# An Association between Genetic Polymorphisms in the Ileal Sodium-dependent Bile Acid Transporter Gene and the Risk of Colorectal Adenomas[1]

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## Abstract

**Epidemiological and experimental studies have implicated bile acids (particularly secondary bile acids) as important factors in the development of colorectal cancer. The ileal sodium-dependent bile acid transporter (ISBT) is a crucial player in the enterohepatic circulation of bile acids. Genetic defects in ISBT may result in malabsorption of bile acids and a loss of bile acids into the large intestine, with a resultant increase in the cytotoxic secondary bile acids in the colon. In a case-control study, we investigated the association between two sequence variations in *SLC10A2*, the gene encoding ISBT, and colorectal adenomas, a precursor lesion of colorectal cancer. The frequency of the missense mutation in codon *171* of exon 3 (a nucleotide transversion from G to T resulting in an alanine to serine substitution) was not significantly different between cases and controls. However, we found a 2-fold higher risk of colorectal adenomas associated with a C→T nucleotide transition in codon *169* of exon 3 (odds ratio = 2.06; 95% confidence interval: 1.10–3.83). Logistic regression analysis using *A171S/169 C→T* haplotypes as the allelic markers showed that among *AA* wild-type homozygotes for *A171S* mutation, this C→T nucleotide transition in codon *169* was associated with a 2.42 times increased risk (odds ratio = 2.42; 95% confidence interval: 1.26–4.63). This initial observation of an association between a polymorphism in the *SLC10A2* gene and the risk of colorectal adenomatous polyps would, if confirmed by other studies, support the role of bile acids in the carcinogenesis of colorectal cancer.**

## Introduction

Epidemiological and experimental studies suggest that bile acids, and secondary bile acids in particular, may play an important role in colorectal carcinogenesis (reviewed in Ref. 1). Bile acids are the major end products of cholesterol metabolism. There are two classes of bile acids. Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized initially in the liver primarily by 7$\alpha$ hydroxylation of cholesterol followed by several intermediate steps. After conjugation with glycine or taurine, they are secreted into bile and stored in the gall bladder. After a meal, they are released into the lumen of the upper small intestine to facilitate the digestion and absorption of dietary fats. Secondary bile acids (mainly sodium deoxycholate and lithocholic acid) are produced in the colon after the deconjugation and dehydroxylation of primary bile acids by the bacteria that dwell in the colonic lumen. Bile acids in the intestine are absorbed and returned to the liver through the portal vein. They are then extracted by hepatocytes and secreted into bile. This is the so-called EHC.[3] EHC is a very efficient way of recycling bile acids. About 95% of the bile acids passing through the intestine are reabsorbed, and only a small fraction escapes the EHC and is lost in the feces. Although the passive absorption of primary and secondary bile acids occurs throughout the intestine, they are primarily absorbed in the ileum by active transport (2, 3). It is reasonable to postulate that any factor that causes an interruption in this active absorption would lead to an increased amount of bile acids passing into the colon. This would result in an increased exposure of the colorectal epithelium to secondary bile acids (either from the direct passage through the ileum-colon junction or from the increased bacterial transformation from primary bile acids), which have been shown to be tumor promoters in animal studies (1). The ISBT, localized at the apical site of ileal enterocytes, is thus far the only molecule identified to be responsible for the active transport of bile acid in the ileum (4). Recently, a gene (*SLC10A2*) encoding ISBT was cloned and mapped to chromosome *13q33. SLC10A2* is composed of 6 exons, which span ~24 kb of DNA sequence, encoding a 348 amino acid membrane glycoprotein with seven transmembrane helices (5). Four mutations, including a 3-nucleotide substitution at the splice donor site for exon 3, missense mutations *A171S* in exon 3, *L243P* in exon 4, and *T262M* in exon 5, were identified in a study of a family with congenital primary bile acid malabsorption by single-stranded conformational polymorphism analysis. Three of the mutations were found to be functional. The 3-nucleotide substitution was predicted to cause exon skipping. Functional studies found that the missense mutations *L243P*

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[3] The abbreviations used are: EHC, enterohepatic circulation; ISBT, ileal sodium-dependent bile acid transporter; LDL, low-density lipoproteins; OR, odds ratio; CI, confidence interval.

and *T262M* could affect the transport activity of ISBT (6). Our study group performed mutation screening of all 6 exons of *SLC10A2* and found 6 more mutations and polymorphisms.[4] We hypothesized that the known functional mutations in *SLC10A2* may be associated with the risk of colorectal cancer by diminishing the efficiency of EHC. Nonfunctional polymorphisms may also be associated with risk of colorectal cancer if they are in linkage disequilibrium with functional alleles. We report here the results of a case-control study of adenomatous polyps, precursors of colorectal cancer, and selected markers of *SLC10A2*.

