



Monozygotic Twins Suffering From Sodium Taurocholate Cotransporting Polypeptide Deficiency: A Case Report

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Sodium taurocholate cotransporting polypeptide (NTCP) is a carrier protein encoded by the human *SLC10A1* gene, acting as the principal transporter of conjugated bile salts from the plasma into hepatocytes. Although NTCP was cloned as early as in 1994 and its function has been studied extensively, clinical description of NTCP deficiency remains rather limited thus far. The patients in this paper were 2 female monozygotic twins, who were referred to our hospital at the age 2 years with the complaint of persistently-raised total bile acids (TBA) for 21 months. At age 3 months, they were both diagnosed to have cholestatic liver disease due to raised serum TBA and direct bilirubin (DBIL) with the fraction >20% of the elevated total bilirubin (TBIL). Thereafter, their jaundice subsided and the DBIL levels recovered gradually, while serum TBA remained raised persistently. In view of their refractory hypercholanemia but negative symptoms and signs, *SLC10A1* genetic analysis was performed for all family members to evaluate the possibility of NTCP deficiency. As a result, the twins were both homozygotes, while the parents, carriers, of the reportedly pathogenic variant c.800C>T (p.Ser267Phe). These findings suggested that NTCP deficiency may be a unique genetic factor causing transient cholestasis in early infancy, as well as, persistent hypercholanemia in pediatric patients.

Keywords: sodium taurocholate cotransporting polypeptide deficiency, *SLC10A1*, bile acid, hypercholanemia, cholestasis

INTRODUCTION

Deficiency of sodium taurocholate cotransporting polypeptide (NTCP) is an inborn error of bile acid metabolism caused by biallelic *SLC10A1* variants, which impairs the NTCP function as the primary transporter of conjugated bile salts from the plasma into hepatocytes (1–4). NTCP was cloned as early as in the year 1994 (5), and thereafter, its function has been studied extensively (6, 7), while a number of *SLC10A1* genetic variants have been identified in humans (1, 7, 8). However, the first patient with NTCP deficiency was just reported very recently by Vaz et al. (9). Since then, several papers about patients with NTCP deficiency have been published (4, 10–14), but the patient number was limited and the laboratory and clinical features of this new disorder still remained open for further investigation.

Herein, we reported two monozygotic twins suffering from NTCP deficiency, who presented with persistent hypercholanemia and transient cholestatic jaundice in their early infancy.

CASE DESCRIPTION

Patient 1. A 2-years-old female infant was referred to the First Affiliated Hospital, Jinan University, due to elevated serum total bile acids (TBA) discovered for 21 months. At the age 3 months, she was admitted to Women and Children’s Hospital of Quanzhou because of jaundice for 2 months. Laboratory test revealed that the serum levels of TBA, direct bilirubin (DBIL), aspartate transaminase (AST) and alanine transaminase (ALT) were all elevated (**Table 1**), and she was thus diagnosed to have cholestatic liver disease. After being treated with intravenous reduced glutathione and ademetionine-1,4-butanedisulfonate for 9 days, which were commonly used in cholestatic patients with elevated alanine transaminase levels, her jaundice was alleviated, but the liver function indices remained abnormal (**Table 1**). Then oral ursodesoxycholic acid was given and she was discharged at the age 3.3 months. During the subsequent follow-up over 20 months, she showed normal anthropometric and neurobehavioral development without abnormal appearance, and her cholestasis resolved completely since the age 7 months. However, persistently raised TBA levels were observed on repeated biochemical analyses (**Table 1**). So she was referred to our hospital for further investigation and management when aged 2 years.

The patient was the elder sister of two monozygotic twins who was delivered by cesarean section at the gestation age of 37 weeks and 2 days with the birth weight 2.25 kg and body length 45.0 cm. Her father is a hepatitis B virus (HBV) carrier who was clinically healthy but with slightly elevated serum TBA level on biochemistry analysis, and her mother was physically and biochemically healthy (**Table 1**). There was no family history of any genetic disease.

