www.impactjournals.com/oncotarget/ **Oncotarget, 2017, Vol. 8, (No. 41), pp: 70854-70864**

Research Paper

Different effection of p.1125Val>Ala and rs11954856 in APC on Wnt signaling pathway

Fei-Feng Li[1,2,*], Zhi-Xun Zhao[3,*], Peng Yan[3], Song Wang[3], Zheng Liu[4], Qiong Zhang[5], Xiao-Ning Zhang[5], Chang-Hao Sun[6], Xi-Shan Wang[3,4], Gui-Yu Wang[2,3] and Shu-Lin Liu[1,2,7]

[1]Systemomics Center, College of Pharmacy, and Genomics Research Center (State-Province Key Laboratories of Biomedicine-Pharmaceutics of China), Harbin Medical University, Harbin, China

[2]Translational Medicine Research and Cooperation Center of Northern China, Heilongjiang Academy of Medical Sciences, Heilongjiang, China

[3]Department of Colorectal Surgery of the Second Affiliated Hospital, Harbin Medical University, Harbin, China

[4]Department of Colorectal Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

[5]Department of Antibiotics, Heilongjiang Province Food and Drug Inspection Testing Institute, Harbin, China

[6]Department of Nutrition and Food Hygiene, Public Health College, Harbin Medical University, Harbin, China

[7]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Canada

[*]These authors have contributed equally to this work

Correspondance to: *Gui-Yu Wang,* ***email:*** *guiywang@gmail.com*
Shu-Lin Liu, ***email:*** *slliu@ucalgary.ca*
Xi-Shan Wang, ***email:*** *wxshan1208@126.com*

Keywords: *colorectal cancer, APC, mutation, Wnt/β-catenin signaling pathway, gene expression*

Received: *May 04, 2017* ***Accepted:*** *May 23, 2017* ***Published:*** *August 05, 2017*

Copyright: Li et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC BY 3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Colorectal cancer (CRC) is among the most common and fatal forms of solid tumors worldwide and more than two thirds of CRC and adenomas patients have *APC* gene mutations. APC is a key regulator in the Wnt/β-catenin signaling pathway but its roles in CRC remains to be elucidated. In this study, we compared *APC* genes between CRC patients and controls to determine possible associations of nucleotide changes in the *APC* gene with the pathways involved in CRC pathogenesis. All participants received physical and enteroscopic examinations. The *APC* gene was sequenced for 300 Chinese Han CRC patients and 411 normal controls, and the expression levels of genes in the signaling pathway were analyzed using Western Blotting. Statistical analyses were conducted using SPSS (version 19.0) software. We found that rs11954856 in the *APC* gene was associated with colorectal cancer and could increase the expression levels of *APC*, *β-catenin*, *TCF7L1*, *TCF7L2* and *LEF1* genes in the pathway in the CRC patients, demonstrating the involvement of APC in the pathological processes leading to CRC.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common and fatal forms of solid tumors in both men and women [1, 2]. Most CRC cases are sporadic, with about 15–25% having a family history [3, 4] and 5% diagnosed with inherited CRC syndrome [5]. Many genetic factors have been reported for their associations with the CRC syndrome [6]. For example, instability of the chromosomes and changes in the CpG island methylator phenotype may cause defects in the pathways, leading to CRC [6–8]. To date, a large number of somatic

mutations have been identified in numerous genes for their involvement in colorectal cancer, such as *KRAS* [9, 10], *PIK3CA* [11], *BRAF* [12], *MYH11* [13], and *PARK2* [14]. Additionally, multiple mutations concurrently appearing in a gene may result in marked predisposition to CRC, such as those in the adenomatous polyposis coli (*APC*) gene, which is associated with familial adenomatous polyposis disease (FAP) [15]. Other examples of connections between CRC and genomic variations include mutations in genes such as *MSH2* [16], *MLH1* [17], *PMS1* [18], *TGFBR2* [19] etc, which are associated with hereditary nonpolyposis colorectal cancer (HNPCC), and those in the *MUTYH* gene, which are associated with autosomal recessive adenomatous polyposis [20]. However, the exact functions of the variations or mutations in these genes remain largely unknown.

APC is a key regulator in the Wnt/β-catenin signaling pathway, modulating the quantity ofβ-catenin in the cells. It plays key roles in several fundamental life processes, such as cell division, signal transduction, and tumor suppression [21, 22]. Several mutations and deletions in the APC gene and methylation in its promoter [23] have been described in FAP, and more than two thirds of CRC and adenomas patients have APC gene mutations [23–25]. Additionally, APC gene mutations have also been reported in tumors of other tissues and organs, such as the liver [4], stomach [5, 8, 26], lung [27], breast [28], and brain [29], further calling for the elucidation of the roles of these genetic changes in carcinogenesis [21, 30, 31].

In a previous study, we identified a novel mutation (p.1125Val>Ala) in the APC gene, which is associated with FAP and sporadic cases of CRC [32]. The polyposis syndrome is one of the most common syndromes associated with CRC [29, 30]. However, as many CRC cases do not present polyposis, and the associations between gene changes and the disease are still not established even though recent technical advances have demonstrated an apparent inheritance pattern of CRC [31, 33], contributions of specific changes in the *APC* gene to CRC have not been fully documented.

In this work, we profiled nucleotide changes of the *APC* gene and found that the SNP rs11954856 was associated with CRC. This SNP increased expression levels of genes in the Wnt/β-catenin signaling pathway in the CRC cancer patients, including *APC*, *β-catenin*, and transcription factor genes *TCF7L1*, *TCF7L2*, *LEF1* in the pathway. Of particular significance, the p.1125Val>Ala mutation of the *APC* gene in the CRC cancer patients increased expression levels of not only genes downstream of *APC* in the Wnt/β-catenin signaling pathway but also a gene upstream of *APC*, *GSK-3β*, for feedback, providing further evidence indicating the involvement of the *APC* gene in the pathogenesis of CRC.

