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Original Article

NR1H4 gene single nucleotide polymorphisms are associated with susceptibility to colorectal polyps, ulcerative colitis and colorectal cancer

Jiayu Mao[1], Xueqi Chen[1], Wenbin Li[1], Jian Li[2], Hong Yang[1], Jiaming Qian[1], Jingnan Li[1]

[1]*Peking Union Medical College Hospital, Beijing, China;* [2]*Henan Cancer Hospital, Henan, China*

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Abstract: Background: Down-regulation of farnesoid X Receptor (FXR, NR1H4 gene), a nuclear receptor of bile acids (BA), has been reported in colorectal carcinoma (CRC). FXR may influence BA homeostasis and thus promote CRC, but underlying mechanism remains unknown. The aim of this study was to examine the possible association of single nucleotide polymorphisms in the NR1H4 gene with susceptibility to colorectal polyps, ulcerative colitis (UC) and CRC. Methods: A total of 284 patients (96 CRC, 144 polyps, 44 UC) at Peking Union Medical College Hospital or Henan Cancer Hospital between January and May 2015 were genotyped for five SNP variants of the NR1H4 gene (rs56163822, rs12313471, rs7138843, rs35724, rs10860603) using Sequenom MassARRAY assays. A total of 504 East Asian samples from the NCBI 1000 Genome Project were used as control. Results: In our study rs56163822 allele T and rs7138843 allele A were found to be significantly associated with polyps, UC and CRC. The rs35724 C allele was found significantly associated with polyps and the genotype CC+CG was more frequent in all groups. SNPs rs12313471, rs10860603 were not associated with any of the colorectal pathologies examined. Conclusions: NR1H4 polymorphisms rs56163822 and rs7138843 were associated with susceptibility to polyps, UC and CRC.

Keywords: Colorectal neoplasms, ulcerative colitis, intestinal polyps, farnesoid X-activated receptor, single nucleotide polymorphism

Introduction

Colorectal cancer incidence and mortality rates vary markedly around the world due to variability in environmental and genetic factors. A high-fat, low-fiber diet has been established as a major risk factor for colon cancer development [1], and cholecystectomy has been associated with right-sided colon cancer [2]. These factors may result in an increased bile acids (BA) load in the intestine, and higher relative concentrations of fecal secondary bile acids, mostly deoxycholic and lithocholic acid [3]. Consequently, resistance to BA-induced DNA oxidative damage, inflammation, nuclear factor-kappa B (NFkB) activation and enhanced cell proliferation has been reported to increase colon tumorigenesis [4].

Bile acids, especially the hydrophobic ones, are endogenous ligands for nuclear receptors like Farnesoid X receptor (FXR; NR1H4 gene), Pregnane X receptor (PXR; NR1I2 gene), and vitamin D receptor (VDR; NR1I1 gene). These receptors regulate BA concentration by modulating BA influx, efflux and detoxification [5, 6]. FXR was first identified as a bile acid nuclear receptor in 1999 [7, 8], as the master regulator of BA homeostasis in gut-liver axis. Since then, it has been shown that FXR controls the BA pool size, through multiple mechanisms, in the intestine. For instance, it has been reported that FXR suppresses BA neosynthesis via inducing expression of fibroblast growth factor 15/19 (FGF15/19). This is important since FGF15 binding to FGF receptor 4 (FGFR4) ultimately results in inhibition of CYP7A1 expression in the liver [9]. Another mechanism by which FXR exerts it function is by interfering with BA transport and reabsorption, through induction of binding proteins and transporters [ileal bile acid binding protein (IBABP) and