Physical examination revealed a body weight of 12.0 kg, height 83.5 cm and head circumference 46.0 cm. No jaundice was observed in the skin and sclera. No stridor, crackles or crepitus was heard in the two lungs, and the heart sound was normal without any murmurs. There was no abdominal distention, and the liver and spleen were non-palpable. Physiological reflexes were normal and no pathological reflexes could be found on nervous system examination. On biochemical analysis, the TBA level reached 173.8 μmol/L (0–10 μmol/L) as other indices were normal (**Table 1**).

Patient 2 was the younger sister of patient 1, who also experienced transient cholestatic jaundice in early infancy and was referred to our hospital with the same complaint of persistently elevated serum TBA levels. As the younger one of two monozygotic twins, her birth weight was 2.30 kg and body length, 46.0 cm. On physical examination at referral, the body weight was 13.0 kg, height 85.0 cm, and head circumference 46.0 cm. No jaundiced skin or sclera was observed. Examinations of the lungs, the heart, the abdomen and nervous system were all normal. Her TBA level was 198.3 μmol/L (0–10 μmol/L), with otherwise normal biochemistry indices (**Table 1**).

Considering their refractory hypercholanemia whist lack of clinical symptoms and signs on physical examination, NTCP deficiency was highly suspected and hence Sanger sequencing of the *SLC10A1* gene was carried out for all family members. As a

TABLE 1 | Biochemical indices of the monozygotic twins and their parents.

Indices (reference range)	Patient 1/Patient 2										Parents			
	3M ^a	3.3M	7M	8M	10M	12.3M	14.2M	15.7M	18.3M	21.6M	24M ^b	27M	Father	Mother
TBIL (2–19 μmol/l)	106.5/73.1	34.9/23.3	11.6/12.7	6.9/7.8	4.4/3.9	4.2/3.9	4.3/3.7	3.8/4.5	3.4/4.0	7.4/7.4	5.2/4.8	10.2/10.1	11.8	7.7
DBIL (0–6 μmol/l)	67.6/45.6	21.7/13.8	2.8/3.5	1.6/2.1	1.5/1.1	1.2/1.3	1.2/1.0	1.1/1.2	1.1/1.3	2.2/1.8	1.6/1.3	2.9/3.2	3.5	2.6
IBIL (2.56–20.9 μmol/l)	38.9/27.5	13.2/9.5	8.8/9.2	5.3/5.7	2.9/2.8	3.0/2.6	3.1/2.7	2.7/3.3	2.3/2.7	5.2/5.6	3.6/3.5	7.3/6.9	8.3	5.1
TBA (0–10 μmol/l)	221/210.8	229/239.1	170.1/144	171.2/138.9	211.4/225	107.4/139.1	187.1/123.2	179.9/276.1	103.0/184.1	104.8/105.4	173.8/198.3	162.6/118	18.5	5.1
ALT (5–40 U/l)	100/95	122/96	68/49	34/25	142/38	21/23	25/24	18/21	20/17	22/17	15/13	19/16	15	19
AST (5–40 U/l)	114/106	112/90	68/56	52/51	76/55	47/50	51/42	36/41	40/45	43/45	35/38	40/37	16	18
GGT (8–50 U/l)	110/112	63/46	17/16	10/9	10/9	7/7	9/8	8/9	14/9	9/12	8/9	7/8	18	12
ALP (20–500 U/l)	391/34.5	385/326	172/164	190/192	184/199	286/277	241/240	179/192	205/245	237/264	165/170	157/172	79	49
TP (60.0–83.0 g/L)	41/40.5	–	57.4/54.3	59.4/58.8	63.6/3.5	62.4/66.9	63.7/61.3	66.4/65.5	62.8/70.2	66/67.8	66.4/70.1	66.8/66.3	70.8	67.3
ALB (35.0–55.0 g/L)	–	–	40.3/38.9	40/40.1	40.5/41.8	42.9/44.4	43.3/41.7	41.3/41.1	39.3/42.7	43.5/44.4	43.2/44.2	43.3/43.4	48.8	45.3

^aFirst visiting to the local hospital.

^bReferral to our clinic.

TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TBA, total bile acids; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; ALB, albumin.

result, the twins were both homozygotes of the variant c.800C>T (p.Ser267Phe), and their parents were both carriers of the same variant (**Figure 1A**). Electrophoresis of the PCR-RFLP products showed that the twins both harbored two additional bands of 164 bp and 65 bp rising from the aforementioned *SLC10A1* variant, which further confirmed the *SLC10A1* genotypes of all family members (**Figure 1B**).

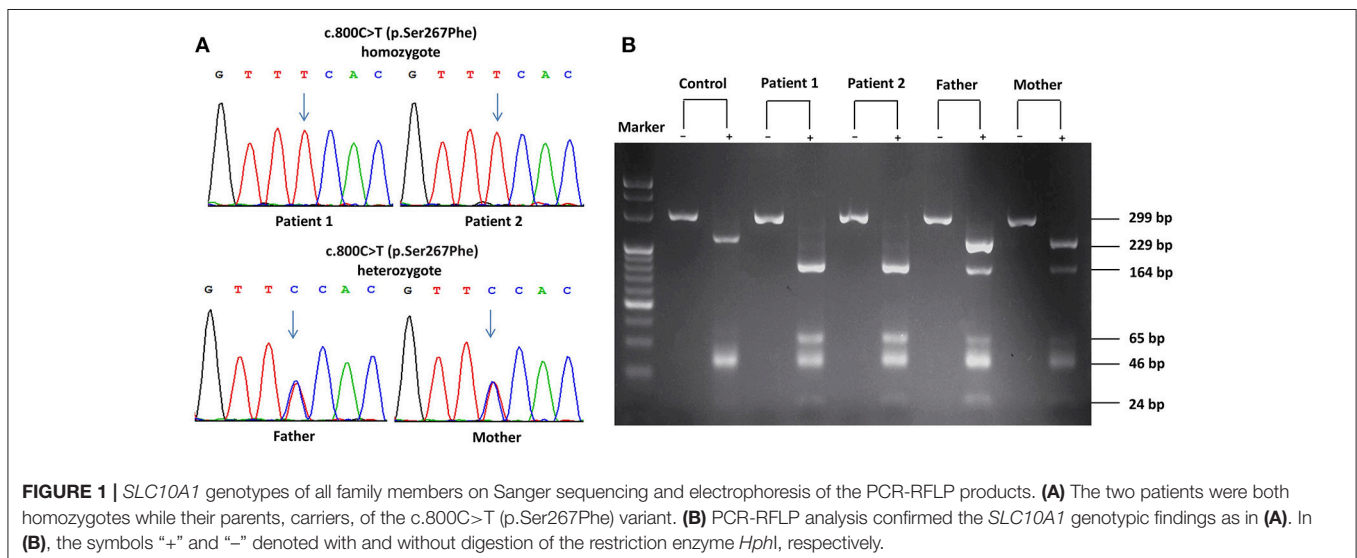
NTCP deficiency was thus definitely diagnosed, and the twins were followed-up in the local hospital, and no specific medication was given. Thus far, the twins were both healthy but the serum TBA levels were still raised (**Table 1**), and their long-term outcomes needed to be observed.

DISCUSSION

In this paper, *SLC10A1* genetic analysis provided reliable evidences for the definite diagnosis of NTCP deficiency for the monozygotic twins. The p.Ser267Phe variant in *SLC10A1* gene had proven pathogenic by functional, bioinformatic, and clinical evidences (4, 10–12). Of note, this *SLC10A1* variant was prevalent in East Asian population, with an allele frequency of 8% in Southern Han Chinese, 12% in Chinese Dai and 11% in Vietnam (http://grch37.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=14:70244693-70245693;v=rs2296651;vdb=variation;vf=1699086) suggesting that this type of hypercholanemia might affect 0.64% of the Southern Han, 1.44% of the Dai Chinese, and 1.21% of the Vietnamese population (11). In addition, other non-synonymous *SLC10A1* variants have been identified previously in different ethnic populations as well, such as p.Ile223Thr and p.Ala64Thr, with an allele frequency of 5% in African Americans and 1% in Korean, respectively (1, 7). Therefore, although patients of NTCP deficiency were rarely reported in the past over 20 years, NTCP deficiency may not be rare worldwide, especially in East Asian population.

