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

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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.
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 China⁴, 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 2013⁵. 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 Asia⁶-⁸. 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 screening⁹. However, only 37.2% of the target population accepted an iFOBT in a CRC screening program conducted in Hangzhou city⁴⁰. 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 systems⁴⁰.

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 CRC⁴¹. 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 lines⁹. 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 D₁⁷. 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, [1α,25-(OH)₂D₃]) locally. Thus, vitamin D hydroxylases play a prominent role in this process. The CYP24A1 gene encodes a vitamin D₃ catabolic enzyme. The expression level of CYP24A1 was found to be significantly higher in CRC tissues⁴²,⁴³. Although the mechanism of this up-regulation is unclear, CYP24A1 may be an interesting candidate biomarker for use in the screening of colorectal cancer.

It is estimated that 35% of CRC risk may be explained by heritable factors⁴⁴. 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 CRC⁴⁵. The SNPs in CYP24A1 gene have been partially determined. Pibiri et al⁴⁶ reported that rs6022990 was nominally associated with left-sided CRC (P = 0.018) in African Americans. Dong et al⁴⁷ 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...
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, controls for each SNP are shown in Table 3. All SNPs have minor allele frequency (MAF) of ≥ 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.

### Table 1 Characteristics of the study population

| Variable                             | Cases | $P$ value |
|--------------------------------------|-------|-----------|
|                                      | Colonic polyps, $n = 144$ | Colonic cancer, $n = 96$ | Ulcerative colitis, $n = 44$ |
| Sex, n (%)                           |       |           |                            |
| 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 |

$^1P$ value was calculated by the $\chi^2$ test; $^2P$ value was calculated by the ANOVA test.

### 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.
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, \( P = 1.5659\times 10^{-26} \). 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[10]. 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-hydroxvitamin 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

| Variable (M/J/M) | 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                 |
|                 | Polyp/1KGeno  | GG vs AG + AA | 2.20E-16                 |
|                 |               | G vs A        | 2.20E-16                 |
|                 | UC/1KGeno     | AG + GG vs AA | 2.20E-16                 |
|                 |               | G vs A        | 2.20E-16                 |
|                 | Cancer/Polyp  | AG + GG vs AA | 0.7998                   |
|                 |               | G vs A        | 0.8036                   |
|                 | Cancer/UC     | AG + GG vs AA | 0.4362                   |
|                 |               | G vs A        | 0.4424                   |
|                 | Polyp/UC      | AG + GG vs AA | 0.3011                   |
|                 |               | G vs A        | 0.3096                   |
| rs6068816 C/T   | Cancer/1KGeno | CT + TT vs CC | 2.20E-16                 |
|                 |               | T vs C        | 2.20E-16                 |
|                 | Polyp/1KGeno  | CT + TT vs CC | 2.20E-16                 |
|                 |               | T vs C        | 2.20E-16                 |
|                 | UC/1KGeno     | CT + TT vs CC | 2.29E-09                 |
|                 |               | T vs C        | 2.20E-16                 |
|                 | Cancer/Polyp  | CT + TT vs CC | 1.0000                   |
|                 |               | T vs C        | 0.4635                   |
|                 | Cancer/UC     | CT + TT vs CC | 1.0000                   |
|                 |               | T vs C        | 0.2786                   |
|                 | 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                 |
|                 | Polyp/1KGeno  | GT + TT vs GG | 0.000472                 |
|                 |               | T vs G        | 4.06E-12                 |
|                 | UC/1KGeno     | GT + TT vs GG | 1.32E-13                 |
|                 |               | T vs G        | 2.40E-05                 |
|                 | Cancer/Polyp  | GT + TT vs GG | 1.0000                   |
|                 |               | T vs G        | 0.9258                   |
|                 | Cancer/UC     | GT + TT vs GG | 0.5180                   |
|                 |               | T vs G        | 0.8971                   |
|                 | Polyp/UC      | GT + TT vs GG | 1.0000                   |
|                 |               | T vs G        | 1.0000                   |
| rs8124792 G/A   | Cancer/1KGeno | GA + AA vs GG | 2.20E-16                 |
|                 |               | A vs G        | 2.20E-16                 |
|                 | Polyp/1KGeno  | GA + AA vs GG | 0.000758                 |
|                 |               | A vs G        | 2.20E-16                 |
|                 | UC/1KGeno     | GA + AA vs GG | 4.75E-05                 |
|                 |               | A vs G        | 2.91E-09                 |
|                 | Cancer/Polyp  | GA + AA vs GG | 1.0000                   |

1KGeno: Control from 1000 Genomes Project; Cancer: Colon cancer cohort; M: Minor allele (i.e., less common in controls); M: Major allele (i.e., more common in controls); Polyp: Colonic polyp cohort; UC: Ulcerative colitis cohort.
half-life of vitamin D$_3^{[11]}$. 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 might not affect the amino acid sequence of 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 $\chi^2$ test may not be sufficiently precise and Fisher’s exact test showed that there appears

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**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) | MAF* | HWE P* | Cases/controls | Genetic model | $P$ for $\chi^2$ test | Odds ratio for Mi/Mj | 95% CI |
|-----------------|------|--------|----------------|---------------|----------------------|---------------------|-------|
| rs4809957 A/G   | 0.395 | 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 |

*MAF 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 $\chi^2$ test | Odds ratio, GT + TT vs GG | 95% CI |
|-----------------|-------|----------|------------------------|--------------------------|-------|
| 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).
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-D\(\text{d}\) 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\(^{25}\). 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 colon 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.

