Vitamin D, Calcium, Dairy Products and Genes Involved in Their Metabolic Pathways in Colorectal Cancer Risk and Survival

by

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Abstract

Background

Vitamin D, calcium, and dairy products are inversely associated with colorectal cancer incidence. These inverse associations may be mediated by the vitamin D binding protein, the vitamin D receptor (VDR), and the calcium sensing receptor (CASR). The purpose of the thesis was to investigate prediagnostic consumption of vitamin D, calcium, and dairy products and genetic variations in genes involved in their metabolic pathways (*GC*, *VDR*, and *CASR*) for their relevance to colorectal cancer risk and survival in the Newfoundland and Labrador population.

Methods

A population-based case-control study identified over 700 incident colorectal cancer cases (including 531 patients with follow-up data on mortality end-points) and 489 matched controls. Data on diet and lifestyle factors were gathered via epidemiological questionnaires. Germline DNA samples were genotyped with the Illumina Omni-Quad 1 Million chip in cases and the Affymetrix Axiom® myDesign™ Array in controls. Multivariable logistic regression examined the associations of these nutrients and genetic variants with colorectal cancer risk. Kaplan-Meier curves and Cox models assessed the relationship with overall survival (all-cause mortality, OS) and disease-free survival (DFS) among colorectal cancer patients.

Results

Results from this study are presented in three related, yet standalone manuscripts: 1) The *GC* rs2282679 polymorphism was not associated with colorectal cancer risk overall but was

correlated with the DFS of colorectal cancer patients (per C allele HR, 1.36; 95% CI, 1.05-1.77). The association of this SNP on DFS was limited to BRAF wild-type tumors. 2) *VDR* and *CASR* genes were associated with DFS and OS of colorectal cancer, respectively, at the gene level. Haplotype analysis within linkage blocks of CASR revealed the G-G-G-G-G-A-C haplotype (rs10222633-rs10934578-rs3804592-rs17250717-A986S-R990G-rs1802757) to be associated with a decreased OS of colon cancer (HR, 3.15; 95% CI, 1.66-5.96). 3) Prediagnostic calcium intake from foods, but not total calcium intake, was negatively associated with all-cause mortality (HR for Q2 vs. Q1, 0.44; 95% CI, 0.26-0.75). An inverse relationship was also seen in a dose-response fashion for prediagnostic cheese intake (P trend=0.029). No evidence for modification by factors known to be associated with colorectal cancer survival was observed.

Conclusions

Our results indicate that genetic variations in *GC*, *VDR* and *CASR* genes are associated with survival after colorectal cancer diagnosis. These findings also suggest a protective role of prediagnostic intakes of cheese and calcium from foods against colorectal cancer progression.

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List of Abbreviations

NL	Newfoundland and Labrador
DBP	Vitamin D-Binding Protein
GC	Vitamin D-Binding Protein Coding Gene
VDR	Vitamin D Receptor
CASR	Calcium Sensing Receptor
SNP	Single-Nucleotide Polymorphism
CRC	Colorectal Cancer
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
FAP	Familial Adenomatous Polyposis
APC	Adenomatous Polyposis Coli
CIN	Chromosomal Instability
MMR	Mismatch Repair
MSI	Microsatellite Instability
CIMP	CpG Island Methylator Phenotype
WCRF	World Cancer Research Fund
AICR	American Institute of Cancer Research
GWAS	Genome-Wide Association Study
TNM	Tumor-Node-Metastasis Stage System
AJCC	American Joint Committee on Cancer
OS	Overall Survival
DFS	Disease-Free Survival
GcMAF	Macrophage-Activating Factor
NFCCS	Newfoundland and Ontario Familial Colorectal Cancer Study
NFCCR	Newfoundland Familial Colorectal Cancer Registry
FFQ	Food Frequency Questionnaire
FHQ	Family History Questionnaire
PHQ	Personal History Questionnaire
HR	Hazard Ratio
CI	Confidence Interval
ICD	International Classification of Disease
OR	Odds Ratio
LD	Linkage Disequilibrium
RCT	Randomized Controlled Trial
25(OH)D	25-Hydroxyvitamin D

Chapter 1 – Introduction

1.1 Background

Colorectal cancer is the development of malignancies from the epithelial cells of the large intestine (colon, rectum and appendix). It is a heterogeneous disease in terms of its clinical, pathological and molecular characteristics [1]. Globally, colorectal cancer is the third most common malignancy, which accounts for nearly 10% of all cancers with about 1.4 million new cases in 2012, the latest year for which world cancer statistics are available [2]. In Canada, colorectal cancer is the second and the third most common cancer diagnosed among men and women, respectively [3]. The incidence and mortality rates of colorectal cancer vary geographically. The highest age-standardized incidence and mortality rates in the country have been observed in the province of Newfoundland and Labrador (NL). According to the estimated Canadian Cancer Statistics (2017) [3], in NL there will be 112.2/100,000 colorectal cancer incidents among males and 76.5/100,000 incidents among females in 2017. The mortality rates for colorectal cancer for males and females in the province will be 47.7 and 26.7 per 100,000 in 2017 [3].

