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CASE REPORT

Analysis of ileal sodium/bile acid cotransporter and related nuclear receptor genes in a family with multiple cases of idiopathic bile acid malabsorption

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Abstract

The etiology of most cases of idiopathic bile acid malabsorption (IBAM) is unknown. In this study, a Swedish family with bile acid malabsorption in three consecutive generations was screened for mutations in the ileal apical sodium-bile acid cotransporter gene (ASBT; gene symbol, SLC10A2) and in the genes for several of the nuclear receptors known to be important for ASBT expression: the farnesoid X receptor (FXR) and peroxisome proliferator activated receptor alpha (PPARα). The patients presented with a clinical history of idiopathic chronic watery diarrhea, which was responsive to cholestyramine treatment and consistent with IBAM. Bile acid absorption was determined using ^{75}Se-homocholic acid taurine (SeHCAT); bile acid synthesis was estimated by measuring the plasma levels of 7α-hydroxy-4-cholesten-3-one (C4). The ASBT, FXR, and PPARα genes in the affected and unaffected family members were analyzed using single stranded conformation polymorphism (SSCP), denaturing HPLC, and direct sequencing. No ASBT mutations were identified and the ASBT gene did not segregate with the bile acid malabsorption phenotype. Similarly, no mutations or polymorphisms were identified in the FXR or PPARα genes associated with the bile acid malabsorption phenotype. These studies indicate that the intestinal bile acid malabsorption in these patients cannot be attributed to defects in ASBT. In the absence of apparent ileal disease, alternative explanations such as accelerated transit through the small intestine may be responsible for the IBAM.

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Key words: Bile acid malabsorption; Diarrhea; Genetics; ^{75}Se-homocholic acid taurine test; Nuclear receptors

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INTRODUCTION

Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine, where they facilitate absorption of fat, fat-soluble vitamins and cholesterol[1]. The bile acids are then reabsorbed from the intestine and returned to the liver *via* the portal venous circulation. The enterohepatic cycling of bile acids is an extremely efficient process, and less than 5% of the intestinal bile acids escape reabsorption and are eliminated in the feces. The ileal apical sodium/bile acid cotransporter (ASBT)[1] mediates the first step in the active uptake of bile acids from the intestine, and defects in ileal ASBT function may be responsible for bile acid malabsorption associated with watery diarrhea. Impaired ileal uptake of bile acids has been documented in several patients[2] and inherited ASBT mutations were demonstrated in congenital primary bile acid malabsorption (PBAM)[3]. However, ASBT mutations are not found in most patients with adult-onset bile acid malabsorption, chronic diarrhea, and a morphological and

functionally normal ileum[4], a more common condition termed idiopathic bile acid malabsorption (IBAM)[5-7]. In this study we examined the association between IBAM and inherited mutations affecting the ASBT and several of the nuclear receptors known to be important for ASBT expression, the farnesoid X receptor (FXR)[8] and peroxisome proliferator activated receptor A (PPARα)[9], in a Swedish family with three generations of bile acid malabsorption.

CASE REPORT

Three family members (subjects 1, 10, and 11) reported chronic diarrhea, occurring especially after meals (Figure 1). Fasting blood samples were obtained from each family member. Informed consent to participate in the study was obtained from each subject and the protocol was approved by the Ethics Committee of Karolinska University Hospital Huddinge. Bile acid absorption was determined using ^{75}Sehomocholic acid taurine (SeHCAT), a synthetic analog of taurocholic acid, as previously described[10]. Briefly, a capsule containing 10 μCi of ^{75}SeHCAT was given orally and retained activity was measured after 3 h and 7 d using an uncollimated gamma counter. Retention of less than 10% of the administered radiolabeled bile acid was considered abnormal. The plasma level of 7α-hydroxy-4-cholesten-3-one (C4) (normal < 19 ng/mL), an intermediate product in the synthesis of bile acids, was measured as described[11]. C4 is a reliable marker for the activity of hepatic cholesterol 7α-hydroxylase, the rate-determining enzyme in bile acid synthesis[12,13].