Table 1. Clinical-pathological characteristics of patients included in the study

	Control	CRC patients	Patients with polyps	UC patients	
Mean age (± SD)		58.8±14	56.1±10.6	55.0±12.3	
Male gender (%)	48.4%	50%	48.6%	47.7%	
Clinical stage					Montreal Classification
1		20		0	E1
2		52		12	E2
3		24		32	E3
					Disease severity
				6	Mild
				31	Moderate
				7	Severe

organic solute transporters a and b (OSTa/OSTb)] [10], and inhibition of others [Na^+-dependent taurocholic cotransporting polypeptide (NTCP), apical sodium dependent bile acid transporter (ASBT) and organic anion transporting polypeptide (OATP) [5, 6]. In addition, FXR might promote BA detoxification by inducing expression of aldo-keto reductase 1B7 (AKR1B7) and cytochrome P450 isoform 3A11 (CYP3A11) [6]. Finally, FXR might influence antibacterial defense in the ileum by controlling the expression of inducible nitric oxide synthase (iNOS), interleukin-18 (IL-18), angiogenin 1 (ANG1) and carbonic anhydrase 12 (CAR12), and repressing expression of the inflammatory genes (IL-1b, IL-6 and macrophage attractant protein-1) (MCP-1) which would in turn promote antimicrobial actions (via elevated levels of nitric oxide, NO) in the colon [11].

Enteric FXR has been found to be downregulated in both mouse and human CRC models during the progression from normal intestinal epithelia to dysplastic lesions [12]. However, the underlying molecular mechanisms are still poorly understood. FXR variants have already been reported to associate with inflammatory bowel disease (IBD) [13, 14], cholelithiasis [15, 16], cholangiocarcinoma [17] and some other liver diseases. The aim of this study was to investigate whether single nucleotide polymorphisms of NR1H4 gene are associated with susceptibility to colorectal polyps, ulcerative colitis (UC) and colorectal cancer (CRC), and further develop its potential role in the progression of polyps and UC to CRC. To the best of our knowledge, this is the first investigation on NR1H4 gene polymorphisms in patients with colonic polyps, and the first investigation to study the relationship between SNPs in NR1H4 gene and colon cancer risk in a Chinese population.

Materials and methods

Study population

A total of 144 patients with polyps (male/females 70/74; age 56.1±10.6 years), 44 patients with UC (male/female 21/23; age 55.0±12.3 years) and 96 patients with CRC (male/female 48/48; age 58.8±14 years), were enrolled in this study. All polyp cases and 13 CRC cases were obtained from Peking Union Medical College Hospital, Beijing, China, while 83 CRC cases were obtained from the Henan Cancer Hospital, China. All were obtained from the Gastroenterology clinics of these two hospitals between January and May 2015. All patients had a confirmed diagnosis, with clinical, endoscopic, radiological and histopathological findings fulfilling standard diagnostic criteria. The patients' Montreal Classification, UC disease severity and CRC clinical stage were also recorded (**Table 1**). All patients provided written informed consent and all DNA samples and data were handled anonymously. The study was approved by the Ethical Committee of the Peking Union Medical College Hospital. Since it's very difficult to collect a normal control group of study participants in our clinic, we downloaded the data of 504 East Asian samples from NCBI as the control group. This group includes 103 (20.4%) CHB (Han Chinese in Bejing, China), 104 (20.6%) JPT (Japanese in Tokyo, Japan), 105 (20.8%) CHS (Southern Han Chinese), 93 (18.5%) CDX (Chinese Dai in Xishuangbanna, China) and 99 (19.6%) KHV

Table 2. Sequences of primers and masses of extension products of MALDI-TOF MS assays