RESULTS

Clinical data

The clinical diagnosis was confirmed by three specialists in CRC in the Second Affiliated Hospital of Harbin Medical University, Harbin, China. There was no history of other systemic abnormalities of these CRC patients or previous tumor or familial history of tumor. All the CRC patients (n = 300, male 183, female 117, the min and max age were 16 and 87 respectively, and the average age was 58.59 years) and unrelated controls (n = 411, male 256, female 155, the min and max age were 50 and 70 respectively, and the average age was 59.39 years) were recruited specifically for this study, and there were no statistical differences in gender or age composition between the two groups (Supplementary Table 1).

SNP gene analyses

Using standard protocols, we extracted the genomic DNA from the peripheral blood leukocytes [34] and sequenced the APC gene to detect SNPs and test the hypothesis that germline common genetic variants in the gene may be associated with the susceptibility to CRC. We analyzed the SNPs distributed on the APC gene from the NCBI database (http://www.ncbi.nlm.nih.gov/) and eventually chose six of them to focus on, including rs11241185, rs11954856, rs2019720, rs2229992, rs2431238 and rs2289484 (Supplementary Figure 1A), based on the finding that the genetic heterozygosity of these six SNPs was high (Supplementary Figure 1B).

Polymorphism-disease association analyses

To test the hypothesized associations between *APC* variations and CRC, we conducted SNP analyses and found that the variant rs11954856 in the APC gene was associated with the risk of CRC in the Chinese Han population (Tables 1 and 2). We further analyzed the genotype frequencies in the CRC and control groups by three genetic models (allelic, dominant and recessive) and found that the variant rs11954856 was associated with the risk of CRC in allelic and dominant models (Table 3). On the other hand, we did not find statistical significance in the other analyzed APC gene SNPs rs11241185, rs2019720, rs2229992, rs2431238 and rs2289484 between the CRC and control groups (data not shown). We conducted the Hardy-Weinberg equilibrium test for the CRC and controls and the result was in line with equilibrium (Table 4). We also compared the genotype frequency of the rs11954856 in the CRC, control groups and the data from the HapMap HCB population, and the frequency in the control group was more consistent with the data from the HapMap HCB population (Table 5).

Table 1: The genotype and allele frequency of rs11954856 variations in 300 Chinese Han sporadic colorectal cancer patients and 411 non-CRC controls

Group		*Genotype frequency (%)*			*Allele frequency (%)*	
Genotype		**G/G**	**G/T**	**T/T**	**G**	**T**
CRC	300	179(59.7)	105(35.0)	16(5.3)	463(77.2)	137(22.8)
Controls	411	283(68.9)	116(28.2)	12(2.9)	682(83.0)	140(17.0)

Table 2: rs11954856 variation within *APC gene* associated with risk of sporadic colorectal cancer in Chinese populations

Variations	*Type*	*Pearson Chi-square*				*Risk*		
		Value	**Min count[a]**	**df**	**Asymp. Sig.**	**OR**	***95%CI-Up***	***95%CI-Low***
rs11954856	Genotype	7.381[a]	11.81	2	**0.025**	--	--	--
	Allele	7.443[a]	116.88	1	**0.006**	0.694	0.533	0.903

a: The minimum expected count; b: Not assuming the null hypothesis; c: Using the asymptotic standard error assuming the null hypothesis; d: Based on normal approximation.

Table 3: SNP rs11954856 variation within *APC gene* associated with risk of sporadic colorectal cancer in allelic and dominant model

Value	*Allelic model*	*Dominant model*	*Recessive model*
ChisQ	7.443	6.436	2.671
P	0.0064	0.0112	0.1022

Table 4: The CRC and controls groups were in line with Hardy-Weinberg equilibrium

Group		*Genotype frequency (%)*			*H-W equilibrium testing*		
Genotype		**G/G**	**G/T**	**T/T**	**0 (HET)**	**E (HET)**	**P**
CRC	300	179(59.7)	105(35.0)	16(5.3)	0.3500	0.3524	0.8708
Controls	411	283(68.9)	116(28.2)	12(2.9)	0.2822	0.2826	1.0000

Table 5: The frequency in control group was more consistent with the data from the HapMap HCB population

Group	*Genotype frequency (%)*		
Genotype	**G/G**	**G/T**	**T/T**
CRC	0.597	0.350	0.160
Controls	0.689	0.282	0.029
HCB data	0.682	0.295	0.023

Gene expression analysis

We used Western blotting analysis to measure the expression levels of *APC* gene and related genes in the Wnt/*β*-catenin signaling pathway, including *β-catenin*, *TCF7L1*, *TCF7L2*, *LEF1*, *MMP7*, *C-myc*, *C-jun*, *CYCLIND1* and *GSK-3β* in both cancer and normal tissues, for the patients who may have the wild or mutation types of the genes, the latter being either heterozygous or homozygous. We used the p.1125Val>Ala mutation in the *APC* gene, which is associated with the FAP syndrome [32], for the positive control. We found that the expression levels of the genes in the Wnt/β-catenin signaling pathway, including *APC*, *β-catenin*, *TCF7L1*, *TCF7L2*, *LEF1*, *MMP7*, *C-myc*, *C-jun*, *CYCLIND1* and *GSK-3β*, were remarkably higher in cancer than non-cancer tissues in the p.1125Val>Ala mutant FAP family members (Figure 1A, 1B). Of significant importance, homozygous variation of the *APC* gene SNP rs11954856 was associated with higher expression levels of the *APC* and*β-catenin* genes in the cancer tissue (Figure 2A, 2B, 2C). Notably, in patients with wild type or heterozygous variation types, the expression levels of the two genes in the normal tissue were higher than those in the cancer tissue (Figure 2A, 2B, 2C). The expression levels of cell cycle proteins MMP7, C-myc, C-jun, CYCLIND1 and β-catenin degradation protein GSK-3β in the Wnt/β-catenin signaling pathway were higher in cancer than in non-cancer tissues in all the wild type and the homozygous and heterozygous variations of the patients (Figure 2A, 2G and Figure 3A, 3B, 3C, 3D, 3E). Conversely, the expression levels of transcription factors TCF7L1, TCF7L2 and LEF1 in the Wnt/β-