Persistent hypercholanemia was the prominent feature of the patients with NTCP deficiency in this paper. Bile acids are synthesized from cholesterol in the liver, secreted into bile which is stored in the gallbladder, and then enter the small intestine during a meal. The majority (>90%) of bile acids are reabsorbed from the intestine and returned to the liver via the portal venous circulation. This circling of bile salts is well-known as enterohepatic circulation (15). NTCP plays a key role in the enterohepatic circulation of bile salts as the major transporter of conjugated bile salts from the plasma compartment into the hepatocyte (5). The p.Ser267Phe variant had been reported to result in an almost complete loss of the function for bile acid uptake, rendering NTCP without taurocholate transporting activity (6, 7, 10). Although the hepatocyte sinusoidal membrane also expresses other sodium-independent bile acid transporters, these transporters play limited roles in bile acid clearance (16). As such, it was not strange for the twins in this paper to present with refractory hypercholanemia.

Of particular note, the father of the twins, who was a carrier of the p.Ser267Phe variant and HBV as well, also exhibited slight hypercholanemia (**Table 1**). Since the reported patients with NTCP deficiency thus far were all compound heterozygotes or homozygotes of *SLC10A1* biallelic variants (4, 9–14), indicating an autosomal recessive disorder, the slightly elevated TBA level in the father could not be explained by his carrier status of the p.Ser267Phe variant. Recently, Yan et al. found that NTCP also functions as a cellular receptor for viral entry of HBV through a specific interaction between NTCP and the pre-S1 domain of HBV large envelope protein (17), and showed that pre-S1 lipopeptide binding blocked the physiological function of NTCP in bile salt transport (6). As such, it is reasonable to speculate that the HBV carrier status of the father might affect the NTCP function to uptake bile acid at the basolateral membrane of the hepatocyte, being responsible for his slight hypercholanemia, as shown in **Table 1**.



Besides the persistent hypercholanemia, the elevated DBIL (67.6 $\mu\text{mol/l}$ and 45.6 $\mu\text{mol/l}$) fraction >20% of TBIL (106.5 $\mu\text{mol/l}$ and 73.1 $\mu\text{mol/l}$) in the twins at 3 months (Table 1) clearly indicated cholestatic jaundice in early infancy (18), and lent support to the concept that NTCP deficiency might work as one of the contributing factors that affect the bilirubin homeostasis, particularly in early infants who have very rich bilirubin sources but immature liver function to uptake, conjugate, and excrete bilirubin (19). Actually, hepatic uptake of bile salts is mediated by sodium-dependent and sodium-independent transporters (20). Sodium-independent uptake of bile salts is principally accomplished by the activity of multiple organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1/1B3), which work as a dimer protein to uptake plasma bilirubin, especially the direct bilirubin, as well as, bile acids into the hepatocyte (21, 22). NTCP is the primary sodium-dependent transporter of conjugated bile salts from the plasma compartment into the hepatocyte (5). Therefore, the elevated plasma bile acids caused by the deficiency of NTCP might increase the burden of the OATP1B1/1B3 to uptake bile acids into the hepatocyte, competitively inhibit its' function to uptake bilirubin, and result in secondary elevation of bilirubin, especially direct bilirubin, giving rise to cholestatic jaundice in infants as in the twins in this study, as well as, in NTCP-deficient patients reported previously (4, 10, 14).

CONCLUSION

In summary, this study reported the clinical and genetic findings of two monozygotic twins with NTCP deficiency. Our findings lent support to the important role of NTCP in bile salt homeostasis, and suggested that NTCP deficiency may be a unique genetic factor causing cholestasis in early infancy, as well as, persistent hypercholanemia at pediatric age.

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INFORMED CONSENT

The authors declare that this study was performed after written informed consent had been obtained from the parents of the twins, which permitted publication of this case report.

ETHICS STATEMENT

This study has been approved by the Committee for Medical Ethics, the First Affiliated Hospital, Jinan University.

AUTHOR CONTRIBUTIONS

H-JT performed data collection and drafted the initial manuscript. Y-ZS conceptualized and designed the study, critically reviewed, and revised the manuscript. MD and J-WQ carried out the genetic analyses and reviewed the manuscript. J-FW treated and followed up the patients and reviewed the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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