SNP	Primer	Sequence	Mass (KDa)
rs56163822	PCR primer 1	5'-ACGTTGGATGGACCACCATAAAGAAAGTGC-3'	
	PCR primer 2	5'-ACGTTGGATGGTGGTAGGTAAATGGGAATG-3'	
	Extension primer	5'-AGATTCATTTTTGATCCCAT-3'	6057
	Analyte G	5'-AGATTCATTTTTGATCCCATC-3'	6304.1
	Analyte T	5'-AGATTCATTTTTGATCCCATA-3'	6328.2
rs12313471	PCR primer 1	5'-ACGTTGGATGGGACATGTTTCATGTCTCTC-3'	
	PCR primer 2	5'-ACGTTGGATGAGTTATCAGGGACACAGTGG-3'	
	Extension primer	5'-GGTGAACAAGATAGGTGTA-3'	5940.9
	Analyte A	5'-GGTGAACAAGATAGGTGTAA-3'	6212.1
	Analyte G	5'-GGTGAACAAGATAGGTGTAG-3'	6228.1
rs7138843	PCR primer 1	5'-ACGTTGGATGGTTTTTCTTTAGTCTAATGG-3'	
	PCR primer 2	5'-ACGTTGGATGGCACTGAATTCTTCTTTTAAC-3'	
	Extension primer	5'-TTTTAAGAAAAAAACCAATGATTAA-3'	7589.1
	Analyte T	5'-TTTTAAGAAAAAAACCAATGATTAAA-3'	7960.3
	Analyte A	5'-TTTTAAGAAAAAAACCAATGATTAAT-3'	8016.2
rs35724	PCR primer 1	5'-ACGTTGGATGCCATCTGGAAAATGGAGACG-3'	
	PCR primer 2	5'-ACGTTGGATGCCATCTGGAAAATGGAGACG-3'	
	Extension primer	5'-CTAATAATCAATGATAATGCTCTT-3'	7309.8
	Analyte C	5'-CTAATAATCAATGATAATGCTCTTC-3'	7557
	Analyte G	5'-CTAATAATCAATGATAATGCTCTTG-3'	7597
rs10860603	PCR primer 1	5'-ACGTTGGATGGAAACAACTCAAGCACAAA-3'	
	PCR primer 2	5'-ACGTTGGATGGATAACTGTCTCTGAGATGC-3'	
	Extension primer	5'-TGTTAATTGAGGCTTTAGAAC-3'	6475.2
	Analyte A	5'-TGTTAATTGAGGCTTTAGAACA-3'	6746.4
	Analyte G	5'-TGTTAATTGAGGCTTTAGAACG-3'	6762.4

(Kinh in Ho Chi Minh City, Vietnam) populations. All SNP genotypes in the control group were in Hardy-Weinberg equilibrium (data not shown, $p > 0.05$).

SNP selection and genotyping

NR1H4 gene SNP genotype data were retrieved from the HapMap Phase II + Phase III database, and the haplotypes were analyzed with restrictive standards [r2 > 0.8 and a minimum allele frequency (MAF) > 0.1] in Haploview 4.2 software. Finally, five NR1H4 variants (rs56163822, rs12313471, rs7138843, rs35724, rs10860603) which were able to cover 80% of the MAF > 0.1 SNPs, were selected for genotyping. Four are tagging SNPs, while -1G > T (rs56163822) was previously reported to affect FXR expression and function [18]. In the East Asian population, the reported minor allele frequencies (MAF) of these five SNPs were 18% (rs56163822 allele T), 46.1% (rs12313471 allele G), 14.9% (rs7138843 allele A), 29.7% (rs35724 allele C) and 28.8% (rs10860603 allele G) (http://www.internationalgenome.org/). All five variants are common SNPs, and have previously been examined in association with IBD [13, 14].

DNA was extracted from the peripheral blood samples from each patient using standard methods. Genotyping was performed by MALDI-TOF Mass Spectrometry for all donors. The DNA from the donors was blinded, coded and tested using a 384-well format SpectroCHIP microarray. PCR primers and single base extension primers for rs56163822, rs12313471, rs7138843, rs35724, rs10860603 were designed using Sequenom Assay design 3.1 software. These primer sequences are shown in **Table 2**. A MALDI-TOF Mass Spectrometer was used for data acquisition from the SpectroCHIP. The results were analyzed using Sequenom MassARRAY RT software.