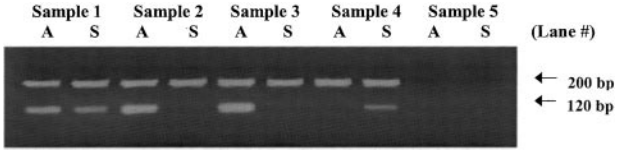


*Fig. 1.* Genotyping of *A171S* polymorphism. The figure shows two DNA fragments of 200 bp and 120 bp that correspond to the products from internal amplification control primers and allele-specific primers, respectively. *Sample 1*, heterozygote *AS; Sample 2* and *Sample 3*, homozygote *AA; Sample 4*, homozygote *SS; Sample 5*, $H_2O$ (negative control).

## Materials and Methods

**Subjects.** Subjects were obtained from a sigmoidoscopy-based case-control study of colorectal adenomatous polyps. A detailed description of this study can be found in a previous publication (7). In brief, cases and controls were selected from subjects who underwent a sigmoidoscopy in either of the two Southern California Kaiser Permanente Medical Centers (Bellflower and Sunset, CA) during the period from January 1, 1991 to August 25, 1993. Cases were diagnosed for the first time with one or more histologically confirmed colorectal adenomas. Controls had no history of polyps and were free of polyps at the sigmoidoscopy and were individually matched to cases by gender, age (within 5-year category), date of sigmoidoscopy (within 3-month category), and Kaiser Permanente Center. Cases and controls were: (*a*) 50–74 years of age at the time of recruitment; (*b*) residents of Los Angles or Orange County; and (*c*) fluent in English. The exclusion criteria were: (*a*) having a history of invasive cancer, inflammatory bowel disease, or familial polyposis; (*b*) having symptoms that suggested gastrointestinal disease; and (*c*) having physical or mental disability that would preclude an interview. The study was approved by the University of Southern California and the Kaiser Permanente Institutional Review Boards. All of the subjects signed informed consents.

**Mutation Detection.** Twenty-eight (a manageable sample size for mutation screening) cases with the lowest serum LDL levels among all of the cases of this case-control study were selected for the purpose of mutation screening. This number of test subjects should allow 80% power to detect polymorphic alleles present at a frequency of >3% (based on the assumption that the sensitivity of mutation detection is ≥90% for sequencing and for nonisotopic RNase cleavage assay). The subject selection strategy was based on the assumption that dysfunctional mutations in *SLC10A2* would lead to a loss of bile acids in the ileum. The loss of bile acids would result in an increased synthesis of bile acids from cholesterol in the liver and, thus, may cause a low serum level of cholesterol. Consequently, choosing cases with low LDL for mutation screening would provide us with the greatest chance of finding at-risk mutations/polymorphisms in *SLC10A2* for colorectal adenomas, under the assumption that their low LDL was not attributable to LDL-lowering drugs. Sequence information on the human *SLC10A2* gene was obtained from GenBank (accession no. U67669-U67674). Direct sequencing was used for the screening of mutations in exons 2 and 3. Nonisotopic RNase cleavage assay was used for mutation screening in exons 1, 4, 5, and 6. Six new mutations/polymorphisms were found. A C to T nucleotide transition in codon *169* of exon 3 (no amino acid change, leucine→leucine) was the most frequent polymorphism found among all of the newly identified mutations/polymorphisms, with an allele frequency of 8.9% (5/56). Among the four mutations reported previously, only *A171S* (a nucleotide transversion from G to T, resulting in an alanine to serine substitution at amino acid position 171) had a relatively high allele frequency of 12.5% (7/56). To have enough statistical power to detect any significant genetic differences between cases with colorectal adenomas and controls, we chose to study only the most frequent polymorphisms, *A171S* and *169 C→T*.