**REFERENCES**

1. Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. *Chin J Cancer Res* 2015; 27: 2-12 [PMID: 25717220 DOI: 10.3978/j.issn.1000-9604.2015.01.06]

2. Feng YJ, Wang N, Fang LW, Cong S, Yin P, Li YC, Zhou MG. [Burdens of disease of colorectal cancer in the Chinese population, in 1990 and 2013]. Zhonghua Liuxingbingxue Zazhi 2016; 37: 768-772 [PMID: 23746009 DOI: 10.3760/cma.j.issn.0254-6450.20.16 0005]

3. Halloran SP, Launoy G, Zappa M; International Agency for Research on Cancer. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition—Faecal occult blood testing. *Endoscopy* 2012; 44 Suppl 3: S65-S87 [PMID: 23021213 DOI: 10.1055/s-0033-1309791]

4. Sung JJ, Ng SC, Chan FK, Chuu HM, Kim HS, Matsuda T, Ng SS, Lau JY, Zheng S, Adler S, Reddy N, Yeoh KG, Tsoi KK, Ching JY, Kuipers EJ, Rabeneck L, Young GP, Steele RJ, Lieberman D, Goh KL; Asia Pacific Working Group. An updated Asia Pacific Consensus Recommendations on colorectal cancer screening. *Gut* 2015; 64: 121-132 [PMID: 24647008 DOI: 10.1136/ gutjnl-2013-30603]

5. Qaseem A, Denberg TD, Hopkins RH Jr, Humphrey LL, Levine J, Sweet DF, Shekelle P; Clinical Guidelines Committee of the American College of Physicians. Screening for colorectal cancer: a guide to clinicians. *Arch Intern Med* 2012; 172: 378-386 [PMID: 22933133 DOI: 10.1001/ archiveinternmed.2012.1990 DOI: 10.1001/archinternmed.2012.1990 DO

6. Meng W, Cai SR, Zhou L, Dong Q, Zheng S, Zhang SZ. Performance value of high risk factors in colorectal cancer screening in China. *World J Gastroenterol* 2009; 15: 6111-6116 [PMID: 20027686 DOI: 10.3748/wjg.v15.i511]

7. Cai SR, Zhang SZ, Zhu HH, Zheng S. Barriers to colorectal cancer screening: a case-control study. *World J Gastroenterol* 2009; 15: 2531-2536 [PMID: 19469005 DOI: 10.3748/wjg.v15.i513]

8. Sano Y, Byeon JS, Li XB, Wong MC, Chiu HM, Rerknimitr PR, Utama M, Heffeter P, Berger W, Kállay E. Effect of 1,25-dihydroxyvitamin D3 on the Wnt pathway in non-malignant colonic cells. *J Steroid Biochem Mol Biol* 2016; 165: 225-230 [PMID: 25777338 DOI: 10.1016/j.jsbmb.2015.12.012]

9. YureklI OT, Solakoglu T, Atalay R, Bolat AD, Akın FE, Selvi E, Buyukasik NS, Ersoy O. Association between serum vitamin D and parathyroid hormone levels in Turkish patients with colorectal polyps. *Acta Gastroenterol Belg* 2015; 78: 206-211 [PMID: 26151689]

10. Del Pinto R, Pietropaoli D, Chaudhary AK, Ferri C, Cominelli F. Association Between Inflammatory Bowel Disease and Vitamin D Deficiency: A Systematic Review and Meta-analysis. *Inflamm Bowel Dis* 2015; 21: 2708-2717 [PMID: 26348474 DOI: 10.1097/MIB.0000000000001056]

11. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr* 2000; 130: 2648-2652 [PMID: 11053501]

12. Di Rosa M, Malaguarnera M, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immuno-modulatory effects of vitamin D3 in human monocoyte and macrophages. *Cell Immunol* 2012; 280: 36-43 [PMID: 23261827 DOI: 10.1016/j.cellimm.2012.10.009]

13. Xue LN, Xu KQ, Zhang W, Wang Q, Wu J, Wang XY. Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn’s disease: a meta-analysis. *Inflamm Bowel Dis* 2013; 19: 54-60 [PMID: 22467262 DOI: 10.1002/WJGNET
Pei FH, Wang YJ, Gao SL, Liu BR, DU YJ, Liu W, Yu HY, Zhao LX, Chi BR. Vitamin D receptor gene polymorphism and ulcerative colitis susceptibility in Han Chinese. *J Dig Dis* 2011; 12: 90-98 [PMID: 21401893 DOI: 10.1111/j.1751-2980.2011.00483.x]

Hughes DJ, McManus R, Neary P, O’morain C, O’sullivan M. Common variation in the vitamin D receptor gene and risk of inflammatory bowel disease in an Irish case-control study. *Eur J Gastroenterol Hepatol* 2011; 23: 807-812 [PMID: 21818054 DOI: 10.1097/MEG.0b013e328349283e]

P-Reviewer: Yeo SG, Sammour T  S-Editor: Qi Y  
L-Editor: Filipodia  E-Editor: Zhang FF