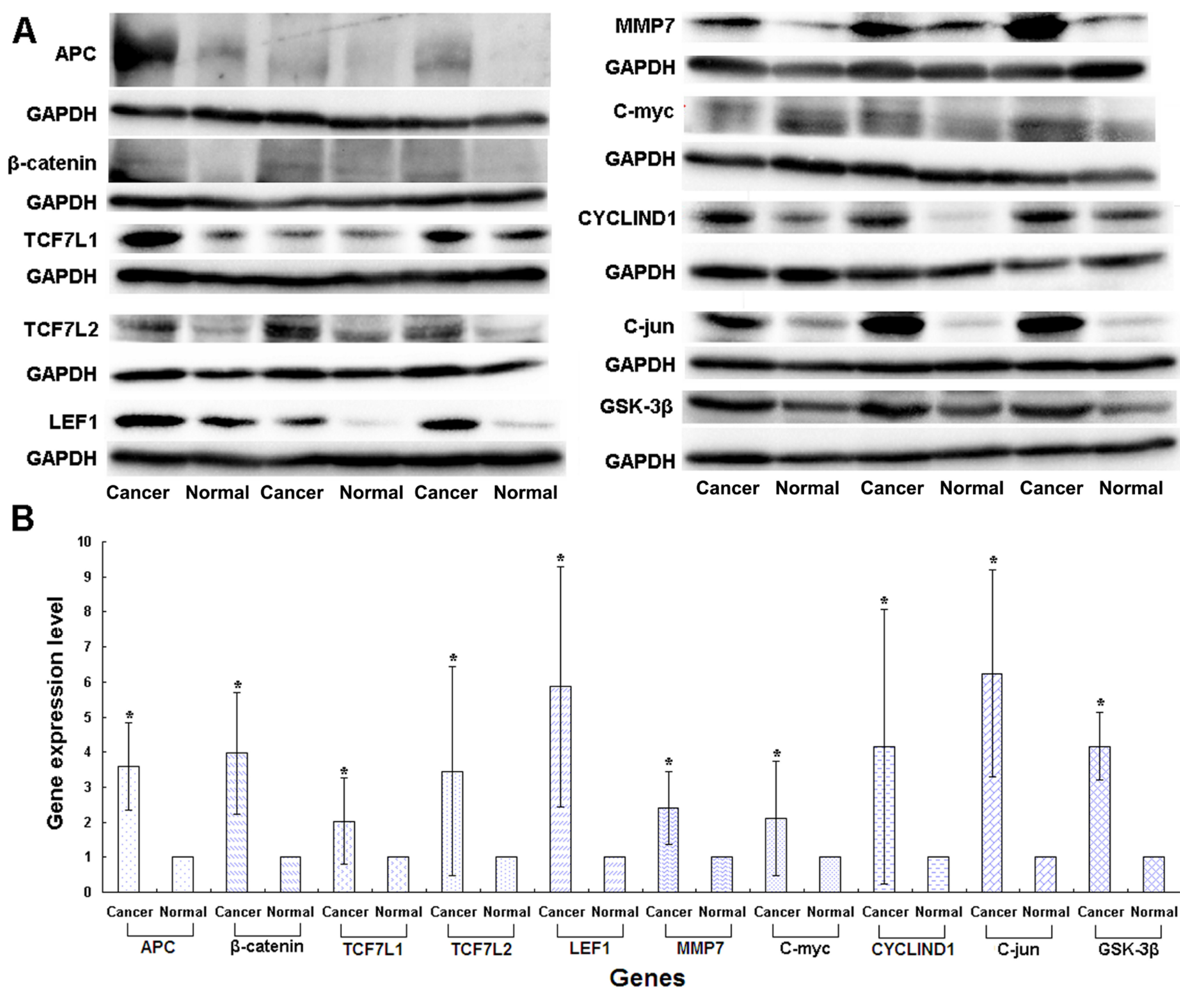


Figure 1: Expression levels of genes in the Wnt/β-catenin signaling pathway in CRC patients detected in the p.1125Val>Ala mutant FAP family patients by Western blotting. **(A)** Original experimental results; **(B)** Numerical experimental results with the band values in the original experimental pictures read by the image J software. The protein expression levels were normalized to GADPH.

catenin signaling pathway were higher in cancer tissue in both homozygous and heterozygous variation types of the patients (Figure 2A, 2D, 2E, 2F).

Comparative analysis of clinical features

We also compared the clinical characteristicsbetween the wild type, heterozygous variant and homozygous variant groups of the CRC patients. We found statistically significant differences between the three groups in stool occult blood and CA199 but not in other clinical characteristics, such as gender composition, age, white blood cell counts, CEA, TNM stage, tumor sites, or pathological types and grades etc. (Table 6). Multiple testing assays on the three groups demonstrated statistically significant differences between the wild type, heterozygous, and homozygous variant groups of the CRC patients in stool occult blood (Table 6). For CA199, however, we found statistically significant differences only between the wild type and heterozygous variant groups (Table 6).

