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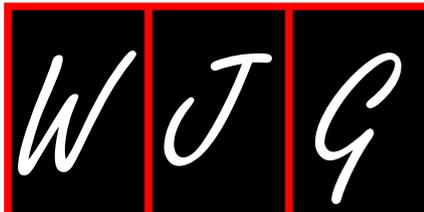
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Case Control Study

Association between *CYP24A1* polymorphisms and the risk of colonic polyps and colon cancer in a Chinese population

Xue-Qi Chen, Jia-Yu Mao, Wen-Bin Li, Jian Li, Hong Yang, Jia-Ming Qian, Jing-Nan Li

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Abstract

AIM

To determine the pathogenesis and potential single nucleotide polymorphisms (SNPs) as screening sites for colonic polyps, colon cancer and ulcerative colitis, and to analyze the possible association between these genetic polymorphisms and the three diseases.

METHODS

We evaluated genetic polymorphisms in 144 newly diagnosed colonic polyp patients, 96 colon cancer patients and 44 ulcerative colitis patients. The four SNPs genotyped were rs4809957, rs6068816, rs6091822 and rs8124792. The control group consisted of 504 East Asians enrolled in the 1000 Genomes Project. Correlations between *CYP24A1* SNPs and the diseases were analyzed by Fisher's exact probability test.

RESULTS

CYP24A1 polymorphisms rs4809957 A/G and rs6068816 C/T showed a statistically significant association with risk of the three diseases, when both the genotypes and allele frequencies were considered. With regard to rs6091822 G/T, all three diseases were related to risk allele carriers (GT + TT) *vs* wild-type (GG), but the associations between the allele frequencies and the diseases were not significant. The risk of colonic polyps and colon cancer was related to the allele frequencies of rs8124792 G/A, and this association remained for genotype frequencies of this SNP.

CONCLUSION

Four SNPs are related to the risk of colonic polyps and colon cancer. G allele in rs6091822 G/T may play an anti-cancer role only if it is homozygous. The A allele, which is a minor component of rs8124792, may be indicated in the diagnosis of colonic polyps or colon cancer rather than ulcerative colitis.

Key words: *CYP24A1*; Single nucleotide polymorphisms; Colonic polyps; Colon cancer

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Core tip: To determine the pathogenesis and potential single nucleotide polymorphisms (SNPs) as screening sites for colonic polyps and colon cancer, we examined four SNPs located in *CYP24A1* in patients with colonic polyps, colon cancer, ulcerative colitis and controls, and found a statistically significant association with risk of the three diseases. Our research represents the first investigation on *CYP24A1* gene polymorphisms in colonic polyp patients. These findings predicted a potential role of *CYP24A1* polymorphisms as biomarkers for population-level screening of colon cancer.

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer incidence and mortality in China. It is the fifth most common tumor and the fifth leading cause of cancer-related deaths in China^[1], and is predicted to increase in the future as the standardized incidence rate of CRC among Chinese people increased by 66.83% from 12.15/100000 in 1990 to 20.27/100000 in 2013^[2]. To date, there are no ideal diagnostic tests, and fecal occult blood, flexible sigmoidoscopy and optical colonoscopy are the main screening methods for CRC

in Europe, the United States and Asia^[3-5]. The value of these screening methods in detecting early cancer and reducing CRC-related mortality is well established.

For population-based CRC screening in China, a two-step screening strategy has been recommended by the Chinese Center for Disease Control and Prevention, the Ministry of Health of China: the immunochemical Fecal Occult Blood Test (iFOBT) and a quantitative high-risk factor questionnaire as the primary screening test, with a full colonoscopy for follow-up screening^[6]. However, only 37.2% of the target population accepted an iFOBT in a CRC screening program conducted in Hangzhou city^[7]. The low participation rate in screening is also a common problem in other countries in Asia. The participation rate is mainly affected by insufficient staff, possible adverse events and differences in government insurance systems^[8].