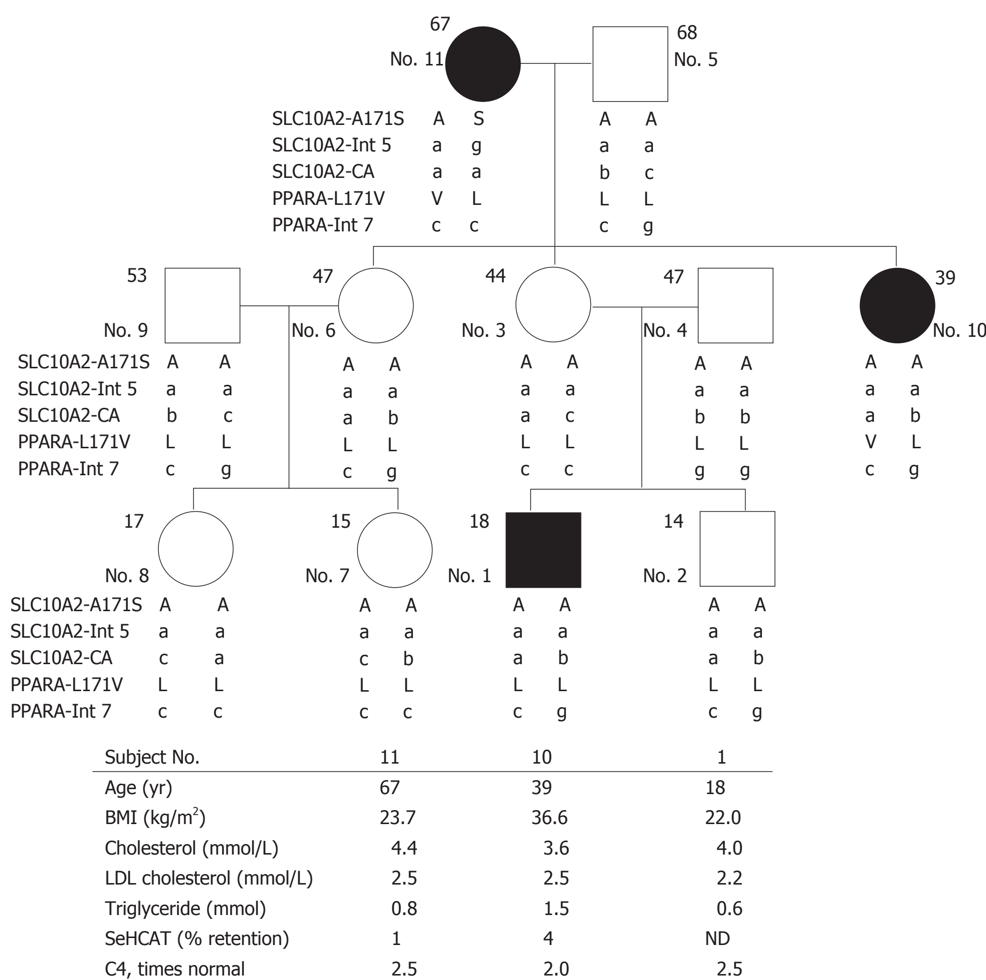
Patient 11 had a history of diarrhea since adulthood with 15 to 20 watery bowel movements per day over the past 10 years. Clinical history was unremarkable except for a cholecystectomy at age 24. Patient 10 had a history of frequent watery diarrhea since her teenage years. Patient 1 reported frequent bowel movements following a meal. In all three patients, celiac disease was excluded; lactose tolerance tests, vitamin B12 absorption, and routine laboratory blood tests including hemoglobin, sedimentation rate and liver function tests were normal. Barium contrast gastrointestinal exams and ileocolonoscopy with mucosal biopsies (patients 10 and 11) were normal. SeHCAT tests (patients 10 and 11) and plasma levels of C4 (patients 1, 10 and 11) were markedly abnormal (Figure 1). Treatment of patients 10 and 11 with cholestyramine (Questran, Bristol-Myers) reduced the stool frequency and improved the stool consistency.

Dysfunctional mutations in the ASBT gene were previously identified in a subject with PBAM[3]. To determine if similar mutations in ASBT are associated with bile acid malabsorption in this family, we employed simple sequence length polymorphism (SSLP) analysis using a dinucleotide repeat linked to the ASBT gene and SSCP analysis to screen for mutations in the ASBT coding and proximal promoter regions. The SSCP primers designed for ASBT intron or exon sequences and PCR amplification conditions have been described previously[3,14]. PCR amplification products were resolved using three different gel electrophoresis conditions, gels contained 10% glycerol, 1 × TBE buffer, and 6% acrylamide, 10% acrylamide (acrylamide: N, N'-methylenebisacrylamide ratio 50:1), or 0.4 × MDE (Mutation Detection Enhancement acrylamide; FMC Bioproducts, Rockland, Maine), in order to increase the assay sensitivity[15,16], and the nucleotide sequence changes responsible for the SSCP band shifts were subsequently identified by PCR amplification and sequencing. No ASBT mutations or polymorphisms were found in patients 1 or 10, whereas patient 11 was heterozygous for two common polymorphisms that do not affect ASBT function[14], a G-to-T transversion in exon 3 that causes an alanine to serine substitution at position 171 (A171S) and an intronic A-to-G transition located 20 bp upstream of exon 6 (int 5). Following SSCP analysis, the two ASBT alleles could then be distinguished in the original proband (patient 11) using a combination of the linked dinucleotide repeat marker and the single nucleotide polymorphisms (A171S and int 5). The affected individuals shared only one ASBT allele, and four unaffected individuals (subjects 2, 3, 6 and 8) also inherited this allele.