DISCUSSION

In this study, we found that the *APC* gene SNP rs11954856 was associated with colorectal cancer and increased the expression levels of genes in the Wnt/β-

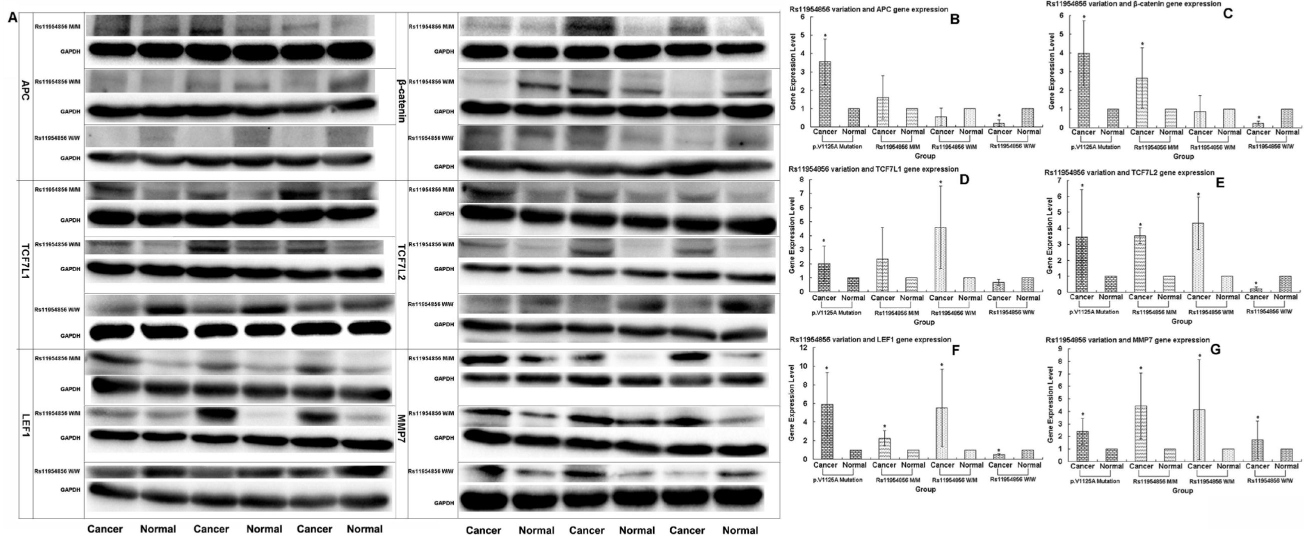


Figure 2: Expression levels of *APC, β-catenin*, *TCF7L1*, *TCF7L2*, *LEF1* and *MMP7* genes detected in patients with rs11954856 wild type or heterozygous or homozygous variations by Western blotting. **(A)** Original experimental results; **(B, C, D, E, F, G)** Numerical experimental results with the band values in the original experimental pictures read by the image J software. The protein expression levels were normalized to GADPH.

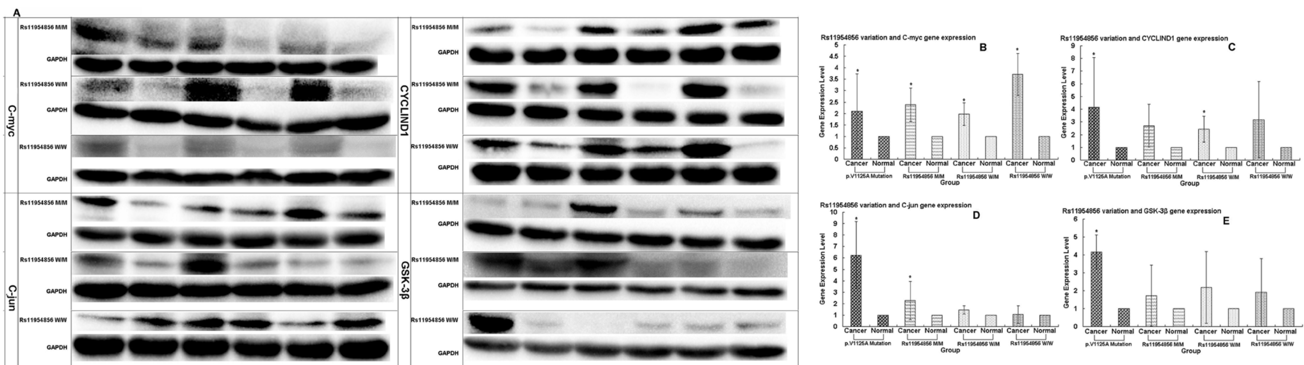


Figure 3: Expression levels of *C-myc, C-jun, CYCLIND1* and *GSK-3β* genes detected in patients with wild type or heterozygous or homozygous variations by Western blotting. **(A)** Original experimental results; **(B, C, D, E)** Numerical experimental results with the band value in the original experimental pictures read by the image J software. The protein expression levels were normalized to GADPH.

Table 6: Comparative analysis of clinical features between wild type, heterozygous variation and homozygous variation groups

Clinical Index	Wild Type	heterozygous variation	homozygous variation	Chi-Square test
Gender (Male/ Female)	105/74	65/40	13/3	P=0.201
Blood in stool (Yes/No)	119/60	56/49	5/11	P=0.005
BSMT	WT-HE P=0.028	HE-HO P=0.100	WT-HO P=0.005	——
Smoking (Yes/No)	64/115	35/70	6/10	P=0.897
Drinking (Yes/No)	59/120	45/60	8/8	P=0.140
Type (Rectum/Colon)	97/82	53/52	11/5	P=0.384
Age	59.64±12.09	59.24±12.79	55.09±15.35	P=0.512
White Blood Cells	6.69±2.35	6.71±2.27	7.83±2.72	P=0.299
NGP	61.54±10.94	60.73±10.04	64.96±14.33	P=0.474
Hemoglobin	126.81±26.31	127.69±23.53	130.64±16.61	P=0.876
Platelets	251.02±86.04	242.45±72.04	244.64±74.08	P=0.760
ALT	16.24±10.23	17.19±11.32	14.36±5.95	P=0.652
AST	18.77±7.78	18.49±8.07	16.00±4.86	P=0.524
Prealbumin (SPA)	180.68±75.07	171.99±64.97	192.30±70.56	P=0.569
Albumin	40.51±6.42	44.85±41.60	40.13±4.97	P=0.471
Creatinine	75.77±19.03	76.71±17.87	80.57±33.00	P=0.720
CEA	17.92±87.83	21.04±71.96	3.58±3.06	P=0.769
CA199	36.95±133.43	107.30±279.63	14.05±12.97	P=0.034
CA199MT	WT-HE P=0.013	HE-HO P=0.138	WT-HO P=0.707	——
TNM Stage (I/II/III/ IV)	25/85/58/11	6/56/38/5	0/12/4/0	P=0.115
Tumor Sites (left/ right)	133/46	73/32	15/1	P=0.117
Pathological Types (PU/PP)	97/82	65/40	10/6	P=0.408
Pathological Grades (H/M/L/MA)	21/148/4/6	9/88/4/4	4/12/0/0	P=0.522