Statistical analysis

Correlations between NR1H4 SNPs and diseases were statistically analyzed using SPSS 22.0

Table 3. Allele frequencies of the NR1H4 SNPs in the cases and control groups

SNP rs56163822 (G→T)					
	Allele T (%)	P	OR	95% CI	Power
Control	14.9%				
CRC	45.8%	2.20E-16**	4.8400	3.4703-6.7504	1
Polyps	44.4%	2.20E-16**	4.5760	3.4240-6.1156	1
UC	56.8%	2.20E-16**	7.5263	4.7699-11.8757	1
SNP rs12313471 (A→G)					
	Allele G (%)	P			Power
Control	18%				
CRC	22.9%	ns			0.256
Polyps	19%	ns			0.069
UC	19.3%	ns			0.055
SNP rs7138843 (T→A)					
	Allele A (%)	P	OR	95% CI	Power
Control	46.1%				
CRC	100%	2.20E-16**	449.5113	27.9368-7232.7580	1
Polyps	100%	2.20E-16**	645.6617	40.1713-10377.5391	1
UC	100%	2.20E-16**	197.3179	12.2071-3189.4945	1
SNP rs35724 (C→G)					
	Allele C (%)	P	OR	95% CI	Power
Control	29.7%				
CRC	50%	9.78E-08	2.3712	1.7331-3.2444	0.977
Polyps	50%	4.08E-10*	2.3712	1.8145-3.0989	0.996
UC	50%	0.0001567	2.3712	1.5214-3.6958	0.81
SNP rs10860603 (G→A)					
	Allele G (%)	P			Power
Control	28.8%				
CRC	22.9%	ns			0.279
Polyps	19%	ns			0.75
UC	19.3%	ns			0.325

MAF, minor allele frequencies. *, P < 1E-9. **, P < 2E-11. OR, odds ratio; CI, confidence interval. ns, not significant.

software. Statistical analysis of alleles and genotype categories were carried out by Fisher's exact test, all tests were two-sided, and $P < 10^{-9}$ was considered statistically significant. Odds ratio (OR) for the association of each polymorphism with the three diseases were analyzed by the Chi-square test. The Bonferroni method was used to correct for multiple testing. All tables show the uncorrected *p* values. Since the predicted frequency of some alleles is low, small sample size may increase the probability of making a type 2 error (also referred as the false negative rate), Statistic power is also included in the table. If the statistical power is < 0.8, influence of small sample size during the analysis could not be ignored.

Results

Population chatacteristics

A total of 144 cases of colonic polyps, 44 of UC, 96 of CRC and 504 controls were enrolled in this study. As is shown in **Table 1**, the case groups and the control group had similar sex and age distributions.

Association analysiss

Table 3 shows the analysis of different allele frequencies of the NR1H4 SNPs, and **Table 4** shows that of genotype frequencies. All of the results using Fisher's exact test, Chi-square test and odds ratio (OR) for the association of

