Int J Clin Exp Med 2016;9(6):9302-9308
www.ijcem.com /ISSN:1940-5901/IJCEM0010846

Original Article

The effects of calcium-sensing receptor (CASR) gene variants on colorectal cancer (CRC) risk and mortality: a systematic review and meta-analysis

Like Zhang, Jianguang Wang, Yanbin Chen, Pengfei Yuan, Dechun Liu

Department of General Surgery, The First Affiliated Hospital of the Henan University of Science and Technology, Luoyang 471003, Henan, China

Received May 30, 2015; Accepted July 20, 2015; Epub June 15, 2016; Published June 30, 2016

Abstract: The role of calcium-sensing receptor (CASR) in colorectal cancer (CRC) have been assessed with several studies, but the conclusions were inconclusive. Thus we performed a meta-analysis to evaluate the impact of CASR polymorphisms on CRC risk and mortality. We searched literature from PubMed, Embase, Cochrane Library, and Web of Science databases. The strength of association between the CASR polymorphisms and CRC risk was assessed by calculating odds ratio (OR) with 95% CI. CASR rs1801725 polymorphism was related to an increased risk of CRC (OR = 1.44; 95% CI, 1.08-1.93; P = 0.01). CASR rs1042636 polymorphism was not found to be associated with CRC risk (OR = 0.90; 95% CI, 0.74-1.10; P = 0.32). As for rs1801726 (OR = 0.90; 95% CI, 0.66-1.23; P = 0.51) and rs12485716 (OR = 0.99; 95% CI, 0.88-1.11; P = 0.82), they were also not associated with CRC risk, respectively. CASR rs4678174 polymorphism was significantly related to decreased risk of CRC (OR = 0.91; 95% CI, 0.83-0.99; P = 0.03). However, CASR rs1801725 polymorphism was not associated with CRC mortality risk. In conclusion, this meta-analysis demonstrates CASR rs1801725 polymorphism and rs4678174 polymorphism were significantly related to the risk of CRC.

Keywords: Colorectal cancer, calcium-sensing receptor, Meta-analysis, polymorphism

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy and the fourth leading cause of cancer death worldwide [1]. Due to screening and removal of premalignant polyps, incidence rates have declined over the last 3 decades [2]. Despite the progress in the diagnosis and treatment of CRC, the mortality from this disease remains high. Approximately 20% of patients with CRC have distant metastasis at the time of presentation [3]. Additionally, among those patients who undergo curative resection of the primary tumor, nearly a third will develop recurrent disease.

The calcium-sensing receptor (CaSR) has been identified as a key molecule in regulating systemic calcium homeostasis in the parathyroid [4]. Aggarwal et al. demonstrate that the CaSR inhibits epithelial-to-mesenchymal transition and the acquisition of a stem cell-like phenotype in the colon of mice lacking the CaSR as well as colorectal cancer cells, identifying the CaSR as a key molecule in preventing tumor progression [5]. Fetahu et al. show that hypermethylation of the CaSR promoter and H3K9 deacetylation, but not H3K4me2 demethylation are important factors that cause silencing of the CaSR in colorectal cancer [6].

The CASR gene encodes a calcium-binding G protein-coupled receptor (GPCR), with an extracellular N-terminal domain (containing the calcium binding sites), joined to the C-terminal domain via a seven transmembrane region (essential for its signaling function). Several studies have demonstrated an increased risk of CRC risk in subjects with the CASR polymorphisms, whereas other studies have reported no association between CASR polymorphisms and CRC risk [7-15]. Therefore, we performed this meta-analysis to assess the role of CASR polymorphisms on CRC risk and mortality.