Thus, it is very important to identify novel molecular signatures as reliable biomarkers of CRC. Their application in a more advanced and easily obtainable primary screening test may improve CRC diagnosis in high-risk populations that require colonoscopy, and may be cost-effective. Albumin, haptoglobin, transferrin, pyruvate kinase (PK) isoenzyme type M2, calprotectin, Ca3 anaphylatoxin and colon-specific antigen (CCSA-3 and CCSA-4) have been reported as alternative biomarkers for the detection of CRC^[3]. In addition, DNA-related markers have received considerable attention.

The association between CRC and vitamin D was observed in humans and confirmed in animal models and cell lines^[9]. Interestingly, many cancers have been found to be associated with low serum level of the precursor 25-hydroxyvitamin D3 (25-D3), but not with serum concentration of the active vitamin D^[10]. This may be due to the extra-renal autocrine/paracrine vitamin D system, which synthesizes and degrades the active 1,25-D3 (Vitamin D3, 1,25-dihydroxyvitamin D3, [1 α ,25-(OH)2D3]) locally. Thus, vitamin D hydroxylases play a prominent role in this process^[11]. The *CYP24A1* gene encodes a vitamin D3 catabolic enzyme. The expression level of *CYP24A1* was found to be significantly higher in CRC tissues^[12,13]. Although the mechanism of this up-regulation is unclear, *CYP24A1* may be an interesting candidate biomarker for use in the screening of colonic cancer.

It is estimated that 35% of CRC risk may be explained by heritable factors^[14]. A single nucleotide polymorphism (SNP) is the most common genetic variation, and may be a reliable biomarker of the genetic background of patients to predict the risk of CRC^[15]. The SNPs in *CYP24A1* gene have been partially determined. Pibiri *et al*^[16] reported that rs6022990 was nominally associated with left-sided CRC ($P = 0.018$) in African Americans. Dong *et al*^[17] found a statistically significant association between rs4809958 and colon cancer risk in patients from three states in the United States. However, the association between *CYP24A1*

Table 1 Characteristics of the study population

Variable	Cases			P value
	Colonic polyps, n = 144	Colon cancer, n = 96	Ulcerative colitis, n = 44	
Sex, n (%)				0.9631 ¹
Males	70 (48.6)	48 (50.0)	21 (47.7)	
Female	74 (51.4)	48 (50.0)	23 (52.3)	
Age in years, mean ± SD	56.1 ± 10.7	58.8 ± 14.1	55.0 ± 12.4	0.1401 ²

¹P value was calculated by the χ^2 test; ²P value was calculated by the ANOVA test.

gene polymorphisms and colonic polyps has never been determined. Given the crucial role of *CYP24A1* in the development of cancer, it is plausible that the *CYP24A1* polymorphisms may affect the risk of colonic polyps and colon cancer.

To determine the pathogenesis and potential SNPs as screening sites for colonic polyps and colon cancer, we conducted a case-control study. In this study, we selected four SNPs located in *CYP24A1*, and examined these SNPs in patients with colonic polyps, colon cancer, ulcerative colitis and controls, analyzing the possible association between these genetic polymorphisms and the three diseases. To the best of our knowledge, this is the first investigation on *CYP24A1* gene polymorphisms in patients with colonic polyps, and the first investigation to study the relationship between SNPs in *CYP24A1* gene and colon cancer risk in a Chinese population.

MATERIALS AND METHODS

Study population

A total of 144 newly diagnosed colonic polyp patients, 96 colon cancer patients and 44 ulcerative colitis patients were enrolled in this study between January and May 2015. Eighty-three of the CRC cases were from Henan Cancer Hospital in Zhengzhou and the others were from Peking Union Medical College Hospital in Beijing, China. All patients had a confirmed diagnosis, fulfilling standard diagnostic criteria according to clinical, endoscopic, radiological and histopathological findings. All patients gave written informed consent. Five milliliter venous blood was collected from each patient to extract DNA, and all DNA samples and data were handled anonymously. DNA was isolated from peripheral blood leukocytes using the standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation, and stored at -80 °C. Demographic and clinical data were collected during in-person interviews using a questionnaire, and included age, sex, ethnicity, residential region and date of diagnosis. The control group consisted of 504 East Asians enrolled in the 1000 Genomes Project, and included Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), Southern Han Chinese

(CHS), Chinese Dai in Xishuangbanna, China (CDX) and Kinh in Ho Chi Minh City, Vietnam (KHV).