Since the nuclear receptor, FXR, is an important regulator of ASBT expression and bile acid metabolism, the coding region of the FXR gene, exons 3 to 11[17], was also analyzed in patients 1, 10, and 11 using SSCP conditions described by Lind *et al*[18]. Sequences for SSCP primers designed for FXR intron and exon sequences are shown in Table 1. Briefly, exons 3-11 were amplified by PCR from genomic DNA, generating fragments varying from 200 to 250 bp in length, except exon 10 (315 bp) and exon 11 (385 bp). Due to its larger size, exon 4 was PCR-amplified using two sets of primers that yielded products of 240 and 300 bp. The fragments were separated on precast polyacrylamide gels visualized by silver staining (GenePhor DNA Separation System, Amersham Bioscience, Uppsala, Sweden). This analysis detected no mutations or polymorphisms in the human FXR gene of these patients.

Table 1 Sequences for the primers for SSCP on human FXR

Exon		Primer
3	Forward	5'-CATTCCCACAGTCACAAACTATTTA-3'
	Reverse	5'-GTAGTTTGTTCTTATTGATATTCAAATG-3'
4 proximal	Forward	5'-GATGACATTCATTCCAGTTTTGTTGTC-3'
	Reverse	5'-AGCTGGCATACGCCTGAGTTCATAT-3'
4 distant	Forward	5'-TCATCCTATTATTCCAACCTGGGTTTC-3'
	Reverse	5'-AGTAAAACCTGAAGGAGAAAACTGCC-3'
5	Forward	5'-GAGGACTTTTTACACTTTTCAGTGT-3'
	Reverse	5'-AATGTAATTGCTTGAAGTGAATACC-3'
6	Forward	5'-GTACTTTCTGTGATTGGTGAAGTCTC-3'
	Reverse	5'-AAACTCAGTTCCTGCCAGTCTTGGC-3'
7	Forward	5'-GATGAATGCACATATAGAAAGAAGGC-3'
	Reverse	5'-CTCCAGAAAATATTAACTTTAAACCCACAT-3'
8	Forward	5'-CAAAGATCTGAGAAATAGTAAGATGG-3'
	Reverse	5'-AGGTAATCTAATCTGTGGGCAC-3'
9	Forward	5'-GTTACTCCTTGATACCAATTTGATTATC-3'
	Reverse	5'-CTCCTAGAAACAAACTCTTTTACCAT-3'
10	Forward	5'-CTAGTTTTACACTGTTTAGTCACTC-3'
	Reverse	5'-ATTTGGATAGCAGAATTATAGGCTAC-3'
11	Forward	5'-CTTACACTTCAAAATAGTTAACGTTGC-3'
	Reverse	5'-GCTCCTTTTTCTCTCTCATATTAATC-3'



Subject No.	11	10	1
Age (yr)	67	39	18
BMI (kg/m^2)	23.7	36.6	22.0
Cholesterol (mmol/L)	4.4	3.6	4.0
LDL cholesterol (mmol/L)	2.5	2.5	2.2
Triglyceride (mmol)	0.8	1.5	0.6
SeHCAT (% retention)	1	4	ND
C4, times normal	2.5	2.0	2.5

BMI: Body mass index; ND: Not determined.

Figure 1 Family pedigree and genetic analysis. Individuals with bile acid malabsorption are indicated by the shaded symbols. The haplotype for each subject is provided below the symbol in the pedigree. The affected subjects' number, age, BMI, SeHCAT results, and C4 results are indicated below the pedigree.

Polymorphisms in the intronic and exonic regions of the PPARα gene (PPARA) have been previously described[19,20], and PPARα is a known regulator of ASBT gene expression[21]. We analyzed two well-characterized polymorphic regions of PPARA, exon 5 and intron 7, in the PBAM family in order to determine if a mutation in this gene could be associated with the disease. Specific primers were employed for PCR amplification of exon 5 (forward: 5'-AGTAAAGCAAGTGCGCTGGT-3'; reverse: 5'-AAGGAAGGGGAACTGAGGAA-3') and intron 7 (forward: 5'-CCTCCCGAGTATCTGGGATT-3'; reverse: 5'-TGAGCTGCCTTTAGATATTGTCA-3'). The PCR products were analyzed for polymorphisms or mutations by denaturing HPLC (D-HPLC) (Transgenomic Wave, Transgenomic, Omaha, Nebraska) and automatic sequencing (automatic sequencer CEQ™8000 XL, Beckman Coulter Inc., Fullerton. CA). PPARA gene analysis did not show any new mutations. Analysis of the L162V polymorphism of exon 5 and G > C polymorphism of intron 7 revealed that PPARA alleles did not segregate with the bile acid malabsorption symptoms (Figure 1).