NGP: neutrophilic granulocyte percentage; BSMT: blood in stool-multiple testing; CA199MT: CA199-multiple testing; PU: pathological ulcerative type; PP: pathological protuberant type; H: pathological high grades; M: pathological moderately grades; L: pathological low grades; MA: mucinous adenocarcinoma.

catenin signaling pathway in the CRC patients (Figure 4). Of remarkable significance, the p.1125Val>Ala mutation in the *APC* gene, previously reported for its association with the FAP syndrome [32], also increased the expression levels of all the genes downstream of *APC* gene in the Wnt/β-catenin signaling pathway in the CRC patients, including *APC*, *β-catenin*, *TCF7L1*, *TCF7L2*, *LEF1*, *MMP7*, *C-myc*, *C-jun*, and *CYCLIND1*, and *GSK-3β* for feedback located upstream of the *APC* gene (Figure 4).

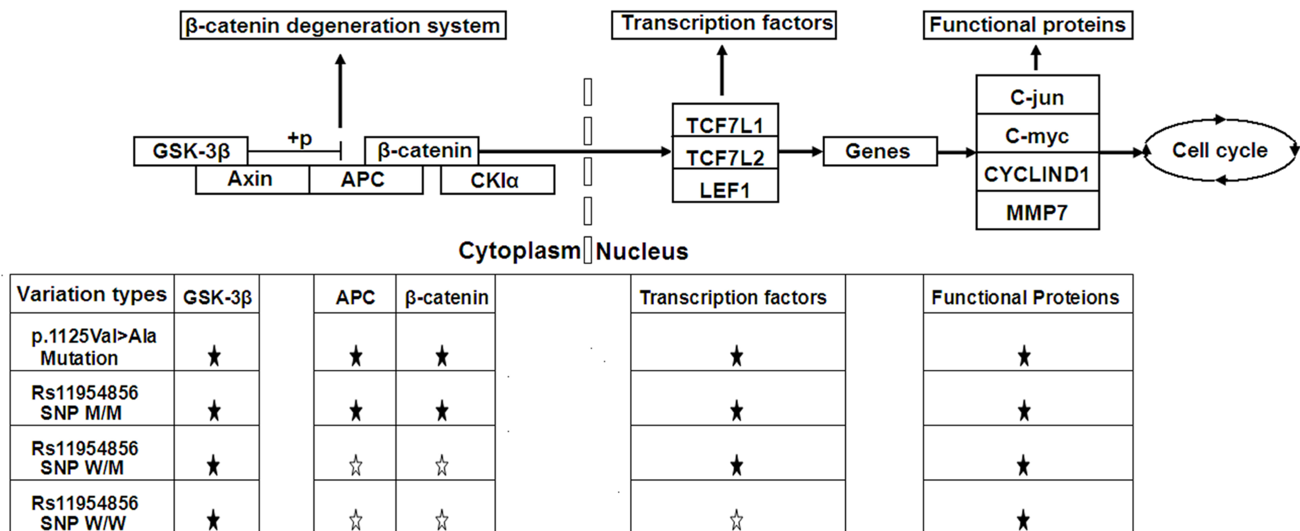
As an important integral part of the Wnt/*β*-catenin signaling pathway, the protein encoded by the *APC* gene plays key roles in tumor suppression by antagonizing the pathway [23]. Loss of APC functions can lead to inappropriate activation of the Wnt/*β*-catenin signaling

pathway and thus may facilitate carcinogenesis [35]. Additionally, APC also has important roles in cell functions, such as migration, adhesion, chromosomal segregation, spindle assembly, apoptosis and differentiation [23]. The sequence about 40 kb upstream of the initiator codon is the promoter of the *APC* gene [36] and its hypermethylation is associated with dysfunction of the Wnt/*β*-catenin signaling pathway [37]. Transcripts bearing the untranslated exon of *APC* are spliced to either exon1 or exon2, which can be detected in mouse embryonic stem cells and all mouse tissues, demonstrating the housekeeping feature of these sequences [36]. We found in this study that the SNP rs11954856, located before the exon1 and within the 3,261 site upstream of the initiating methionine of the *APC* gene, was associated with colorectal cancer.

Compared to the p.1125Val>Ala mutation in the *APC* gene, which increases the expression of not only the genes downstream of the *APC* gene in the signaling pathway but also the *GSK-3β* gene upstream of the *APC* gene, rs11954856 increased the expression of only *APC* and *β-catenin* in the CRC cancer patients, with lesser influences on those of the transcription factors *TCF7L1*, *TCF7L2* and *LEF1*. The amino acids from 1265 to 2035 in the APC protein form the domain for bindingβ-catenin, which is important for β-catenin degradation [38, 39]. Since the variations in the binding domain may increase APC binding withβ-catenin, this domain has been believed to have key roles in the pathogenesis of CRC especially FAP [40]. The results of the present work provided new support for the involvement of APC in the pathogenesis of CRC.

The distinct influences between the SNP rs11954856 and mutation p.1125Val>Ala on the expression levels of genes in the Wnt/β-catenin signaling pathway in the CRC cancer patients may be associated with their different locations in the *APC* gene or different features of particular SNPs/mutations or both. Nucleotide changes may inactivate *APC* gene and in the meantime activate or stabilize *β-catenin*, resulting in the permanent activation of the Wnt/β-catenin signaling pathway, and the silenced *β-catenin* may lead to decreased colonosphere formation, which is an important feature in the tumorigenesis [41]. When *TCF/LEF* and *C-myc* are hyperactivated, the formation of colonospheres is increased [42]. Such events in stem cells may result in increased tumorigenesis and cause CRC [43]. Our findings in the present work are consistent with the previous reports [41–43], such as the *APC* gene p.1125Val>Ala mutation increasing the expression levels of all the genes in the Wnt/β-catenin signaling pathway in the CRC cancer patients (Figure 4).