SNP selection and genotyping

The following four SNPs were genotyped: rs4809957, rs6068816, rs6091822 and rs8124792. All were single nucleotide substitutions, previously identified within the *CYP24A1* gene (www.ncbi.nlm.nih.gov/snp/). These SNPs have minor allele frequency (MAF) of $\geq 5\%$ in the Hap-Map CHB population. Genotyping was carried out using Sequenom MassARRAY assays and TYPER4.0 software (SEQUENOM Inc., San Diego, CA, United States). Primer sequences for PCR and single-base extension were designed by Assay Design 3.1 (SEQUENOM Inc.). Multiplex PCR was performed to amplify DNA isolated from the peripheral blood. PCR reactions were treated with shrimp alkaline phosphatase to neutralize unincorporated dNTPs. A single-base extension reaction was performed after PCR. Reactions were subjected to a 3-fold dilution with H₂O, and fragments were purified with resin, spotted onto Sequenom SpectroCHIP microarrays and scanned by MALDI-TOF mass spectrometry. The laboratory staff who conducted the genotyping assays was blinded to the patients' information. All reported P values were uncorrected unless stated otherwise.

Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, United States). Correlations between *CYP24A1* SNPs and diseases were analyzed by Fisher's exact probability test. All tests were two-sided, and $P < 10^{-9}$ was considered statistically significant.

RESULTS

Population characteristics

A total of 144 incident cases of colonic polyps, 96 of colon cancer, 44 of ulcerative colitis and 504 controls were enrolled in this study. As shown in Table 1, the case groups and the control group had similar sex and age distributions.

Association analysis

Results of the analysis by genotype categories using Fisher's exact test are shown in Table 2. Table 3 shows the results of the analysis by alleles using the Chi-square test and odds ratio (OR) for the association of each polymorphism with the three diseases. The MAF and test for Hardy-Weinberg equilibrium in the controls for each SNP are shown in Table 3. All SNPs met quality-control measures for the Hardy-Weinberg equilibrium.

Rs4809957 A/G and rs6068816 C/T showed a statistically significant association with the risk of colonic polyps, colon cancer and ulcerative colitis,

Table 2 Association between the selected single nucleotide polymorphisms and risks of colon cancer, polyp and ulcerative colitis

