, telmisartan, rosuvastatin, and alogliptin, while the other patients were not taking any drugs. Except for slight elevations in serum alanine aminotransferase and alkaline phosphatase levels, laboratory data were normal. Liver biopsy denied the presence of liver cirrhosis or cholestasis in all patients.

Sequencing of all *SLCO1B1* and *SLC10A1* exons after obtaining written informed consent did not reveal any pathogenic variants, whereas all patients were homozygous for the L1 insertion in intron 5 of *SLOC1B3* (Table 1B). We confirmed the lack of hepatic OATP1B3 expression by immunohistochemistry in three patients (Fig. 1).

Discussion

OATP1B3 and NTCP can transport ICG *in vitro*.[5] An unrevealed deficiency of NTCP in addition to the absence of OATP1B3 might have contributed to the

lack of ICG uptake in our patients. However, this seems unlikely because NTCP deficiency–associated hypercholanemia[4] was not observed (Table 1A). ICG is reported to accumulate in some hepatocellular carcinoma nodules after intravenous injection. Hepatocellular carcinoma tissues with ICG accumulation had stronger OATP1B3 expression than those without ICG accumulation, suggesting the involvement of this transporter in ICG uptake.

We conclude that OATP1B3 is the major hepatic transporter for ICG uptake and that its deficiency profoundly impairs ICG clearance. A solitary defect in OATP1B3 does not appear to cause conjugated hyperbilirubinemia as reported.[2] The allele frequency of the L1 insertion in *SLCO1B3* was 0.054 in a Japanese population,[3] suggesting that homozygosity occurs in approximately 1 in 400 individuals. Because OATP1B3 is involved in the hepatic uptake of various drugs such as statins, bosentan, olmesartan, erythromycin, and docetaxel, the risk of drug-induced toxicity is likely increased in these individuals.

Patients with impaired function of a specific transporter, such as those reported here, can provide important insights into understanding the complicated hepatic transport systems.

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REFERENCES

1) Namihisa T, Nambu M, Kobayashi N, Kuroda H. Nine cases with marked retention of indocyanine green test and normal sulfobromophthalein test without abnormal liver histology: constitutional indocyanine green excretory defect. Hepatogastroenterology 1981;28:6-12.
2) van de Steeg E, Stranecky V, Hartmannova H, Noskova L, Hrebicek M, Wagenaar E, et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. J Clin Invest 2012;122:519-528.
3) Kagawa T, Oka A, Kobayashi Y, Hiasa Y, Kitamura T, Sakugawa H, et al. Recessive inheritance of population-specific intronic LINE-1 insertion causes a Rotor syndrome phenotype. Hum Mutat 2015;36:327-332.
4) Vaz FM, Paulusma CC, Huidekoper H, de Ru M, Lim C, Koster J, et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. HEPATOLOGY 2015;61:260-267.
5) de Graaf W, Hausler S, Heger M, van Ginhoven TM, van Cappellen G, Bennink RJ, et al. Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. J Hepatol 2011;54:738-745.