The main features of the gastrointestinal epithelium cells include rapid cell proliferation and differentiation [43], so mutations may accumulate quickly, with some contributing to carcinogenesis. Additionally, other factors, such as advanced age [44], smoking [45], unhealthy lifestyle and polluted environment also play important roles in the pathogenesis of CRC syndrome [6]. The genes in the Wnt/β-catenin signaling pathway are affected by multiple genetic factors. For example, the expression of *C-myc* can be suppressed by hyperactivation of cell checkpoint genes such as *CDKN1A*, *CDKN1B* and *CDKN2B* [46, 47]. Therefore, the increased expression levels of cell cycle functional proteins MMP7, C-myc,



Variation types	GSK-3β	APC	β-catenin	Transcription factors	Functional Proteions
p.1125Val>Ala Mutation	★	★	★	★	★
Rs11954856 SNP M/M	★	★	★	★	★
Rs11954856 SNP W/M	★	☆	☆	★	★
Rs11954856 SNP W/W	★	☆	☆	☆	★

Figure 4: Schematic diagrams of Wnt/β-catenin signaling pathway. Shown here are influences of rs11954856 and the p.1125Val>Ala mutation in *APC* gene on the expression levels of genes in the pathway in CRC patients. ★ denotes statistically significant and ☆ denotes no statistical differences.

C-jun and CYCLIND1 in the Wnt/β-catenin signaling pathway that we observed here in the cancer tissue (Figure 4) may have contributions also from other genes or factors.

In conclusion, we found that the *APC* gene SNP rs11954856 was associated with CRC by increasing expression levels of genes in the Wnt/β-catenin signaling pathway in the CRC cancer patients, compared to the p.1125Val>Ala mutation in *APC*, which increased expression levels of not only all the genes downstream of *APC* in the Wnt/β-catenin signaling pathway in CRC but also *GSK-3β* upstream of *APC*. Recently, some researchers have found that the SNP rs11954856 is associated with the risk of ovarian and gallbladder cancers [48, 49]. All such findings indicate the importance of the SNP rs11954856 in pathogenesis of cancer and may help develop new strategies for the treatment of cancers or new biomarkers for evaluation or diagnosis of colorectal cancer.

MATERIALS AND METHODS

Study population

We included 300 sporadic CRC cases and 411 normal controls (Supplementary Table 1) for this study, which were assembled at the Department of Colorectal Surgery and Medical Examination Center of the Second Affiliated Hospital of Harbin Medical University, Harbin, China. We obtained a written informed consent from each participant or guardian, and this work has been reviewed and approved by the Ethics Committee of Harbin Medical University. We also confirmed that all experiments were performed in accordance with relevant guidelines and regulations, consistent with the 1975 Declaration of Helsinki. Medical histories were recorded in detail for all the enrolled participants, and all participants received physical and enteroscopic examinations.

DNA analysis

We extracted genomic DNA from peripheral blood leukocytes of each participant using standard protocols [50]. The *APC* gene was amplified by polymerase chain reaction (PCR) with the primers (Supplementary Table 2) for analysis of SNPs. PCR products were sequenced using standard protocols [51] for genotype analysis.

APC SNP genotyping and statistical analysis

The variations of rs11241185, rs11954856, rs2019720, rs2229992, rs2431238 and rs2289484 within the *APC* gene (Supplementary Figure 1A) were determined for 300 sporadic colorectal cancer cases and 411 normal controls. We amplified the *APC* gene and sequenced the PCR products to determine the genotypes (Supplementary Figure 1B).

The statistical analyses were conducted using the SPSS software (version 19.0) and PLINK v1.07 software (http://pngu.mgh.harvard.edu/Bpurcell/plink/) by the methods as previous reported [52, 53]. P values less than 0.05 were considered statistically significant. The Hardy-Weinberg equilibrium test of the CRC and control populations was conducted with the online software OEGE [54].

Western blotting analysis

Proteins of the tumor and normal tissues near the tumor were extracted using standard protocols, and the contents were determined by the BCA protein assay kit (from BOSTER) and ELISA. The proteins were separated by 8% SDS-PAGE and transferred to PVDF membrane. The membranes were then incubated with the primary antibodies against the proteins, including APC (No.ab58, Abcam, Cambridge, USA), β-catenin (No.ab32572, Abcam, Cambridge, USA), TCF7L1 (No.ab133360, Abcam, Cambridge, USA), TCF7L2 (No.ab76151, Abcam, Cambridge, USA), LEF1 (No. ab137872, Abcam, Cambridge, USA), C-myc (No. sc40, Santa, California, USA), C-jun (No.ab32137, Abcam, cambridge, USA), CYCLIND1(No.ab134175, Abcam, cambridge, USA), MMP7 (No.ab205525, Abcam, cambridge, USA), GSK-3β (No.sc53931, Santa, California USA) and GAPDH (No.ta08, ZSGB-BIO, Beijing China) in 5% non-fat milk in TBST at room temperature for two hours. After washing for three times using TBST, the membranes were incubated with secondary antibodies (No.zdr5306 and 5307, ZSGB-BIO, Beijing China) at room temperature for two hours. Then the membranes were developed using the enhanced chemiluminescence plus reagent and imaged using the Bio-Rad gel imaging system [55]. Finally, the band values were read using the image J software.

Author contributions

Conceptualization: FF L, SL L; methodology: FF L, SL L, CH S, XS W, GY W; software: FF L, SL L, Q Z; formal analysis: FF L, SL L, Q Z; investigation: ZU Z, P Y, S W, Z L, XN Z; resources: ZU Z, P Y, S W, Z L, XS W, GY W; data curation: FF L, SL L, CH S, XS W, GY W; writing: FF L, SL L; funding acquisition: FF L, SL L, XS W, GY W.

ACKNOWLEDGMENTS

The authors thank the patients and their families for their cooperation and participation in this study.

CONFLICTS OF INTEREST

All the authors have declared that no competing interest exist.