Variable (MJ/MI)	Cases/controls	Genetic model	P for Fisher's test
rs4809957 A/G	Cancer/1KGeno	AG + GG vs AA	2.20E-16
		G vs A	2.20E-16
		GG vs AG + AA	2.20E-16
	Polyp/1KGeno	AG + GG vs AA	2.20E-16
		G vs A	2.20E-16
		GG vs AG + AA	2.20E-16
	UC/1KGeno	AG + GG vs AA	2.20E-16
		G vs A	2.20E-16
		GG vs AG + AA	9.37E-09
	Cancer/Polyp	AG + GG vs AA	0.7998
		G vs A	0.8036
		GG vs AG + AA	1.0000
	Cancer/UC	AG + GG vs AA	0.4362
		G vs A	0.4424
		GG vs AG + AA	1.0000
	Polyp/UC	AG + GG vs AA	0.3011
		G vs A	0.3096
		GG vs AG + AA	1.0000
rs6068816 C/T	Cancer/1KGeno	CT + TT vs CC	2.20E-16
		T vs C	2.20E-16
		TT vs CT + CC	2.20E-16
	Polyp/1KGeno	CT + TT vs CC	2.20E-16
		T vs C	2.20E-16
		TT vs CT + CC	2.20E-16
	UC/1KGeno	CT + TT vs CC	2.29E-09
		T vs C	2.20E-16
		TT vs CT + CC	2.20E-16
	Cancer/Polyp	CT + TT vs CC	1.0000
		T vs C	0.4635
		TT vs CT + CC	0.4546
	Cancer/UC	CT + TT vs CC	1.0000
		T vs C	0.2786
		TT vs CT + CC	0.2658
	Polyp/UC	CT + TT vs CC	1.0000
		T vs C	0.06703
		TT vs CT + CC	0.06164
rs6091822 G/T	Cancer/1KGeno	GT + TT vs GG	2.20E-16
		T vs G	1.06E-08
		TT vs GT + GG	0.000472
	Polyp/1KGeno	GT + TT vs GG	2.20E-16
		T vs G	4.06E-12
		TT vs GT + GG	0.001364
	UC/1KGeno	GT + TT vs GG	1.32E-13
		T vs G	2.40E-05
		TT vs GT + GG	0.2411
	Cancer/Polyp	GT + TT vs GG	1.0000
		T vs G	0.9258
		TT vs GT + GG	0.5180
	Cancer/UC	GT + TT vs GG	1.0000
		T vs G	0.8971
		TT vs GT + GG	0.3094
	Polyp/UC	GT + TT vs GG	1.0000
		T vs G	1.0000
		TT vs GT + GG	0.5456
rs8124792 G/A	Cancer/1KGeno	GA + AA vs GG	2.20E-16
		A vs G	2.20E-16
		AA vs GA + GG	0.000758
	Polyp/1KGeno	GA + AA vs GG	2.20E-16
		A vs G	2.20E-16
		AA vs GA + GG	4.75E-05
	UC/1KGeno	GA + AA vs GG	4.90E-09
		A vs G	2.91E-09
		AA vs GA + GG	0.06359
	Cancer/Polyp	GA + AA vs GG	1.0000

Cancer/UC	A vs G	1.0000
	AA vs GA + GG	1.0000
	GA + AA vs GG	1.0000
Polyp/UC	A vs G	1.0000
	AA vs GA + GG	1.0000
	GA + AA vs GG	1.0000
	A vs G	1.0000
	AA vs GA + GG	1.0000

1KGeno: Control from 1000 Genomes Project; Cancer: Colon cancer cohort; MI: Minor allele (*i.e.*, less common in controls); MJ: Major allele (*i.e.*, more common in controls); Polyp: Colonic polyp cohort; UC: Ulcerative colitis cohort.

when both the genotypes and allele frequencies were considered. The minimum OR for rs4809957 G when compared with A in ulcerative colitis patients was 0.008, 95%CI: 0.001-0.055, $P = 1.5659E-26$. ORs for rs6068816 C vs T in all diseases were high (OR = 32.086, 95%CI: 16.238-63.403 for colon cancer; OR = 48.918, 95%CI: 24.888-96.150 for colonic polyps; and OR = 18.260, 95%CI: 8.350-39.932 for ulcerative colitis). For rs6091822, all three diseases were related to minor allele carriers (GT + TT) vs major allele homozygotes (GG), but other types of associations (T vs G and TT vs GT + GG) were not significant. The frequencies and distributions of the genotypes and ORs for these associations are shown in Table 4. Risks of colonic polyps and colon cancer were both related to allele frequencies of rs8124792 G/A, and this association remained for genotype frequencies for this SNP. In ulcerative colitis patients, the difference in the distribution was not significant.

