Hypothyroidism Associated with ATP8B1 Deficiency

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Objective To examine whether hypothyroidism is an extrahepatic feature of ATPase, aminophospholipid transporter, class I, type 8B, member 1 (ATP8B1) deficiency.

Study design Children with normal γ-glutamyltransferase cholestasis (n = 47; 13 patients with ATP8B1 deficiency, 19 with ATP-binding cassette, subfamily B (MDR/TAP), member 11 (ABCB11) deficiency, and 15 without either *ATP8B1* or *ABCB11* mutations) were enrolled. Clinical information and thyroid function test results were retrospectively retrieved from clinical records and compared. Hypothyroidism was diagnosed by clinical-biochemistry criteria (thyroid function test results).

Results Three out of 13 patients with ATP8B1 deficiency were diagnosed as hypothyroid and 2 as subclinically hypothyroid. The frequency of hypothyroidism and subclinical hypothyroidism was significantly higher than in patients with ABCB11 deficiency (5/13 vs 0/19, $P = .006$) and in patients without *ATP8B1* or *ABCB11* mutations (5/13 vs 0/15, $P = .013$). Thyroid function test results normalized after hormone replacement in all 5 patients, with no relief of cholestasis.

Conclusions As hypothyroidism can be another extrahepatic feature of ATP8B1 deficiency, thyroid function should be monitored in these patients. *(J Pediatr 2015;167:1334-9)*.

ATPase, aminophospholipid transporter, class I, type 8B, member 1 (ATP8B1) deficiency usually presents with intrahepatic cholestasis either as benign recurrent intrahepatic cholestasis (BRIC) (BRIC type 1; Online Mendelian Inheritance in Man (OMIM#243300) or progressive familial intrahepatic cholestasis (PFIC) (PFIC type 1; OMIM#211600). It is caused by mutations in *ATP8B1*.[1-3] ATP-binding cassette, subfamily B (MDR/TAP), member 11 (ABCB11) deficiency, presents either as BRIC type 2 (OMIM#605479) or PFIC type 2 (OMIM#601847), and is caused by mutations in *ABCB11*, which encodes bile salt export pump (BSEP). BSEP is exclusively expressed in the canalicular membrane of the liver.[4,5] Both ATP8B1 and ABCB11 deficiencies are characterized by intrahepatic cholestasis with normal serum γ-glutamyltransferase (GGT) activity.

ATP8B1 encodes familial intrahepatic cholestasis 1 protein, also named ATP8B1, a membrane P-type ATPase. Familial intrahepatic cholestasis 1 protein (or ATP8B1) is possibly an aminophospholipid flippase.[2,3] It is expressed in many tissues, including the liver, pancreas, small intestine, bladder, stomach, and prostate, and localizes to the apical membrane of many epithelial cells, including the canalicular membrane of hepatocytes.[6-9] This may explain extrahepatic features of ATP8B1 deficiency, such as pancreatitis, secretory diarrhea, hearing loss, and growth retardation. These may persist after liver transplantation.[10-14]

We noticed hypothyroidism in patients who were diagnosed as having ATP8B1 deficiency. Given the widespread expression of ATP8B1, we speculated that hypothyroidism might be another extrahepatic feature of ATP8B1 deficiency. We compared the frequency of hypothyroidism in patients with ATP8B1 deficiency with that of patients with ABCB11 deficiency and that of patients with unexplained cholestasis to explore whether there was an association between hypothyroidism and ATP8B1 deficiency.

Methods

Between October 2009 and May 2015, a total of 94 patients with intrahepatic cholestasis and normal serum GGT activity presented at the Center for Pediatric Liver Diseases at Children's Hospital of Fudan University and the Department of Pediatrics at Jinshan Hospital of Fudan University. None of these patients had an obvious

ABCB11	ATP-binding cassette, subfamily B (MDR/TAP), member 11	MRP2	Multidrug resistance-associated protein 2
ATP8B1	ATPase, aminophospholipid transporter, class I, type 8B, member 1	OMIM	Online Mendelian Inheritance in Man
BRIC	Benign recurrent intrahepatic cholestasis	PCR	Polymerase chain reaction
BSEP	Bile salt export pump	PFIC	Progressive familial intrahepatic cholestasis
GGT	γ-glutamyltransferase	TJP2	Tight junction protein 2
L-T4	Levo-thyroxine	TSH	Thyroid-stimulating hormone

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cause of liver disease identified after an extensive workup to exclude infective, metabolic, and surgically remediable causes of conjugated jaundice.[15] Among them, 47 patients underwent both *ATP8B1* and *ABCB11* sequencing; 13 underwent *ATP8B1* sequencing and 34 underwent *ABCB11* sequencing only.

We reviewed the data for all 94 patients. Patients who satisfied these inclusion criteria were further studied: (1) onset of conjugated jaundice before age 3 months; (2) serum GGT levels consistently <100U/L; (3) at least 1 detailed documentation of thyroid function available; (4) without indices of severe hypoglycemia or liver failure; and (5) disease-causing mutations detected in either *ATP8B1* or *ABCB11*, or in neither of them.