DISCUSSION

The enterohepatic circulation efficiently conserves bile acids, thereby maintaining bile flow and adequate intraluminal bile acid concentrations for micellular solubilization and absorption of lipids[22]. Defective small intestinal absorption leads to increased concentrations of dihydroxy bile acids reaching the colon, where they alter water and electrolyte movement leading to secretory diarrhea[23,24]. Three types of intestinal bile acid malabsorption are generally recognized[25]. Type Ⅰ bile acid malabsorption is the most common form and is caused by ileal resection, ileal disease such as Crohn's disease, ileal bypass, and radiation enteritis[26,27]. Type Ⅲ bile acid malabsorption is associated with conditions such as cholecystectomy, peptic ulcer surgery, chronic pancreatitis, celiac disease, diabetes mellitus, cystic fibrosis, and the use of various drugs[28].

In contrast to types Ⅰ and Ⅲ, type Ⅱ bile acid malabsorption (also called primary or idiopathic bile acid malabsorption) is not associated with obvious ileal disease. A very rare congenital form of type Ⅱ bile acid malabsorption (primary bile acid malabsorption) exhibiting refractory infantile diarrhea, steatorrhea, and growth failure[2,25] was found to be associated with inherited mutations in the ASBT gene[3]. However, most patients with adult-onset idiopathic bile acid malabsorption appear to have a normal ASBT gene[4] and the etiology is still obscure. The identification of a family with idiopathic bile acid malabsorption in three consecutive generations offered a rare occasion to further evaluate association of this syndrome with inherited mutations affecting

the ASBT. These patients were diagnosed with IBAM on the basis of clinical presentation, low SeHCAT test values, increased bile acid synthesis, and response to cholestyramine treatment. Analysis of these individuals and unaffected family members conclusively demonstrated that the intestinal bile acid malabsorption in these subjects is not due to inherited defects in the ASBT gene. In addition, we also looked for polymorphisms of PPARα and FXR, two nuclear receptors known to be important for the regulation of the ASBT. To our knowledge, polymorphism analysis of the human FXR has not been described previously and no mutations of the FXR gene were found in the present study. Likewise, no association between PPARA and IBAM was found in this family.

There is increasing evidence emerging in support of IBAM etiologies other than defective ileal uptake of bile acids. Earlier studies had provided evidence for an increased ileal uptake of bile acids[29] as well as an expanded bile acid pool in some patients with type Ⅱ bile acid malabsorption[28]. Very recently, Bajor *et al*[30] demonstrated elevated *in vitro* bile acid uptake and ASBT protein expression in ileal biopsies from patients with bile acid malabsorption, abnormal SeHCAT-retention values, and elevated plasma C4 levels. This apparent increase in ASBT activity and expression could be explained by accelerated small bowel transit in IBAM patients, thereby reducing the contact time between the luminal contents and the mucosa. In support of this hypothesis, a more rapid small bowel transit has been reported for patients with IBAM[31]. The etiology of the postulated accelerated small bowel transit in these patients is not clear. However, more rapid small bowel transit has been noted in subjects with elevated BMI[31,32] and in subjects consuming high fat diets[33], suggesting dysregulation of gut motility under these conditions. The rapid small bowel transit is predicted to reduce the opportunity for ileal absorption, leading to decreased levels of bile acids in the ileal enterocytes and increased ASBT expression. Previous *in vitro* studies have shown that the human ASBT promoter is negatively regulated by bile acids through an FXR dependent mechanism[8]. The decreased enterocyte levels of bile acids are also predicted to reduce the FXR-dependent induction of FGF19 expression, thereby increasing hepatic bile acid synthesis and plasma C4 levels. FGF19 is an ileal enterocyte derived factor that mediates repression of the hepatic cholesterol 7α-hydroxylase gene and bile acid synthesis[34,35].

In conclusion, the present findings further argue against defective ileal uptake of bile acids as the direct cause of IBAM and support the exploration of alternative explanations such as reduced contact time with the ileal mucosa due to changes in small intestinal motility.

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