FINANCIAL SUPPORT

There are no financial disclosures from any authors.

FUNDING

This work was supported by grant from Chinese Postdoctoral Science Foundation (2016M601460), and grants of National Natural Science Foundation of China (NSFC81271786, 81030029, 81671980), Health and Family Planning Commission of Heilongjiang province Foundation (2016-189), Heilongjiang Innovation Research Foundation for College Students (201610226096), Heilongjiang province postdoctoral Science Foundation (LBH-Z14175). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics approval

Ethics Committee of Harbin Medical University.

Informed consent

Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Knopperts AP, Nielsen M, Niessen RC, Tops CM, Jorritsma B, Varkevisser J, Wijnen J, Siezen CL, Heine-Broring RC, van Kranen HJ, Vos YJ, Westers H, Kampman E, et al. Contribution of bi-allelic germline MUTYH mutations to early-onset and familial colorectal cancer and to low number of adenomatous polyps: case-series and literature review. Fam Cancer. 2013; 12:43-50.
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014; 64:9-29.
3. Lovett E. Family studies in cancer of the colon and rectum. Br J Surg. 1976; 63:13-18.
4. Stephenson BM, Finan PJ, Gascoyne J, Garbett F, Murday VA, Bishop DT. Frequency of familial colorectal cancer. Br J Surg. 1991; 78:1162-1166.
5. Tops CM, Wijnen JT, Hes FJ. Introduction to molecular and clinical genetics of colorectal cancer syndromes. Best Pract Res Clin Gastroenterol. 2009; 23:127-146.
6. Schweiger MR, Hussong M, Rohr C, Lehrach H. Genomics and epigenomics of colorectal cancer. Wiley Interdiscip Rev Syst Biol Med. 2013; 5:205-219.
7. Kok-Sin T, Mokhtar NM, Ali Hassan NZ, Sagap I, Mohamed Rose I, Harun R, Jamal R. Identification of diagnostic markers in colorectal cancer via integrative epigenomics and genomics data. Oncol Rep. 2015; 34:22-32.
8. Jarvinen HJ. Hereditary cancer: guidelines in clinical practice. Colorectal cancer genetics. Ann Oncol. 2004; 15:iv127-131.
9. Kranenburg O. The KRAS oncogene: past, present, and future. Biochim Biophys Acta. 2005; 1756:81-82.
10. Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. Nat Genet. 2008; 40:600-608.
11. Gustin JP, Karakas B, Weiss MB, Abukhdeir AM, Lauring J, Garay JP, Cosgrove D, Tamaki A, Konishi H, Konishi Y, Mohseni M, Wang G, Rosen DM, et al. Knockin of mutant PIK3CA activates multiple oncogenic pathways. Proc Natl Acad Sci U S A. 2009; 106:2835-2840.
12. Millington GW. Mutations of the BRAF gene in human cancer, by Davies et al. (Nature 2002; 417: 949-54). Clin Exp Dermatol. 2013; 38:222-223.
13. Alhopuro P, Phichith D, Tuupanen S, Sammalkorpi H, Nybondas M, Saharinen J, Robinson JP, Yang Z, Chen LQ, Orntoft T, Mecklin JP, Jarvinen H, Eng C, et al. Unregulated smooth-muscle myosin in human intestinal neoplasia. Proc Natl Acad Sci U S A. 2008; 105:5513-5518.
14. Veeriah S, Taylor BS, Meng S, Fang F, Yilmaz E, Vivanco I, Janakiraman M, Schultz N, Hanrahan AJ, Pao W, Ladanyi M, Sander C, Heguy A, et al. Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. Nat Genet. 2010; 42:77-82.
15. Homma MK, Li D, Krebs EG, Yuasa Y, Homma Y. Association and regulation of casein kinase 2 activity by adenomatous polyposis coli protein. Proc Natl Acad Sci U S A. 2002; 99:5959-5964.
16. Lamers MH, Perrakis A, Enzlin JH, Winterwerp HH, de Wind N, Sixma TK. The crystal structure of DNA mismatch repair protein MutS binding to a G x T mismatch. Nature. 2000; 407:711-717.
17. Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, et al. Mutation of a mutL homolog in hereditary colon cancer. Science. 1994; 263:1625-1629.
18. Liu T, Yan H, Kuismanen S, Percesepe A, Bisgaard ML, Pedroni M, Benatti P, Kinzler KW, Vogelstein B, Ponz de Leon M, Peltomaki P, Lindblom A. The role of hPMS1 and hPMS2 in predisposing to colorectal cancer. Cancer Res. 2001; 61:7798-7802.
19. Tannergard P, Liu T, Weger A, Nordenskjold M, Lindblom A. Tumorigenesis in colorectal tumors from patients with

hereditary non-polyposis colorectal cancer. Hum Genet. 1997; 101:51-55.

20. Sampson JR, Dolwani S, Jones S, Eccles D, Ellis A, Evans DG, Frayling I, Jordan S, Maher ER, Mak T, Maynard J, Pigatto F, Shaw J, Cheadle JP. Autosomal recessive colorectal adenomatous polyposis due to inherited mutations of MYH. Lancet. 2003; 362:39-41.

21. Bodmer WF, Bailey CJ, Bodmer J, Bussey HJ, Ellis A, Gorman P, Lucibello FC, Murday VA, Rider SH, Scambler P, Sheer D, Solomon E, Spurret NK. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature. 1987; 328:614-616.

22. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut. 2007; 56:417-425.

23. Hanson CA, Miller JR. Non-traditional roles for the Adenomatous Polyposis Coli (APC) tumor suppressor protein. Gene. 2005; 361:1-12.

24. Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. Hum Mutat. 1993; 2:425-434.

25. Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Mori T, Nakamura Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Hum Mol Genet. 1992; 1:229-233.

26. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Clendenning M, Sotamaa K, Prior T, Westman JA, Panescu J, Fix D, Lockman J, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol. 2008; 26:5783-5788.

27. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004; 96:261-268.