DISCUSSION

The function of vitamin D is traditionally recognized in calcium and phosphate homeostasis. However, the protective role of vitamin D against various cancers has been highlighted in recent research. The association between CRC and reduced serum vitamin D3 levels has been widely observed^[9]. Vitamin D exerts its biological functions in its active form, vitamin D3. Vitamin D3 binds the nuclear vitamin D receptor (VDR), and then regulates hundreds of genes. Therefore, vitamin D3 has an influence on cell proliferation, differentiation, apoptosis, DNA repair mechanisms, inflammation and immune function^[9]. It is confusing that the serum concentration of the active 1,25-D3 does not show a constant relationship with CRC, but low serum level of the precursor 25-D3 does^[10]. The *in situ* autocrine/paracrine vitamin D system in colon cells or colon cancer cells may be an important contributor in the onset and progression of colon cancer, rather than the serum level of vitamin D3 which is mainly affected by the kidneys.

CYP24A1 encodes the enzyme 25-hydroxyvitamin D3 24-hydroxylase, a key enzyme that catabolizes 1,25(OH)2D3 to the less active form 25-D3, which is considered the main enzyme determining the biological

Table 3 Associations between the selected single nucleotide polymorphisms and colon cancer, colonic polyp and ulcerative colitis, and odds ratio for the association of each polymorphism with these diseases

Variable (MJ/Mi) ^a	MAF ^a	HWE P ^a	Cases/controls	Genetic model	P for χ^2 test	Odds ratio for MI/MJ	95%CI	
rs4809957 A/G	0.393	0.476	Cancer/1KGeno	G vs A	2.50E-48	0.021	0.009	0.048
			Polyp/1KGeno	G vs A	1.64E-64	0.026	0.014	0.048
			UC/1KGeno	G vs A	1.57E-26	0.008	0.001	0.055
rs6068816 C/T	0.388	0.434	Cancer/1KGeno	T vs C	8.00E-47	32.086	16.238	63.403
			Polyp/1KGeno	T vs C	8.72E-68	48.918	24.888	96.150
			UC/1KGeno	T vs C	3.83E-22	18.260	8.350	39.932
rs6091822 G/T	0.284	0.208	Cancer/1KGeno	T vs G	3.72E-09	2.524	1.844	3.457
			Polyp/1KGeno	T vs G	1.37E-12	2.596	1.984	3.395
			UC/1KGeno	T vs G	0.00001	2.645	1.696	4.125
rs8124792 G/A	0.281	0.616	Cancer/1KGeno	G vs A	1.26E-13	0.083	0.036	0.188
			Polyp/1KGeno	G vs A	2.93E-18	0.086	0.044	0.170
			UC/1KGeno	G vs A	2.58E-07	0.062	0.015	0.256

^aMAF and HWE were calculated among controls only. 1KGeno: Control from 1000 Genomes Project; Cancer: Colon cancer cohort; MI: Minor allele (*i.e.*, less common in controls); MJ: Major allele (*i.e.*, more common in controls); Polyp: Colonic polyp cohort; UC: Ulcerative colitis cohort.

Table 4 Frequencies and distributions of rs6091822 G/T and odds ratio for the association

Variable (MJ/Mi)		Cases			Controls			P for χ^2 test	Odds ratio, GT + TT vs GG	95%CI	
		GG	GT	TT	GG	GT	TT				
rs6091822 G/T	Cancer/1KGeno	0	142	2	262	198	44	1.30E-20	0.010	0.001	0.069
	Polyp/1KGeno	0	96	0				9.75E-29	0.006	0.001	0.046
	UC/1KGeno	0	42	1				1.53E-10	0.021	0.003	0.154

MJ: Major allele (*i.e.*, more common in controls); MI: Minor allele (*i.e.*, less common in controls).

half-life of vitamin D3^[11]. One study found that the expression level of *CYP24A1* was aberrantly increased in CRC tissues both at the mRNA and protein levels compared with corresponding non-cancerous tissues from CRC patients^[12], and another study revealed that the expression level of *CYP24A1* was absent or very low in normal colon mucosa^[13].

The mechanism of this up-regulation is unclear. However, there are several hypotheses. *CYP24A1* expression is highly induced by 1,25-D3 in a VDR-retinoid X receptor-dependent manner, and a meta-analysis showed that very often VDR levels do not correlate with *CYP24A1*^[11]. Approximately 50 different polymorphisms of *CYP24A1* have been identified, but are only partially characterized. None of the four selected SNPs in our study have previously been investigated in colon cancer patients.