**Genotyping.** *A171S* and *169 C→T* genotyping was performed on 1013 subjects who provided a blood specimen. PCR amplification of specific alleles was used as the genotyping technique (8). For *A171S* genotyping, the forward primer for the *A* allele was 171cg (5′-CATCTTTAGGTACATCTCTGGTCG-3′).[5] The forward primer for the *S* allele was 171ct (5′-CATCTTTAGGTACATCTCTGGTCT-3′).[5] The reverse primer was 3R (5′-GGTTTTGATTGTCACAGTGGAATA-3′). Another primer set was used as an internal amplification control for *A171S* genotyping. The sequences were (5′-CTCTTAAGGGCAGTTGTGAG-3′) for the forward primer 5F and (5′-TTCCTACTGTGGTTGCTTCC-3′) for the reverse primer 5R. DNA (20 ng) was added to a final volume of 25 μl containing 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl, and 0.1% Triton X-100), 0.3 mM deoxynucleoside triphosphate mixture, 80 nM of each primer, 1.5 unit of Taq DNA Polymerase (Promega, Madison, WI), and 2.3 mM $MgCl_2$. A PCR program of 35 cycles (94°C denaturing for 30 s, 55°C annealing for 30 s, and 72°C extension for 30 s) was conducted using PTC-100 thermal cycler (MJ Research, Waltham, MA), preceded by a 5-min denaturing at 94°C and an 85°C hot start stage and followed by an extension step of 72°C for 5 min. The product sizes were 200 bp for the internal amplification control and 120 bp for either the *A* allele or the *S* allele. PCR products were loaded onto a 2.5% native agarose gel. The PCR products of primers 171cg and 3R were loaded in *Lane A*. The products of primers 171ct and 3R from the same sample were loaded in the adjacent *Lane S*. We scored samples with the presence of a 120-bp band in *Lane A* and the absence of 120 bp in *Lane S* as homozygous *AA*; we scored samples with the presence of 120-bp band in *Lane S* and the absence of 120-bp band in *Lane A* as homozygous *SS*; we scored samples with the presence of 120-bp band in both *Lane*s *A* and *S* as heterozygous *AS* (Fig. 1). Samples without the 200-bp PCR products in either *Lane A* or *Lane S* were considered not scorable because of poor PCR amplification, and these samples were reamplified. The same method was used for the genotyping of *169 C→T*. The forward primers used were

[4] S. Xue. Polymorphisms in the ileal sodium-dependent bile acid transporter gene (SLC10A2), manuscript in preparation.

[5] The underlined is the position of the mismatch between primers or the mismatch between primer and genomic sequence.

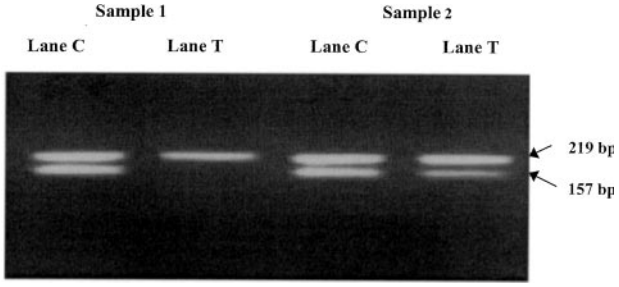


*Fig. 2.* Genotyping of polymorphism *169 C→ T*. The figure shows two DNA fragments of 219 bp and 157 bp that correspond to the products from internal amplification control primers and allele-specific primers, respectively. *Sample 1,* homozygote *CC; Sample 2,* heterozygote *CT*.

169wt (5′-CCTTCTGTCATCTTTAGGTACATCAC-3′)[5] for allele *C* and 169 M (5′-CCTTCTGTCATCTTTAGGTACATCAT-3′)[5] for allele *T*. The reverse primer was the same one as that used in *A171S* genotyping. The primers used for internal control of PCR amplification were X5F (5′-AAAACATGTGCTCTCTTAACATCTTC-3′) and X5R2 (5′-TCATGAAATGGGATTGGC-3′) for *169 C→T* genotyping. The PCR reactions were carried out in a final volume of 25 μl containing 20 ng of DNA, 1 × PCR buffer, 0.3 mM deoxynucleoside triphosphate mixture, 125 nM of internal amplification control primers, 80 nM of allele-specific primers, 1.5 unit of Taq DNA Polymerase (Promega), and 3.5 mM $MgCl_2$. The same PCR profile as in *A171S* genotyping (except for an annealing temperature of 58°C) was used for the genotyping of the *169 C→ T* polymorphism. PCR products were then loaded onto a 2.5% native agarose gel. The PCR product from X5F and X5R2 had a size of 219. In addition to this band, a 157-bp product in *Lane C* but not in *Lane T* indicates a sample homozygous for genotype *CC*. Samples with a 157-bp in *Lane T* but not in *Lane C* were scored as *TT* homozygotes. Samples with a 157-bp band seen in both lanes were called *CT* heterozygotes (Fig. 2). With each batch of samples being genotyped, a negative control ($H_2O$) and a positive control (sample ascertained to have the mutant allele) were included. Lab personnel assigning genotypes were blind to the case-control status of the subjects. All of the genotyping results were independently confirmed by a second reader. Sequencing was performed on all of the samples with ambiguous readings.