Clinical information was retrospectively extracted from clinical records. Information regarding family history of congenital hypothyroidism, maternal intake of antithyroid drugs, and maternal hypothyroidism was prospectively collected by telephone, e-mail, or direct follow-up. This study was approved by the ethics committee of Children's Hospital of Fudan University.

Mutation Sequencing and Analysis

ATP8B1 and *ABCB11* were sequenced and analyzed as described.[16,17] Mutations were considered disease-causing on the basis of previous reports, if protein-truncating, or if predicted to be disease-causing or damaging using Mutation Taster, Polyphen-2, or SIFT (available at: http://www.mutationtaster.org/, http://genetics.bwh.harvard.edu/pph-2/, and http://sift.jcvi.org/, respectively).

Immunostaining for GGT and BSEP Expression

Immunostaining for GGT and BSEP expression was performed in liver-biopsy materials, when available, from patients with heterozygous *ATP8B1* or *ABCB11* mutations or novel missense mutations. Immunostaining for the multidrug resistance-associated protein 2 (MRP2) and the tight junction protein 2 (TJP2) was also performed as control. The pathologist (M.D.) was not aware of the patients' genetic results at assessment. The typical immunohistochemical pattern for ATP8B1 deficiency was defined as absent canalicular GGT expression in centrilobular areas but preserved expression in periportal areas. The typical immunohistochemical pattern for ABCB11 deficiency was defined as absent canalicular BSEP expression. Control MRP2, TJP2 expression were seen in all patients.

Diagnosis of Hypothyroidism

Hypothyroidism was diagnosed according to serum thyroid function test results with age-normal reference ranges. The finding of an elevated serum thyroid-stimulating hormone (TSH) level and a low free T4 or total T4 confirmed the diagnosis of primary hypothyroidism.[18] Elevated TSH with normal free T4 permitted the diagnosis of subclinical hypothyroidism.[19] Because subclinical hypothyroidism in children is often a self-remitting process and its treatment in children should be considered only when TSH values are higher than 10 mIU/L,[20] only children with such TSH values were further analyzed.

Diagnosis of ATP8B1 and ABCB11 Deficiency

We categorized patients as having possible ATP8B1 or ABCB11 deficiency and definite ATP8B1 or ABCB11 deficiency according to the diagnosis criteria following. Possible ATP8B1 or ABCB11 deficiency was defined as in whom at least one predicted disease-causing mutations were detected in *ATP8B1* or *ABCB11*, respectively. Definite ATP8B1 or ABCB11 deficiency was defined as in whom known disease-cause mutations or predicted protein-truncating mutations were detected in both alleles of *ATP8B1* or *ABCB11*, respectively; or in whom the immunostaining of GGT and BSEP were consistent with typical patterns of ATP8B1 deficiency or ABCB11 deficiency on the basis of a possible diagnosis (ie, patients with only 1 mutated allele of *ATP8B1* or *ABCB11*, or with either 1 or 2 novel missense mutations).

Whole Exome Sequencing and Sanger Validation

Homozygosity for *ATP8B1* mutation in 1 patient with hypothyroidism (patient 1034) suggested parental consanguinity. To exclude homozygous mutation in genes associated with primary heritable hypothyroidism, whole exome sequencing was conducted.

Genomic DNA sample was sheared by sonication. The sheared genomic DNA was then hybridized with NimbleGen 2.0 probe sequence capture array of Roche, (http://www.nimblegen.com/products/seqcap/ez/v2/index.html) to enrich the exonic DNA (Joy Orient, Beijing, China). The libraries were first tested for enrichment by quantitative polymerase chain reaction (PCR) and for size distribution and concentration using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, California). The samples were then sequenced on an Illumina Hiseq2500 (Illumina, San Diego, California). Two parallel reactions were done for each sample. Exon-enriched DNA was sequenced by the Illumina hiseq2500 platform following the manufacturer's instructions (Illumina). Raw image files were processed by the BclToFastq (Illumina) for base calling and generating the raw data. The low-quality variations were filtered out using the quality score ≥20. The sequencing reads were aligned to the National Center for Biotechnology Information human reference genome (hg19) using Burrows-Wheeler Aligner. Samtools and Pindel were used to analyze single nucleotide polymorphism and Indel of the sequence. Sanger sequencing was used to confirm the mutation in the proband (PCR primers sequences are available on request). PCR product was sequenced by ABI 3730XL and analyzed by DNASTAR software (DNASTAR, Madison, Wisconsin) and compared with messenger RNA template (*IGSF1*: NM_001170961.1).

Statistical Analyses

Exact Fisher test was performed to analyze the data by Stata11.0 (StataCorp, College Station, Texas). Values for $P < .05$ were defined as statistically significant.