Table 4. NR1H4 SNP genotype frequencies in cases and control groups

SNP rs56163822 (G→T)											
	WT [GG]	Het [GT]	Hom [TT]	P (GG)	OR	95% CI	Power (GG)	P (GG+GT)	OR	95% CI	Power (GG+GT)
Control	370 (73.4%)	118 (23.4%)	16 (3.2%)								
CRC	26 (27%)	52 (54.2%)	18 (18.8%)	2.20E-16**	0.3689	0.2344-0.5807	1	2.74E-07	0.8391	0.6071-1.1599	0.998
Polyps	47 (32.6%)	66 (45.8%)	31 (21.5%)	2.20E-16**	0.4416	0.3116-0.6344	1	2.28E-11*	0.8105	0.615-1.068	1
UC	9 (20.5%)	20 (45.5%)	15 (34%)	4.49E-12**	0.2786	0.1343-0.5779	1	3.89E-10*	0.6807	0.4191-1.1056	1
SNP rs12313471 (A→G)											
	WT [AA]	Het [AG]	Hom [GG]	P (AA)			Power (AA)	P (AA+AG)			Power (AA+AG)
Control	346 (68.7%)	135 (26.8%)	23 (4.5%)								
CRC	59 (61.5%)	30 (31.3%)	7 (7.2%)	0.191			0.346	0.303			0.221
Polyps	96 (66.7%)	41 (28.5%)	7 (4.9%)	0.6851			0.1	0.8248			0.047
UC	28 (63.6%)	15 (34.1%)	1 (2.3%)	0.5022			0.135	0.7112			0.003
SNP rs7138843 (T→A)											
	WT [TT]	Het [TA]	Hom [AA]	P (TT)	OR	95% CI	Power (TT)	P (TT+TA)	OR	95% CI	Power (TT+TA)
Control	150 (29.8%)	243 (48.2%)	111 (22%)								
CRC	0	0	96	7.06E-14**	0.0174	0.0011-0.2814	1	2.20E-16**	0.0066	0.0004-0.1073	1
Polyps	0	0	138	2.20E-16**	0.0121	0.0007-0.1956	1	2.20E-16**	0.0046	0.0003-0.0746	1
UC	0	0	42	9.50E-7	0.0394	0.0024-0.6447	1	2.20E-16**	0.151	0.0009-0.2459	1
SNP rs35724 (C→G)											
	WT [CC]	Het [CG]	Hom [GG]	P (CC)			Power (CC)	P (CC+CG)	OR	95% CI	Power (CC+CG)
Control	45 (8.9%)	209 (41.5%)	250 (49.6%)								
CRC	0	96	0	4.65E-4			1	2.20E-16**	1.9843	1.44-2.7342	1
Polyps	0	144	0	1.71E-5			1	2.20E-16**	1.9843	1.5059-2.6146	1
UC	0	43	0	0.03911			1	1.41E-12**	1.9843	1.2667-3.1082	0.914
SNP rs10860603 (G→A)											
	WT [GG]	Het [GA]	Hom [AA]	P (GG)			Power (GG)	P (GG+GA)			Power (GG+GA)
Control	45 (8.9%)	200 (39.7%)	259 (51.4%)								
CRC	7 (7.2%)	30 (31.3%)	59 (61.5%)	0.696			0.089	0.0749			0.53
Polyps	7 (4.8%)	41 (28.5%)	96 (66.7%)	0.121			0.44	0.001226			0.94
UC	1 (2.3%)	15 (34.1%)	28 (63.6%)	0.1607			0.376	0.1561			0.409

WT, wildtype; Het, heterozygous SNP carrier; Hom, homozygous SNP carrier. *, P < 1E-9. **, P < 2E-11.

each polymorphism with the different groups were shown in the tables.

The NR1H4 SNP variants rs56163822 G/T and rs7138843 T/A were found to be significantly associated with the risk of polyps, UC and CRC, even when considering Bonferroni corrected significance level of P < 2E-11. Upon statistical analysis of the allele and genotype frequencies, we observed that rs56163822 allele T and rs7138843 allele A were more frequent in cases of polyps, UC and CRC than controls. As is shown in **Table 3**, the frequency of rs56163822 T in control group is 14.9%, and its frequency in all the cases of polyps, UC and CRC was significantly higher (rs56163822 T: OR = 4.576, 95% CI: 3.4240-6.1156 for polyps; OR = 7.5263, 95% CI: 4.7699-11.8757 for UC; OR = 4.84, 95% CI: 3.4703-6.7504 for CRC). Similarly, the frequency of rs7138843 A in control group is 46.1%, and it's more frequent in examined groups (rs7138843 A: OR = 645.6617, 95% CI: 40.1713-10377.5391 for polyps; OR = 197.3179, 95% CI: 12.2071-3189.4945 for UC; OR = 449.5113, 95% CI: 27.9368-7232.758 for CRC). In addition, no significant difference was observed between different examined groups.