**Statistical Methods.** Tests for Hardy-Weinberg equilibrium were performed in each ethnic group by comparing observed genotype frequencies with the expected ones using a $\chi^2$ test. Because missing data resulted in the existence of unmatched cases and controls in the dataset (7), unconditional logistic regression was used to estimate the odds ratios for each of the two loci (codon *171* and *169*). Ethnicity and all of the matching variables: gender, age (categorical variable with 5-year intervals), date of sigmoidoscopy (categorical variable with 3-month intervals), and Kaiser Center (Bellflower or Sunset) were adjusted for in the logistic regression model. Odds ratios for haplotypes of the two loci were also estimated. All of the *P*s resulted from two-sided tests.

## Results

This study included 458 cases of colorectal adenoma and 504 controls with both A171S and 169 C→T genotypes available. The demographic characteristics of the subjects are listed in Table 1. Overall, the mean age of subjects was 62 years. There were more males than females. More than half of the study subjects were non-Hispanic whites, followed by African-Americans, Hispanic whites, and Asian or Pacific Islanders. These demographic characteristics were fairly comparable for cases and controls.

*Table 1* Characteristics of subjects with colorectal adenoma and controls

| | Colorectal adenoma cases ($n$ = 458) | Controls ($n$ = 504) |
|---|---|---|
| Gender | | |
| Male | 296 (67.1%) | 338 (64.6%) |
| Female | 162 (32.9%) | 166 (35.4%) |
| Ethnicity | | |
| Non-Hispanic white | 256 (55.9%) | 273 (54.2%) |
| African-American | 77 (16.8%) | 85 (16.9%) |
| Hispanic white | 80 (17.5%) | 91 (18.1%) |
| Asian/Pacific Islander | 45 (9.8%) | 55 (10.9%) |
| Mean age (SD) | 62.0 (6.81) | 62.0 (6.77) |

*Table 2* Associations between the risk of colorectal adenoma and genotypes of *A171S* and *169 C → T*

| Genotype | No. of cases (%) | No. of controls (%) | Odds ratio (95% CI)[a] |
|---|---|---|---|
| *A171S* | | | |
| *AA* | 343 (74.9) | 386 (76.6) | 1.00 |
| *AS* | 100 (21.8) | 104 (20.6) | 1.12 (0.81–1.54) |
| *SS* | 15 (3.3) | 14 (2.8) | 1.22 (0.58–2.60) |
| Total | 458 | 504 | $P$ = 0.41[b] |
| *169 C → T* | | | |
| *CC* | 428 (93.4) | 487 (96.6) | 1.00 |
| *CT* | 30 (6.6) | 17 (3.4) | 2.06 (1.10–3.83) |
| Total | 458 | 504 | |

[a] Odds ratio after the matching variables (age, gender, date of sigmoidoscopy, and Kaiser Center) and ethnicity were adjusted. Additional adjustment of LDL (categorical variable by quintiles) did not change the odds ratios appreciably.
[b] Test for trend.

Among all of the healthy controls, allele *S* at locus *171* had a frequency of 13.1% and allele *T* at locus *169* had a frequency of 1.7%. The genotype frequencies for both loci were found to be in Hardy-Weinberg equilibrium in each ethnic group (data not shown).

Adjusted odds ratios for *A171S* and *169 C→T* genotypes are shown in Table 2. The *A171S* genotypes were not significantly associated with risk of colorectal adenoma (*P* for trend test, 0.41). However, for the *169 C→T* polymorphism, *CT* heterozygotes were at an ~2-fold higher risk, compared with *CC* homozygotes.

Although the *169 C→T* polymorphism itself is not functional, it may be a marker for some unidentified functional at-risk alleles in the *SLC10A2* gene. Nevertheless, if the linkage disequilibrium between this marker and the at-risk allele is not perfect, using the marker allele will cause misclassification of the at-risk allele and result in attenuated odds ratios. Therefore, we also examined *A171S/169C→T* haplotypes, which may be better markers than either *A171S* or *169C→T* alone.