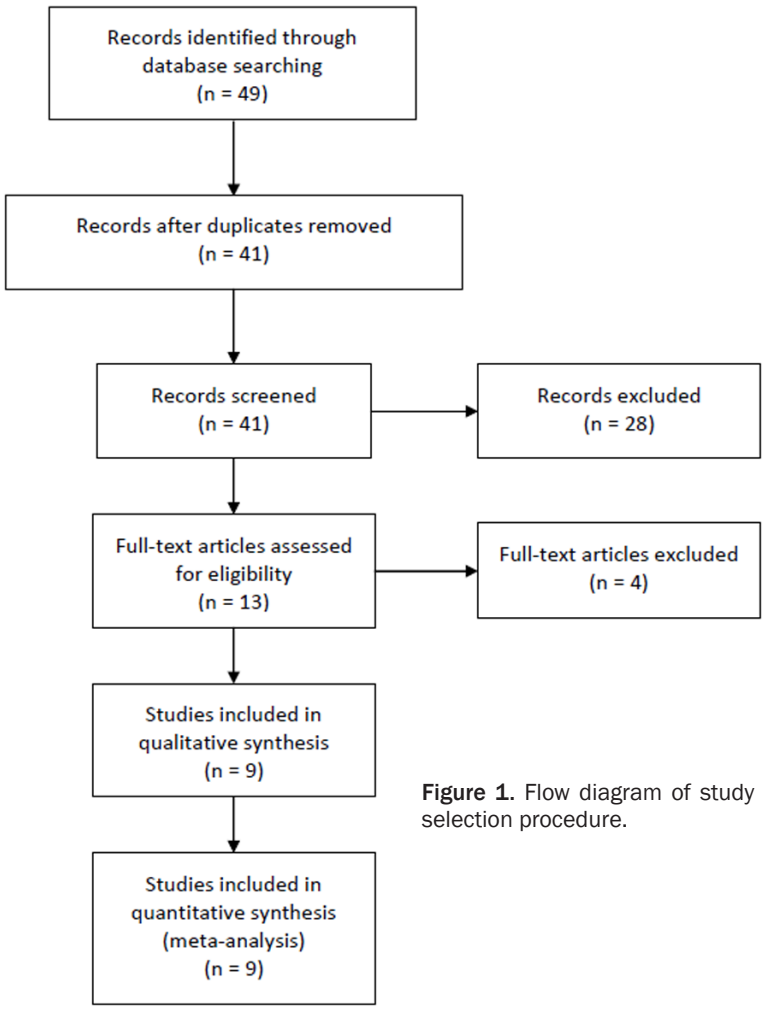

Figure 1. Flow diagram of study selection procedure.

Materials and methods

Search for publications

We searched literature from PubMed, Embase, Cochrane Library, and Web of Science databases with the terms: "calcium-sensing receptor", "CASR", "colorectal neoplasm", "colorectal cancer", "CRC" with all possible combinations. And reference lists of review articles, bibliographies, or some other relevant studies were also searched manually for finding the additional eligible studies. The last search was performed on Apr 17, 2015.

Inclusion and exclusion criteria

Articles were included in this meta-analysis if (1) they described a case-control study; (2) the case group consisted of CRC patients and the control group included healthy individuals; (3) the outcome measures reported CASR polymorphisms and CRC risk or mortality; (4) the study was a high-quality study (i 7 points according to the Newcastle-Ottawa Scale evaluation standard for case-control studies [16]; (5) it was written in English or Chinese. After reading the title and abstract, we excluded a study if it was an animal or in vitro experiment, was not related to CRC, did not contain data on CASR, or was not a case-control study, case reports, and studies consisting of duplicate data. Disagreement was resolved by discussion between the authors.

Data extraction

Data were independently abstracted by two investigators. Differences among evaluators were resolved by discussion and rereading with the third investigator. The following information was extracted from each included study using a standardized data collection protocol: first author, year of publication, ethnicity of participants, age and gender, site of CRC, numbers of cases and controls, and CASR polymorphisms.

Statistical analysis

The strength of association between the CASR polymorphisms and CRC risk was assessed by calculating odds ratio (OR) with 95% CI. A statistical test for heterogeneity was performed based on the Q statistic. The $P > 0.10$ of the Q-test indicated a lack of heterogeneity among studies. If heterogeneity was observed among the studies, the random-effects model was used to estimate the pooled OR (the DerSimonian and Laird method). Otherwise, the fixed-effects model was adopted (the Mantel-Haenszel method). Stratified analysis was performed by site of CRC. All statistical tests were performed with the software Review Manager 5.1. A P value < 0.05 was considered statistically significant.