The rs35724 allele C was found to be significantly associated with polyps, comparing its frequency of 50% to that of 29.7% in control group (OR = 2.3712, 95% CI: 1.8145-3.0989). In the genotype analysis, genotypes CC+CG were found to be significantly more frequent in all examined patient groups compared to controls. As is shown in **Table 4**, the frequency of genotypes CC+CG in control gourp is 50.4%, and it's more frequent in the cases of polyps, UC and CRC (OR = 1.9843, 95% CI: 1.5059-2.6146 for polyps; OR = 1.9843, 95% CI: 1.2667-3.1082 for UC; OR = 1.9843, 95% CI: 1.44-2.7342 for CRC). However, no significant difference was observed between the different examined groups of patients.

Finally the rs12313471 and rs10860603 SNPs were not associated with either group of patients in our study, however the statistic power of the analysis of these two SNPs was below 0.8.

Discussion

Environmental factors such as elevated bile acid loads play an important role in the development of colorectal neoplasms. Although FXR downregulation has been reported in CRC the underlying mechanism is still poorly understood [5, 6]. In addition, FXR mRNA expres0 sion has been reported to be down-regulated in ApcMin/+ mice, FAP patients and an APC-Mutated colon cancer cell line [12]. Moreover FXR expression in CRC tumors is significantly inversely correlated with tumor stage and clinical outcome, local recurrence and metastatic disease [19]. As FXR controls the BA pool size through multiple mechanisms, some studies indicate that it might influence BA transport and reabsorption during tumorigenesis by decreasing OSTa/OSTb activity [20], and increasing IBABP expression [21]. Furthermore, Bailey *et al*. reported that FXR expression might be regulated by DNA methylation and KRAS signaling [22]. Other tumor-suppressive pathways, such as reduced miR-22-silenced cyclin A expression [23] and Src-mediated cross-talk-mediated EGFR induction have been proposed to play a role in intestinal tumorigenesis [24].

In this study we examined the possible association of NR1H4 gene SNPs with susceptibility to polyps, UC and CRC. Five NR1H4 SNPs, rs56163822, rs12313471, rs7138843, rs35724 and rs10860603, were analyzed, all of which have been previously studied in other diseases. All five variants were previously studied in association with IBD, and rs12313471 allele G was found to be more prevalent in UC patients in a Dutch population [13]. In addition, rs56163822 allele T was found to be more prevalent in UC patients in a European population [14]. However, neither variant was found to be significantly associated with disease when considering a corrected significance. The NR1H4 variant rs35724 was identified as potential gender-dependent susceptibility factor for cholelithiasis in a German cohort [16], while rs35724 genotypes CC+CG were underrepresented in cholelithiasis patients after they were stratified by gender. In addition, rs7138843 genotypes AT+TT and rs56163822 genotypes GT+TT were wound to be less frequent in cholelithiasis patients, and were inversely associated with cholelithiasis in a Mexican population and were thus suggested to play a protective role [15]. rs56163822 T allele was also found to be less common in patients with intrahepatic cholestasis of pregnancy (ICP) than in the control group in a British population, although this difference did not reach statistical signifi-

cance [18]. In a large Dutch study population, among the groups of patients with rs35724 genotype GG and rs10860603 genotype AA, the body mass index and the waist circumference was lower than that of other genotypes [25], while the LDL cholesterol (LDL-C) and total cholesterol of Chinese patients with the rs56163822 genotypes GT+TT responded better to rosuvastatin treatment [26]. Furthermore the association between NR1H4 SNPs rs56163822, rs12313471 and rs10860603 and acute pancreatitis (AP) was investigated, and among them a trend was observed only for the rs10860603 allele A which was found to be associated with AP occurrence and severity, though not statistically significantly so after correction [27].

Single nucleotide polymorphism rs12313471 is located in the 5' region of the NR1H4 gene, rs7138843 is located in intron 7, while rs35724 and rs10860603 are located in intron 9 of the NR1H4 gene. Whether these SNPs affect the expression and/or molecular function of FXR is not currently known. As the SNP rs12313471 is located in the 5' region, it may alter a binding site for a transcription factor and may therefore affect NR1H4 gene expression. The intronic SNPs rs7138843, rs35724, and rs10860603 could potentially influence splicing of the FXR mRNA. The SNP rs56163822 lies adjacent to the translation initiation site, and was shown to reduce FXR expression and FXR-dependent promoter activation, which may impair FXR function or expression [14].