In the present study, rs4809957 showed a statistically significant association with the risk of colonic polyps, colon cancer and ulcerative colitis, when both genotypes and allele frequencies were considered. The minimum OR for rs4809957 G when compared with A in ulcerative colitis patients was 0.008, 95%CI: 0.001-0.055, $P = 1.5659E-26$. Our results indicate that the G allele is a strong protective factor, especially for ulcerative colitis, while the ORs in colonic polyp and colon cancer patients were similar.

These findings are consistent with the distribution of this polymorphism in non-small cell lung cancer (NSCLC)^[18]. Rs4809957, located in the 3' untranslated

region which is adjacent to the polyA microsatellite repeat, possibly affects the stability of *CYP24A1* mRNA. Rs4809957 has not been found to affect the function or structure of protein encoded by the enzyme. It is possible that the mechanisms protecting the colon from inflammation or carcinogenesis are different, but this requires further study.

Rs6068816 also showed a statistically significant association with the risk of these three diseases. ORs for rs6068816 C vs T in all diseases were high (OR = 32.086, 95%CI: 16.238-63.403 for colon cancer; OR = 48.918, 95%CI: 24.888-96.150 for colonic polyps; and OR = 18.260, 95%CI: 8.350-39.932 for ulcerative colitis). Thus, these findings indicate that rs6068816 T is a strong risk factor for colon cancer and colonic polyps. Inconsistently, the T of rs6068816 is a weak protective factor in NSCLC (TT vs CT + CC, adjusted OR = 0.40, 95%CI: 0.26-0.60)^[18]. Changes in rs6068816 would not affect the amino acid sequence of the *CYP24A1* expression product, but may affect intron splicing.

For rs6091822, the risk of all three diseases was related to allele carriers (GT + TT) vs major allele homozygotes (GG), but other types of associations (T vs G and TT vs GT + GG) were not significant. The frequencies and distributions of rs6091822 G/T and the OR for the association are described in Table 4. As there were expected frequency numbers less than 5, the χ^2 test may not be sufficiently precise and Fisher's exact test showed that there appears

to be no difference in the distribution of TT in the different groups. Our results suggest that the G allele plays a novel anti-cancer role, especially when homozygous, and the presence of minor allele T, even in heterozygotes, can contribute to the presence of colonic polyps or even colon cancer. Rs6091822 has been reported to have a correlation with breast cancer, but this finding was not constant in a different cohort^[19]. The biological behavior of rs6091822 deserves further investigation.

In our study, the risk of colonic polyps and colon cancer was related to the allele frequencies of rs8124792 G/A, and this association remained for genotype frequencies of this SNP. In ulcerative colitis patients, the difference in the distribution was not significant. The A allele of rs8124792 may indicate the diagnosis of colonic polyps or colon cancer rather than ulcerative colitis. However, this conclusion was derived from a small number of participants, and a large-scale study is needed to verify this finding.

Our findings supported the associations between SNPs on *CYP24A1* and the risk of colonic polyps and colon cancer, and predicted a potential role of *CYP24A1* polymorphisms as biomarkers for population-screening of colonic polyps and colon cancer. In China, a two-step screening method has been used. iFOBT and a questionnaire of high-risk factors are used in the first step. If the iFOBT is positive or the questionnaire reports high-risk factors, a colonoscopy is suggested as the second step. The addition of SNPs testing as primary screening may further decrease the number of high-risk subjects entering the second step and undergoing colonoscopy, thus reducing the medical cost and the rates of complications of colonoscopy. However, the sensitivity and specificity of SNPs tests deserve further investigation before it is applied in clinical practice.