Table 1. Characteristics of the included studies

First author	Year	Ethnicity	Age	Gender	Site	Case (n)	Control (n)	Mortality	Polymorphism	HWE	Quality
Speer	2002	Caucasian	62	Mixed	Rectal	56	112	NA	rs1801725	Yes	7
Peters	2002	Caucasian	55-74	Mixed	CRC	716	729	NA	rs1801725, rs1042636, rs1801726	Yes	7
Bácsi	2008	Caucasian	61	Mixed	CRC	278	260	Yes	rs1801725	Yes	8
Dong	2008	Caucasian	64.9	Mixed	Colon	1600	1949	NA	rs1801725, rs1042636, rs1801726, rs10934578, rs12485716, rs2270916, rs4678174	Yes	9
Jenab	2009	Caucasian	NA	Mixed	Rectal and Colon	1160	1160	NA	rs1801725	Yes	8
Jacobs	2010	Caucasian	53.5	Mixed	Rectal and Colon	1802	2874	NA	rs1042636, rs12485716, rs1801726, rs4678174	Yes	8
Fedirko	2012	Caucasian	62	Mixed	CRC	1202	--	Yes	rs1801725	--	7
Kim	2013	Asian	> 30	Mixed	Rectal and Colon	420	815	NA	rs10934578, rs12485716, rs2270916, rs4678174	Yes	7
Mahmoudi	2014	Caucasian	55.1	Mixed	CRC	350	510	NA	rs1801725	Yes	8

CRC, colorectal cancer; HWE, Hardy-Weinberg equilibrium; NA, not available.

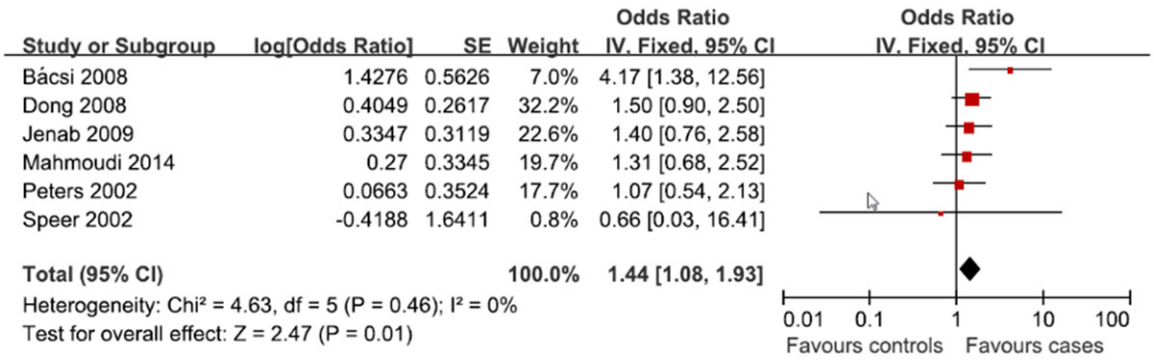

Figure 2. Forest plot of odds ratio (OR) for the association between CASR rs1801725 polymorphism and CRC risk.

Table 2. Results of meta-analysis

Polymorphism	Model	OR (95% CI)	*P* Value	I^2 (%)	*P* Value
rs1801725	F	1.44 (1.08-1.93)	0.01	0	0.46
Rectal	F	1.65 (0.58-4.67)	0.35	0	0.55
Colon	F	1.41 (0.92-2.15)	0.11	0	0.67
rs1042636	F	0.90 (0.74-1.10)	0.32	0	0.68
Colon	F	0.85 (0.66-1.09)	0.20	0	0.88
rs1801726	F	0.90 (0.66-1.23)	0.51	0	0.44
Colon	F	0.88 (0.52-1.47)	0.62	0	0.86
rs12485716	F	0.99 (0.88-1.11)	0.82	0	0.73
Rectal	F	0.92 (0.75-1.14)	0.44	0	0.86
Colon	F	0.96 (0.82-1.12)	0.60	0	0.50
rs4678174	F	0.91 (0.83-0.99)	0.03	19	0.29
Rectal	F	0.86 (0.70-1.04)	0.12	0	0.74
Colon	F	0.93 (0.83-1.03)	0.17	0	0.73

F, fixed-effects model.

Results

Characteristics of studies

The initial search identified 49 articles. Of these articles, 41 relevant studies were selected for detailed evaluation after the exclusion of duplicates and a review of the remaining titles and abstracts. Finally, 9 studies were included in our analysis (**Figure 1**). Of these 9 publications, 8 articles evaluated the association between CASR polymorphisms and CRC risk and 2 were for prognosis of CRC. The clinical features of eligible studies were listed in **Table 1**. Totally, 7584 CRC patients and 8409 controls were included in this study. All of these studies got 6 scores or more in quality assessment, and were classified as high quality studies.