Because there were no subjects genotyped as double heterozygotes (*AS, CT*), *A171S/169 C→T* haplotypes could be inferred from *A171S* and *169 C→T* genotypes for all of the subjects. Using the most frequent allelotype, *AC/AC*, as the baseline, we estimated the adjusted odds ratio for each of the other allelotypes (Table 3). A significantly increased risk of colorectal adenomas was found to be associated with the *AC/AT* allelotype (OR, 2.42; 95% CI, 1.26–4.63), indicating

*Table 3* Association between the risk of colorectal adenoma and haplotypes of *A171S* and *169 C → T*

| *A171S* | *169 C → T* | Haplotype | No. of cases (%) | No. of controls (%) | Odds ratio (95% CI)[a] |
|---|---|---|---|---|---|
| *AA* | *CC* | *AC/AC* | 313 (68.3) | 371 (73.6) | 1.00 |
| *AS* | *CC* | *AC/SC* | 100 (21.8) | 104 (20.6) | 1.17 (0.85–1.61) |
| *SS* | *CC* | *SC/SC* | 15 (3.3) | 12 (2.4) | 1.50 (0.68–3.28) |
| | | | | | $P = 0.18$[b] |
| *AA* | *CT* | *AC/AT* | 30 (6.6) | 15 (3.0) | 2.42 (1.26–4.63) |
| *SS* | *CT* | *SC/ST* | 0 (0) | 2 (0.4) | NA[c] |

[a] Odds ratio after the matching variables (age, gender, date of sigmoidoscopy, and Kaiser Center) and ethnicity were adjusted.
[b] Test for trend.
[c] NA, not applicable. Not estimated because of empty cell.

that among *AA* homozygotes for *A171S*, the *T* allele was associated with the risk. There was an increase in risk estimate associated with the *T* allele in Table 3 compared with Table 2. Analyses found that the change in the risk group (with two controls and no cases reclassified in Table 3) could only explain part of this increase in odds ratios (data not shown), suggesting that this increase in risk estimates was at least partly attributable to the fact that haplotypes may be better markers for the yet-to-be identified at-risk allele.

The effect of the *T* allele could not be evaluated among *AS* heterozygotes or *SS* homozygotes because of small numbers. For the *A171S* polymorphism, among carriers of *CC* genotype at the *169* locus, the magnitudes of odds ratios for *S* alleles were also found to be higher than the ones we reported in Table 2, although the trend was still not significant.

Race-specific allele frequencies for these two sequence variations were examined. The mutant allele *S* was found to be most common in the Asian/Pacific Islander ethnic group, with a frequency of 25.5%. Hispanic whites had a frequency of 13.7%. The frequency for this allele was similar for the other two ethnic groups (about 10%). As to the *T* allele at locus *169*, American-Africans had the highest frequency (4.7%), followed by non-Hispanic whites (1.5%), and Hispanic whites (0.5%). This *T* allele was not found among Asian/Pacific Islanders. We were unable to conduct informative analyses in each of the ethnic groups because of small race-specific sample sizes. Analyses on non-Hispanic whites (the largest ethnic group in this study) alone produced very similar odds ratios to the ones reported in Tables 2 and 3. We also observed the same pattern of an increase in the magnitude of odds ratios for *S* alleles among carriers of *CC* genotype, although the trend test was still not significant. The haplotype *AC/AT* was still associated with a significantly increased risk (OR, 2.74; 95% CI, 1.07–7.00).

## Discussion

Bile acids, especially secondary bile acids, have been implicated in the etiology of colorectal cancer. Although the precise mechanism by which secondary bile acids increase colorectal cancer risk is not clear, the cytotoxicity of secondary bile acids has been indicated as one mechanism behind their tumor-promoting activity (9, 10). Secondary bile acids were found to be able to cause the disruption of the cell membrane of colonic mucosa cells in rats and humans (11, 12). *In vitro*, they were also found to cause DNA damage in both bacterial and mammalian cells (13).

It is generally accepted that the damage and the death of colorectal epithelial cells would in turn stimulate the proliferation of crypt cells to compensate for the dead cells. Together with higher level of deoxycholic acid and lithocholic acid in fecal water, colonic epithelial hyperproliferation was observed in patients with colonic adenomas or cancers and was considered to be a biomarker of increased colon cancer risk (14, 15).