Our research represents the first investigation on *CYP24A1* gene polymorphisms in colonic polyp patients. In our study, the polymorphisms had similar distributions to those in colon cancer. This is concordant with the onset and progression of colonic polyps and colon cancer. Most cancers without family aggregation and precancerous lesions in colon tissues are due to abnormal activation of the Wnt/ β -catenin signaling pathway. 1,25-D₃ can down-regulate this signaling pathway in not only cancer tissues in CRC patients^[20] but also in the non-malignant cell line LT97, which harbors an adenomatous polyposis coli mutation^[21]. Few studies have focused on the associations between serum vitamin D and colorectal polyps, and different to the situation in CRC patients, one study found that serum vitamin D levels were not different between the colorectal polyp and control groups^[22]. The role of SNPs in colonic polyps requires further study.

Inflammatory bowel disease (IBD) is significantly associated with having higher odds for vitamin D deficiency^[23]. Several *in vivo* and *in vitro* studies have examined the role of vitamin D in immune-mediated

diseases such as IBD^[24,25]. The consequences of vitamin D deficiency on the gastrointestinal tract include, but are not limited to, decreased colonic bacterial clearance^[24], reduced expression of tight junctions in the intestinal epithelium, and elevated T helper 1 cell-driven inflammation at the gut level^[23]. However, it is unclear whether this is the result of IBD-related malabsorption due to intestinal mucosal damage, or whether it is a possible contributor to disease onset and progression. Several SNPs in the VDR gene appear to confer susceptibility to ulcerative colitis in Asians, but do not have a statistically significant effect on IBD risk in Europeans^[26-28]. Our study demonstrated that SNPs in the vitamin D-related gene *CYP24A1* are associated with ulcerative colitis in Asians. Furthermore, this association is similar with that for colonic polyps and colon cancer. This suggests that SNPs participate in the onset or progression of ulcerative colitis, and are not only the result of ulcerative colitis-related malabsorption. Although the mechanism is unclear, it may be similar to the way in which vitamin D affects the risk of colonic polyps and colon cancer.

There are some limitations in the present study that must be considered. Firstly, although we present the results of several novel associations, we cannot rule out the possibility that some of these associations may be due to chance, or the possibility of genetic pleiotropy and linkage disequilibrium. Further trials with a larger study population are needed. Secondly, our findings cannot be generalized to the general population, as we included only patients from two hospitals in China as cases and East Asians as controls. Thirdly, we did not include cancer staging information and ulcerative colitis severity in our analysis, and inclusion of these factors may help to identify differences.

In conclusion, we evaluated the associations between rs4809957, rs6068816, rs6091822 and rs8124792, and the risk of colon cancer, colonic polyps and ulcerative colitis. We demonstrated that these four SNPs were related to colon cancer, colonic polyps and ulcerative colitis. In future studies, we will identify both population replication and functional validation to confirm our findings.

COMMENTS

Background

Colorectal cancer (CRC) is a leading cause of cancer incidence and mortality in China and it is very important to identify novel molecular signatures as reliable biomarkers of CRC. Given the crucial role of *CYP24A1* in the development of cancer, it is plausible that the *CYP24A1* polymorphisms may affect the risk of colonic polyps and colon cancer and may be an interesting candidate.

Research frontiers

CYP24A1 polymorphisms have been partially determined, but the association between *CYP24A1* gene polymorphisms and colonic polyps has never been determined.

Innovations and breakthroughs

This research is the first investigation on *CYP24A1* gene polymorphism in

colonic polyp patients. In this study, the polymorphisms had similar distributions to those in colon cancer. This is concordant with the onset and progression of colonic polyps and colon cancer. At the same time, none of the four selected SNPs in our study have previously been investigated in colon cancer patients.

Applications

The addition of SNP testing as primary screening may further decrease the number of high-risk subjects entering the second step and undergoing colonoscopy, thus reducing the medical cost and the complications of colonoscopy.

Peer-review

The authors have investigated the association between CYP24A1 polymorphisms and colon cancer, polyps and ulcerative colitis. They found some significant correlations on direct comparisons. The study is well conducted and expertly written.

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