CASR polymorphisms and CRC risk

As shown in **Figure 2**, CASR rs1801725 polymorphism was related to an increased risk of CRC (OR = 1.44; 95% CI, 1.08-1.93; P = 0.01). Subgroup analyses were performed according to site of CRC. The results showed that CASR rs1801725 polymorphism was not associated with rectal cancer risk (OR = 1.65; 95% CI, 0.58-4.64; P = 0.35) and colon cancer risk (OR = 1.41; 95% CI, 0.92-2.15; P = 0.11). CASR rs1042636 polymorphism was not found to be associated with CRC risk (OR = 0.90; 95% CI, 0.74-1.10; P = 0.32) and colon cancer risk (OR = 0.85; 95% CI, 0.66-1.09; P = 0.20). As for rs1801726 (OR = 0.90; 95% CI, 0.66-1.23; P = 0.51) and rs12485716 (OR = 0.99; 95% CI, 0.88-1.11; P = 0.82), they were also not associated with CRC risk, respectively. In addition, these two polymorphisms were not related to the risk of rectal cancer and colon cancer (**Table 2**). As shown in **Figure 3**, CASR rs4678174 polymorphism was significantly related to decreased risk of CRC (OR = 0.91; 95% CI, 0.83-0.99; P = 0.03). Furthermore, this polymorphism was marginally associated with the risk of rectal cancer and colon cancer.

CASR polymorphisms and CRC mortality risk

Two studies evaluated the association between CASR polymorphisms and CRC mortality risk. In the study by Bácsi et al., CASR rs1801725 polymorphism did not show any association with DFS or OS [9]. Similarly, the study by Fedirko et al. also suggested that CASR (rs1801725) genotype was not associated with survival in CRC [13].

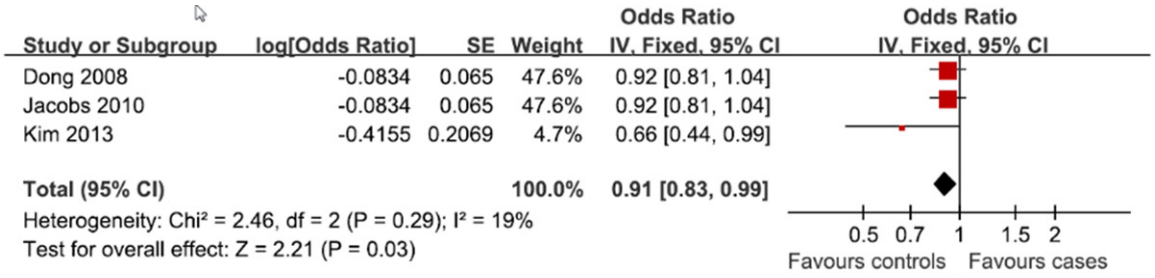

Figure 3. Forest plot of odds ratio (OR) for the association between CASR rs4678174 polymorphism and CRC risk.

Discussion

Human and animal studies have shown that Ca2 + prevents crypt hyperproliferation, suppresses dysplasia and protects the colon from malignant transformation [17]. The CaSR has been proposed as a potential mediator of these effects [18]. However, evidence for a causal relation between CASR polymorphisms and colorectal tumorigenesis is still missing. In this meta-analysis, we found that CASR rs1801725 polymorphism and rs4678174 polymorphism was significantly related to the risk of CRC. However, CASR rs1801725 polymorphism was not associated with CRC mortality risk.

The CaSR is expressed abundantly in normal colonic epithelium and lost in colon cancer, but its exact role on a molecular level and within the carcinogenesis pathway is yet to be described [19]. MacLeod suggested that both inflammation and Wnt/β-catenin signaling are increased in the epithelia of 'rescued' CaSR/PTH double knockout colons, and the capacity for non-canonical Wnt signaling through Wnt5a/Ror2 engagement is reduced [20]. CASR methylation was detected in 69% of colorectal cancer tissues and 90% of lymph node metastatic tissues and was significantly correlated with reduced CASR expression [21]. A previous study shows that serum calcium is associated with SNPs in or near the CASR gene on 3q13. The top hit is rs1801725, a missense variant, explaining 1.26% of the variance in serum calcium [23].