More recent studies suggested that a preferential survival of mutated apoptosis-resistant colonic cells resulting from a long-term exposure to bile acids may be a putative explanation of the link between neoplasm formation and bile acids. Apoptosis is a mechanism for natural cell death. The ability to undergo apoptosis is important to eliminate unrepaired DNA damage caused by damaging agents such as bile acids. It was observed that mucosal biopsies from colorectal cancer patients had less susceptibility to bile acid-induced apoptosis compared with mucosa from cancer-free controls. It is hypothesized that the selective survival of apoptosis-resistant cells may lead to an accumulation and retention of DNA damage and mutations (including those that are carcinogenic), which may result in a greater chance of cancer development (16).

Descriptive epidemiological studies have repeatedly reported that population groups at high risk of colorectal cancer had an elevated excretion of fecal bile acids, often accompanied by an increased ratio of secondary bile acids to primary bile acids (17–20). Some case-control studies also reported that patients with colorectal cancer or polyps had significantly higher levels of fecal deoxycholic acid and lithocholic acid than controls (15, 21). However, other case-control studies did not find significant differences in the concentration of fecal bile acids (total bile acids, primary bile acids, or secondary bile acids) between patients with colorectal adenomas and controls (22, 23).

The substantial inter- and intra-individual variation in the fecal bile acids may be a major cause of the inconsistency of these case-controls studies. There are many variables that could affect the fecal bile acid profile extensively, such as age, dietary habits, transit time, stool size, hepatic function, intestinal disease, and possible previous bowel surgery. For example, it was found that the concentration of fecal secondary bile acids increased with advancing age and was significantly higher in elderly subjects compared with young adults (1). If the cases were older than the controls (which may likely be the case if age is not matched or adjusted for in a case-control study on cancer), we would expect a positively biased result. In other studies, the proportion of secondary bile acids in total fecal bile acids was strongly correlated with both transit time and stool weight (21). Also, the collection and storage of fecal samples and the chemical method chosen to measure the fecal bile acids may make the measurements from different studies incomparable. The reported normal range for fecal bile acid reported in previous studies was very wide and depends on the measurement methods (24). Day-to-day variation in fecal bile acid excretion as high as 400% was found by Setchell *et al.* (24). Even within one stool sample, a sampling error for fecal bile acid measurement may occur because of the lack of mixing of colonic contents. On the basis of these findings, a 3-day or 5-day sample collection has been recommended, which is not feasible for large-scale epidemiological studies and may not be a good indicator for long-term bile acid exposure.

In contrast to fecal samples, serum bile acid levels show much less variation. Results from epidemiology studies based on serum bile acids were more consistent. Higher serum concentration of deoxycholic acid (major secondary bile acids in serum) was seen in patients with adenoma than in healthy controls (25, 26), and this difference still existed at 6-months and 12-months of follow-up (27). However, as the authors

noted, serum bile acids may still be affected by many factors (such as the dietary habits, transit time, the absorption of bile acid by the ileocytes and the colonic mucosa, and the hepatic clearance, gallstones). So it may not be a good indicator for lifetime bile acid exposure either.

The finding of polymorphisms in genes involved in bile acid metabolism may provide us with a more reliable way to assess the bile acid hypothesis in epidemiological studies.

To our knowledge, our study is the first one to estimate the risk of colorectal adenoma associated with genetic variation in *SLC10A2*, the gene encoding the ISBT. ISBT is one of the key molecules in the enterohepatic circulation of bile acids and the only recognized bile acid transporter in the apical membrane of the intestine. It acts in the ileum, where a significant portion of the bile acids is reabsorbed from the intestine and then re-enters the enterohepatic circulation. The functional significance of *SLC10A2* in the bile acid metabolism is demonstrated by the study conducted by Oelkers *et al.* (6). In their study, three functional mutations in *SLC10A2* were found to be responsible for primary bile acid malabsorption, a disorder associated with congenital diarrhea, steatorrhea, bile acid malabsorption, and low plasma cholesterol levels. The association between polymorphisms in the *SLC10A2* gene and colorectal cancer risk observed in our study is supported by an experimental study conducted by Kanamoto *et al.* (28). They reported that the elimination of the ileal sodium/bile acid transporter by ileal resection increased the incidence and the total number of colon tumors in rats.