28. Lynch HT, Lynch JF. What the physician needs to know about Lynch syndrome: an update. Oncology (Williston Park). 2005; 19:455-463; discussion 463-454, 466, 469.

29. Farrington SM, Tenesa A, Barnetson R, Wiltshire A, Prendergast J, Porteous M, Campbell H, Dunlop MG. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. Am J Hum Genet. 2005; 77:112-119.

30. Cleary SP, Cotterchio M, Jenkins MA, Kim H, Bristow R, Green R, Haile R, Hopper JL, LeMarchand L, Lindor N, Parfrey P, Potter J, Younghusband B, Gallinger S. Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study. Gastroenterology. 2009; 136:1251-1260.

31. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadwick RB, Kaariainen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med. 1998; 338:1481-1487.

32. Li FF, Liu Z, Yan P, Shao X, Deng X, Sam C, Chen YG, Xu YP, Wang XS, Wang GY, Liu SL. Identification of a novel mutation associated with familial adenomatous polyposis and colorectal cancer. Int J Mol Med. 2015; 36:1049-1056.

33. Burt RW, Bishop DT, Cannon LA, Dowdle MA, Lee RG, Skolnick MH. Dominant inheritance of adenomatous colonic polyps and colorectal cancer. N Engl J Med. 1985; 312:1540-1544.

34. Tan ZX, Li FF, Qu YY, Liu J, Liu GR, Zhou J, Zhu YL, Liu SL. Identification of a known mutation in Notch 3 in familiar CADASIL in China. PLoS One. 2012; 7:e36590.

35. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev. 2007; 21:2525-2538.

36. Karagianni N, Ly MC, Psarras S, Chlichlia K, Schirrmacher V, Gounari F, Khazaie K. Novel adenomatous polyposis coli gene promoter is located 40 kb upstream of the initiating methionine. Genomics. 2005; 85:231-237.

37. Wang ZK, Liu J, Liu C, Wang FY, Chen CY, Zhang XH. Hypermethylation of adenomatous polyposis coli gene promoter is associated with novel Wnt signaling pathway in gastric adenomas. J Gastroenterol Hepatol. 2012; 27:1629-1634.

38. Sturt NJ, Gallagher MC, Bassett P, Philp CR, Neale KF, Tomlinson IP, Silver AR, Phillips RK. Evidence for genetic predisposition to desmoid tumours in familial adenomatous polyposis independent of the germline APC mutation. Gut. 2004; 53:1832-1836.

39. Schiessling S, Kihm M, Ganschow P, Kadmon G, Buchler MW, Kadmon M. Desmoid tumour biology in patients with familial adenomatous polyposis coli. Br J Surg. 2013; 100:694-703.

40. Polakis P. The adenomatous polyposis coli (APC) tumor suppressor. Biochim Biophys Acta. 1997; 1332:F127-147.

41. Kanwar SS, Yu Y, Nautiyal J, Patel BB, Majumdar AP. The Wnt/beta-catenin pathway regulates growth and maintenance of colonospheres. Mol Cancer. 2010; 9:212.

42. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den Born M, et al. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell. 2002; 111:241-250.

43. Abetov D, Mustapova Z, Saliev T, Bulanin D. Biomarkers and signaling pathways of colorectal cancer stem cells. Tumour Biol. 2015; 36:1339-1353.

44. U.S. Preventive Services Task Force. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2008; 149:627-637.

45. Morrison DS, Batty GD, Kivimaki M, Davey Smith G, Marmot M, Shipley M. Risk factors for colonic and rectal cancer mortality: evidence from 40 years' follow-up in the

Whitehall I study. J Epidemiol Community Health. 2011; 65:1053-1058.

46. Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell. 2000; 103:295-309.
47. Siegel PM, Shu W, Massague J. Mad upregulation and Id2 repression accompany transforming growth factor (TGF)-beta-mediated epithelial cell growth suppression. J Biol Chem. 2003; 278:35444-35450.
48. Mostowska A, Pawlik P, Sajdak S, Markowska J, Pawalowska M, Lianeri M, Jagodzinski PP. An analysis of polymorphisms within the Wnt signaling pathway in relation to ovarian cancer risk in a Polish population. Mol Diagn Ther. 2014; 18:85-91.
49. Yadav A, Gupta A, Yadav S, Rastogi N, Agrawal S, Kumar A, Kumar V, Misra S, Mittal B. Association of Wnt signaling pathway genetic variants in gallbladder cancer susceptibility and survival. Tumour Biol. 2016; 37:8083-8095.
50. Deng X, Zhou J, Li FF, Yan P, Zhao EY, Hao L, Yu KJ, Liu SL. Characterization of nodal/TGF-lefty signaling pathway gene variants for possible roles in congenital heart diseases. PLoS One. 2014; 9:e104535.
51. Li FF, Wang X, Zhu M, Xiao H, Yang Q, Shao X, Feng H, Lin G, Liu L. Identification of a location at chromosome 19p in a big Chinese family with charcot-marie-tooth disease. J Mol Biomark Diagn. 2013; 4: 144. https://doi.org/10.4172/2155-9929.1000144.
52. Li FF, Zhou J, Zhao DD, Yan P, Li X, Han Y, Li XS, Wang GY, Yu KJ, Liu SL. Characterization of SMAD3 gene variants for possible roles in ventricular septal defects and other congenital heart diseases. PLoS One. 2015; 10:e0131542.
53. Li FF, Han Y, Shi S, Li X, Zhu XD, Zhou J, Shao QL, Li XQ, Liu SL. Characterization of transcriptional repressor gene MSX1 variations for possible associations with congenital heart diseases. PLoS One. 2015; 10:e0142666.
54. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol. 2009; 169:505-514.
55. Li FF, Yan P, Zhao ZX, Liu Z, Song DW, Zhao XW, Wang XS, Wang GY, Liu SL. Polymorphisms in the CHIT1 gene: associations with colorectal cancer. Oncotarget. 2016; 7:39572-39581. https://doi.org/10.18632/oncotarget.9138.