To our knowledge, this was the first meta-analysis to investigate the association between CASR polymorphisms and CRC. The inclusion of online database added strength to our study. However, our study had some limitations. Firstly, many of the original studies did not adjust for potentially important confounders. Secondly, because a lack in the original data of the reviewed studies, other subgroup analysis could not conducted, such as age and sex. In spite of these, our meta-analysis had some advantages. First, a substantial number of cases and controls were pooled from different studies, which greatly increased the statistical power compared with individual studies. Second, no significant heterogeneity was found, indicating that our results were moderately robust.

In conclusion, this meta-analysis demonstrates CASR rs1801725 polymorphism and rs4678174 polymorphism were significantly related to the risk of CRC. More prospective studies with high-quality designs should be performed to further validate these findings.

Disclosure of conflict of interest

None.

Address correspondence to: Dechun Liu, Department of General Surgery, The First Affiliated Hospital of The Henan University of Science and Technology, Luoyang 471003, Henan, China. Tel: 86-0379-64830493; E-mail: dechunliu@126.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Akin O, Brennan SB, Dershaw DD, Ginsberg MS, Gollub MJ, Schöder H, Panicek DM, Hricak H. Advances in oncologic imaging: update on 5 common cancers. CA Cancer J Clin 2012; 62: 364-93.

[3] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96.

[4] Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J,

Hebert SC. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. Nature 1993; 366: 575-80.
[5] Aggarwal A, Prinz-Wohlgenannt M, Gröschel C, Tennakoon S, Meshcheryakova A, Chang W, Brown EM, Mechtcheriakova D, Kállay E. The calcium-sensing receptor suppresses epithelial-to-mesenchymal transition and stem cell-like phenotype in the colon. Mol Cancer 2015; 14: 61.
[6] Fetahu IS, Höbaus J, Aggarwal A, Hummel DM, Tennakoon S, Mesteri I, Baumgartner-Parzer S, Kállay E. Calcium-sensing receptor silencing in colorectal cancer is associated with promoter hypermethylation and loss of acetylation on histone 3. Int J Cancer 2014; 135: 2014-23.
[7] Speer G, Cseh K, Mucsi K, Takács I, Dworak O, Winkler G, Szödy R, Tislér A, Lakatos P. Calcium-sensing receptor A986S polymorphism in human rectal cancer. Int J Colorectal Dis 2002; 17: 20-4.
[8] Peters U, Chatterjee N, Yeager M, Chanock SJ, Schoen RE, McGlynn KA, Church TR, Weissfeld JL, Schatzkin A, Hayes RB. Association of genetic variants in the calcium-sensing receptor with risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2004; 13: 2181-6.
[9] Bácsi K, Hitre E, Kósa JP, Horváth H, Lazáry A, Lakatos PL, Balla B, Budai B, Lakatos P, Speer G. Effects of the lactase 13910 C/T and calcium-sensor receptor A986S G/T gene polymorphisms on the incidence and recurrence of colorectal cancer in Hungarian population. BMC Cancer 2008; 8: 317.
[10] Dong LM, Ulrich CM, Hsu L, Duggan DJ, Benitez DS, White E, Slattery ML, Caan BJ, Potter JD, Peters U. Genetic variation in calcium-sensing receptor and risk for colon cancer. Cancer Epidemiol Biomarkers Prev 2008; 17: 2755-65.
[11] Jenab M, McKay J, Bueno-de-Mesquita HB, van Duijnhoven FJ, Ferrari P, Slimani N, Jansen EH, Pischon T, Rinaldi S, Tjønneland A, Olsen A, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Engel P, Kaaks R, Linseisen J, Boeing H, Fisher E, Trichopoulou A, Dilis V, Oustoglou E, Berrino F, Vineis P, Mattiello A, Masala G, Tumino R, Vrieling A, van Gils CH, Peeters PH, Brustad M, Lund E, Chirlaque MD, Barricarte A, Suárez LR, Molina E, Dorronsoro M, Sala N, Hallmans G, Palmqvist R, Roddam A, Key TJ, Khaw KT, Bingham S, Boffetta P, Autier P, Byrnes G, Norat T, Riboli E. Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. Cancer Epidemiol Biomarkers Prev 2009; 18: 2485-91.
[12] Jacobs ET, Martínez ME, Campbell PT, Conti DV, Duggan D, Figueiredo JC, Haile RW, LeRoy EC, Poynter JN, Thompson PA, Baron JA. Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry. Carcinogenesis 2010; 31: 1412-6.
[13] Fedirko V, Riboli E, Tjønneland A, Ferrari P, Olsen A, Bueno-de-Mesquita HB, van Duijnhoven FJ, Norat T, Jansen EH, Dahm CC, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Racine A, Lukanova A, Teucher B, Boeing H, Aleksandrova K, Trichopoulou A, Benetou V, Trichopoulos D, Grioni S, Vineis P, Panico S, Palli D, Tumino R, Siersema PD, Peeters PH, Skeie G, Brustad M, Chirlaque MD, Barricarte A, Ramón Quirós J, Sánchez MJ, Dorronsoro M, Bonet C, Palmqvist R, Hallmans G, Key TJ, Crowe F, Khaw KT, Wareham N, Romieu I, McKay J, Wark PA, Romaguera D, Jenab M. Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European ppulations. Cancer Epidemiol Biomarkers Prev 2012; 21: 582-93.
[14] Kim KZ, Shin A, Kim J, Park JW, Park SC, Choi HS, Chang HJ, Kim DY, Oh JH. Association between CASR polymorphisms, calcium intake, and colorectal cancer risk. PLoS One 2013; 8: e59628.
[15] Mahmoudi T, Karimi K, Arkani M, Farahani H, Nobakht H, Dabiri R, Asadi A, Zali MR. Parathyroid hormone gene rs6256 and calcium sensing receptor gene rs1801725 variants are not associated with susceptibility to colorectal cancer in Iran. Asian Pac J Cancer Prev 2014; 15: 6035-9.
[16] Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomized Studies in Meta-Analysis. Available online: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
[17] Wang JL, Lin YW, Chen HM, Kong X, Xiong H, Shen N, Hong J, Fang JY. Calcium prevents tumorigenesis in a mouse model of colorectal cancer. PLoS One 2011; 6: e22566.
[18] Kállay E, Kifor O, Chattopadhyay N, Brown EM, Bischof MG, Peterlik M, Cross HS. Calcium-dependent c-myc proto-oncogene expression and proliferation of Caco-2 cells: a role for a luminal extracellular calcium-sensing receptor. Biochem Biophys Res Commun 1997; 232: 80-3.
[19] Rogers AC, Hanly AM, Collins D, Baird AW, Winter DC. Review article: loss of the calcium-sensing receptor in colonic epithelium is a key event in the pathogenesis of colon cancer. Clin Colorectal Cancer 2012; 11: 24-30.
[20] MacLeod RJ. Extracellular calcium-sensing receptor/PTH knockout mice colons have increased Wnt/β-catenin signaling, reduced