In our study, the cases and controls were ascertained through sigmoidoscopy. Controls had no history of polyps and had no adenomas detected by sigmoidoscopy at the time of enrollment. However, the entire colon was not examined in the controls, which means adenomas may exist in the right colon of controls that were beyond the reach of a sigmoidoscope. It is estimated that about 15–17% of subjects who have no polyps detected by sigmoidoscopy have adenomas beyond the reach of the sigmoidoscope (29). Under the assumption that bile acids play the same roles in left- and right-sided adenomas, this misclassification of disease status would result in a bias toward the null hypothesis. However, if differences exist in the etiology of left- *versus* right-sided adenomas, then there is no bias, and our study result only applies to left-sided adenomas. Some studies suggest that bile acids may have differential effects within the colon. For example, in a case-control study by Todoroki *et al.* (30), a moderate increase in risk associated with cholecystectomy (which increases the load of secondary bile acids in the intestine) was only observed for proximal colon cancer, not for distal colon cancer.

In summary, the positive association between the genetic polymorphism (169 C→T) of *SLC10A2* and colorectal adenomas supports the role of bile acids in the etiology of colorectal adenomas. However, cautions have to be taken when using this genetic polymorphism as a marker for colorectal adenoma risk, because the linkage disequilibrium between this marker allele and the presumed functional at-risk allele may differ by ethnic groups. Functional studies of the *SLC10A2* gene are under way to identify the functional genetic variation(s). Although the association we observed is biologically plausible, we recognize that the association may also be attributable to chance. Given the biological rationale for an effect of this gene, we believe this initial observation warrants confirmation by other studies.

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## References

1. Nagengast, F. M., Grubben, M. J. A. L., and van Munster, I. P. Role of bile acids in colorectal carcinogenesis. Eur. J. Cancer, *31A:* 1067–1070, 1995.

2. Christl, S. U., Bartran, H. P., Paul, A., Kelber, E., Scheppach, W., and Kasper, H. Bile acid metabolism by colonic bacteria in continuous culture: effects of starch and pH. Ann. Nutr. Metab., *41:* 45–51, 1997.

3. Danielsson, H., and Sjovall, J. (eds.). Sterols and Bile Acids, pp. 303–305. New York: Elsevier Science Publishing Co., Inc., 1985.

4. Dawson P. A., and Oelkers P. Bile acid transporters. Curr. Opin. Lipidol., *6:* 109–114, 1995.

5. Wong, M. H., Rao, P. N., Pettenati, M. J., and Dawson, P. A. Localization of the ileal sodium-bile acid cotransporter gene (SLC10A2) to human chromosome 13q33. Genomics, *33:* 536–540, 1996.

6. Oelkers, P., Kirby, L. C., Heubi, J. E., and Dawson, P. A. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J. Clin. Investig., *99:* 1880–1887, 1997.

7. Haile, R. W., Witte, J. S., Longnecker, M. P., Probst-Hensch, N., Chen, M., Harper, J., Frankl, H. D., and Lee, E. R. A sigmoidoscopy-based case-control study of polyps: macronutrients, fiber and meat consumption. Int. J. Cancer, *73:* 497–502, 1997.

8. Sommer, S. S., Groszbach, A. R., and Bottema, C. D. K. PCR amplification of specific alleles (PASA) is a general method for rapidly detecting known single-base changes. Biotechniques, *12*: 82–87, 1992.

9. Newmark, H. L, Wargovich, M. J., and Bruce, W. R. Colon cancer and dietary fat, phosphate and calcium: a hypothesis. J. Natl. Cancer Inst., *72:* 1323–1325, 1984.

10. Govers, M. J. A. P., Termont, D. S. M. L., Lapre, J. A., Kleibeuker, J. H., Vonk, R. J., and van der Meer, R. Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. Cancer Res., *56:* 3270–3275, 1996.

11. Rafter, J. J., Eng, V. W., Furrer, R., Medline, A., and Bruce, W. R. Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. Gut, *27:* 1320–1329, 1986.

12. Valhouny, G. V., Satchithanandram, S., and Lightfood, F. Morphological disruption of colonic mucosa by free or cholestyramine-bound bile acids. Dig. Dis. Sci., *29:* 439–442, 1984.

13. Kandell, R. L., and Bernstein, C. Bile salt/acid induction of DNA damage in bacterial and mammalian cells: implication for colon cancer. Nutr. Cancer, *16:* 227–238, 1991.