In our study rs56163822 allele T and rs7138843 allele A were observed significantly less frequently in the healthy population when compared to patients with polyps, UC or CRC, suggesting that rs56163822, which was previously reported to reduce FXR function, and rs7138843, within the NR1H4 intron 7, may contribute to the pathogenesis of these diseases. The case of the common SNP rs35724 allele is more complicated as its allele and genotype frequency differs between patients with CRC and UC, where only the CC+CG genotype was significantly associated with all examined groups when compared to the control group, perhaps illustrating the type of Mendelian inheritance where the allele C is the recessive variant. Furthermore, both the C allele and CC+CG genotype were found to be significantly more frequent in patients with polyps than the control group. For rs12313471 and rs10860603, no significant differences between the study groups were observed. However perhaps the sample size was too small to reach a statistical significance since the statistical power of these two groups is < 0.8.

In conclusion, all five NR1H4 SNPs analyzed in this study have been previously examined in other diseases particularly in association with IBD, since long duration of chronic inflammatory colitis like IBD (especially ulcerative colitis) may promote the development of colorectal cancer, our study attempts to explore their association and the potential mechanism. Some of our results for the UC patients differ from previous studies. rs56163822 allele T was previously reported to be more prevalent in the European UC population [14], which is in accordance to our results, but not significant in a Dutch UC population [13]. The variant rs7138843 was not significantly correlated with UC in the European population [14], while in our study the A allele was more prevalent even after Bonferroni correction. The rs12313471 G allele was reported as more frequent in the Dutch UC population [13], while in our study the prevalence of this allele did not differ significantly between case and controls. Nevertheless, since our study was performed in a different population these findings are not surprising.

FXR expression has been reported to be lower in colorectal carcinomas than the peritumoral nonneoplastic mucosa [22]. Furthermore, FXR expression was downregulated at the transcriptional level in colon adenomas and reduced in virtually all stages of adenocarcinoma, correlating inversely with tumor stage and clinical outcome [19, 22]. The mechanism by which FXR suppresses tumor growth is still not defined, but may involve protection of colonic epithelium from inflammation and amelioration of BA toxicity by upregulation of intracellular BA binding proteins and efflux transporters, and downregulation of influx transporters and *de novo* synthesis of BAs [20, 21, 28]. However, FXR can perform additional anti-tumorigenic functions independent of BA homeostasis regulation [29]. For example, FXR deficiency increases susceptibility to colon cancer by increasing epithelial permeability to bacteria, promoting

WNT/β-catenin signaling, increasing intestinal inflammation, and FXR protects against genotoxic compounds [22]. Furthermore, FXR expression was positively correlated with collagen IV and vimentin expression, markers of a mesenchymal cell phenotype [30], and negatively correlated with levels of PI3K, cyclin E1, and other oncogenic signaling mediators [31], suggesting that FXR is either a strong marker of cell differentiation or that FXR regulates these signaling pathways. Bailey *et al*. [22] also reported that both FXR and inhibition of DNA methylation and KRAS silencing increased FXR expression, which may contribute to FXR silencing. Lax *et al*. [19] have reported that FXR expression was not associated with and Ki-67 and cyclin D1.

In conclusion, in our study SNPs in NR1H4 were found to be associated with CRC, polyps and UC, particularly the SNPs rs56163822, rs7138843. Taken together, our findings warrant further study of the influence of FXR on bile acid homeostasis and metabolism, as well as its potential role in intestinal inflammation in the development and progression of polyps and UC to CRC.

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Disclosure of conflict of interest

The authors are from two different institutions, Author Jian Li is a member of Henan Cancer Hospital.

Address correspondence to: Jingnan Li, Peking Union Medical College Hospital, Beijing, China. Tel: 0086-136-0123-5229; E-mail: lijn2008@126.com

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