non-canonical Wnt signaling, and increased susceptibility to azoxymethane-induced aberrant crypt foci. Lab Invest 2013; 93: 520-7.

[21] Hizaki K, Yamamoto H, Taniguchi H, Adachi Y, Nakazawa M, Tanuma T, Kato N, Sukawa Y, Sanchez JV, Suzuki H, Sasaki S, Imai K, Shinomura Y. Epigenetic inactivation of calcium-sensing receptor in colorectal carcinogenesis. Mod Pathol 2011; 24: 876-84.

[22] Kapur K, Johnson T, Beckmann ND, Sehmi J, Tanaka T, Kutalik Z, Styrkarsdottir U, Zhang W, Marek D, Gudbjartsson DF, Milaneschi Y, Holm H, Diiorio A, Waterworth D, Li Y, Singleton AB, Bjornsdottir US, Sigurdsson G, Hernandez DG, Desilva R, Elliott P, Eyjolfsson GI, Guralnik JM, Scott J, Thorsteinsdottir U, Bandinelli S, Chambers J, Stefansson K, Waeber G, Ferrucci L, Kooner JS, Mooser V, Vollenweider P, Beckmann JS, Bochud M, Bergmann S. Genome-wide meta-analysis for serum calcium identifies significantly associated SNPs near the calcium-sensing receptor (CASR) gene. PLoS Genet 2010; 6: e1001035.

[23] Bray I, Brennan P, Boffetta P. Projections of alcohol- and tobacco-related cancer mortality in Central Europe. Int J Cancer 2000; 87: 122-8.