14. Lipkin, M. Biomarkers of increased susceptibility to gastrointestinal cancer: new application to studies of cancer prevention in human subjects. Cancer Res., *48:* 235–245, 1988.

15. Stadler, J., Yeung, K. S., Furrer, R., Marcon, N., Himal, H. S., and Bruce, W. R. Proliferative activity of rectal mucosa and soluble fecal bile acids in patients with normal colons and in patients with colonic polyps or cancer. Cancer Lett., *38:* 315–320, 1988.

16. Garewal, H., Bernstein, H., Bernstein, C., Sampliner, R., and Payne, C. Reduced bile acid apoptosis in "normal" colorectal mucosa: a potential biological marker for cancer risk. Cancer Res., *59:* 2353–2357, 1999.

17. Hill, M. J., Crowther, J. S., and Drasar, B. S. Bacteria and etiology of cancer of the large bowel. Lancet, *1:* 95–100, 1971.

18. Wynder, E. L., and Reddy, B. S. Metabolic epidemiology of colorectal cancer. Cancer, *34:* 801–806, 1974.

19. Reddy, B. S. Diet and excretion of bile acids. Cancer Res., *41:* 3766–3768, 1981.

20. McKeigue, P. M., Adelstein, A. M., Marmot, M. G., Henly, P. H., Owen, R. W., Hill, M. J., and Thompson, M. H. Diet and fecal steroid profile in a South Asian population with a low colon-cancer rate. Am. J. Clin. Nutr., *50:* 151–154, 1989.

21. Imray, C. H. E., Radley, S., Barker, G., Hendrickse, C. W., Konovan, I. A., Lawson, A. M., Baker, P. R., and Neoptolemos, J. P. Faecal unconjugated bile acids in patients with colorectal cancer or polyps. Gut, *33:* 1239–1245, 1992.

22. Breuer, N. F., Dommes, P., Jaelel, S., and Goebell, H. Fecal bile acid excretion pattern in colonic cancer patients. Dig. Dis. Sci., *30*: 852–859, 1985.

23. De Kok, T. M. C., van Faassen, A., Glinghammar, B., Pachen, D. M. F. A., Eng, M., Rafter, J. J., Baeten, C. G. M. I., Engels, L. G. J. B., and Kleinjans, J. C. S. Bile acid concentrations, cytotoxicity, and pH of fecal water from patients with colorectal adenomas. Dig. Dis. Sci., *44*: 2218–2225, 1999.

24. Setchell, K. D. R., Ives, J. A., Cashmore, G. C., and Lawson, A. M. On the homogeneity of stools with respect to bile acid composition and normal day-to-day variations: a detailed qualitative and quantitative study using capillary column gas chromatography-mass spectrometry. Clin. Chim. Acta., *162:* 257–275, 1987.

25. Bayerdorffer, E., Mannes, G. A., Richter, O., Ochsenkuhn, T., Wiebecke, B., Koepke, W., and Paumgartner, G. Increased serum deoxycholate acid levels in men with colorectal adenomas. Gastroenterology, *104:* 145–151, 1993.

26. Bayerdorffer, E., Mannes, G. A., Ochsenkuhn, T., Dirschedl, P., Wiebecke, B., and Paumgartner, G. Unconjugated secondary bile acids in the serum of patients with colorectal adenomas. Gut, *36*: 268–273, 1995.

27. Bayerdorffer, E., Mannes, G. A., Ochsenkuhn, T., Dirschedl, P., and Paumgartner, G. Variation of serum bile acids in patients with colorectal adenomas during a one-year follow-up. Digestion, *55:* 121–129, 1994.

28. Kanamoto, R., Azuma, N., Suda, H., Seki, T., Tsuchihashi, Y., and Iwami, K. Elimination of $Na^+$-dependent bile acid transporter from small intestine by ileum resection increase colonic tumorigenesis in the rat fed deoxycholic acid. Cancer Lett., *145:* 115–120, 1999.

29. Foutch, P. G., Mai, H., Pardy, K., Disario, J. A., Manne, R. K., and Kerr, D. Flexible sigmoidoscopy may be ineffective for secondary prevention of colorectal cancer in asymptomatic average-risk men. Dig. Dis. Sci., *36:* 924–928, 1991.

30. Todoroki, E., Friedman, G. D., Slattery, M. L., Potter, J. D., and Samowitz, W. Cholecystectomy and the risk of colon cancer. Am. J. Gastroenterol., *94*: 41–46, 1999.

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