The high rates of the disease in NL may be attributable in part to the high rate of familial colorectal cancer in this population [4]. However, all identified genetic variants explain only a minor proportion of colorectal cancer heritability. Both genetic and environmental factors may contribute considerably to colorectal cancer and to the regional differences in colorectal cancer incidence and mortality. Evidence from both observational and experimental studies support a role of diet and other environmental factors in colorectal cancer etiology. It is estimated that over 70% of colorectal cancer may be preventable through moderate modifications in diet and lifestyle habits [5].

High intakes of vitamin D, calcium, and dairy products are generally linked with a

reduced risk of colorectal cancer. The inverse associations between dairy products and colorectal cancer have mainly been attributed to their high content of calcium, which may induce apoptosis [6], inhibit heme-associated colon carcinogenesis [7], and reduce cell proliferation directly [8] or indirectly through binding proinflammatory secondary bile acids and fatty acids to render them inert [9, 10]. As adequate vitamin D level is crucial for proper calcium absorption, the anticancer effects of diary products and calcium are strongly dependent on vitamin D status.

Although the inverse associations between intakes of vitamin D, calcium and dairy products and colorectal cancer have been consistently reported, their impact on survival after colorectal cancer diagnosis is largely unknown. The few published studies on pre- or post-diagnostic consumption of dairy products in relation to colorectal cancer survival have yielded inconclusive results. In the Japan Collaborative Cohort Study [11], high pre-diagnostic yogurt intake was associated with reduced risk of rectal cancer mortality in males but not in females. The Cancer Prevention Study-II Nutrition Cohort Study [12] reported that only post-diagnosis intake of dairy products were relevant to survival in patients with colorectal cancer, whereas others [10, 12, 13] yield null associations. Findings for dietary vitamin D (or 25-hydroxyvitamin D), calcium and colorectal cancer survival are also conflicting [12, 14, 15].

It has been suggested that vitamin D mediates its action through binding to vitamin D-binding protein (DBP) and the vitamin D receptor (*VDR*). As the major carrier protein in systemic circulation, DBP reversibly binds and transports vitamin D metabolites to different target organs, including the colorectum, thereby influencing the bioavailability of active 25-hydroxyvitamin D (25(OH)D) [16, 17]. VDR, a member of the nuclear receptor superfamily, could bind to vitamin D as well, enabling the transactivation of target genes that promote cellular differentiation [18], induce apoptosis [19], and inhibit angiogenesis and proliferation [20]. Thus, both *GC (*the gene encoding DBP*)* and *VDR* have been implicated in colorectal

carcinogenesis. Another gene that influences vitamin D and calcium metabolism is the calcium sensing receptor (*CASR*), which is essential for calcium homeostasis and cellular growth kinetics [21, 22]. In the *CASR* promoter region, vitamin D response elements have been discovered, providing evidence at the molecular level for a potential interaction between vitamin D and calcium in the development of colorectal cancer [23, 24]. Recent studies have also demonstrated a critical role of *CASR* as a tumor suppressor in the large intestine [25]. Expression of this receptor has been shown to be reduced in colon cancer cells as compared to normal colonic epithelial cells [26].

Current molecular studies have identified numerous single-nucleotide polymorphisms (SNPs) in the *GC*, *VDR* and *CASR* genes, but only a handful that are considered potentially functional have been examined in relation to CRC risk, including rs2282679 in *GC*; FokI (rs10735810) [22, 27], BsmI (rs1544410) [22, 28], ApaI (rs7975232) [27, 29], and TaqI (rs731236) [29] in *VDR*; and A986S (rs1801725) [24, 30] and R990G (rs1042636) [24] in *CASR*.

Several studies have linked one or more of these variants to colorectal cancer and, particularly, the VDR BsmI bb (GG) [22, 28] and CASR A986S (TT) genotypes [31] were related to an increased risk of colorectal cancer. However, very few relevant studies on colorectal cancer survival have been published and none of these were from the Canadian population [15, 31-34]. Limited evidence shows no association of polymorphisms in *GC*, *VDR* and *CASR* with survival after colorectal cancer diagnosis, but can be criticized for limited power or incomplete coverage of the variation within the gene [15, 31]. In addition, little is known regarding how pre- or post-diagnostic dietary factors could interact with *GC*, *VDR* and *CASR* genotypes to influence colorectal cancer prognosis [15]. This is important especially because long-term eating habits prior to diagnosis may affect post-diagnosis diet, and because cancer patients may have a strong desire to make positive changes, and may

benefit from recommendations for a healthy diet and supplement use as a complement to their therapy [15, 35].

Together, it remains unclear whether the consumption of vitamin D, calcium, dairy products, and variants of metabolism-related genes will influence patients' survival after a diagnosis of colorectal cancer. Novel research is clearly needed to explore these associations.

1.2 Research purposes

Using data collected from the Newfoundland Familial Colorectal Cancer Registry (NFCCR), the thesis aimed to:

1) Investigate the associations of the *GC* rs2282679 variant with colorectal cancer risk and overall (all-cause death) and disease-free survival;
2) Explore if the relationship between the *GC* SNP rs2282679 and colorectal cancer is modified by tumor microsatellite instability (MSI), *BRAF* Val600Glu mutation status, and dietary intakes of vitamin D, calcium, milk, and total dairy products;
3) Examine the association of genetic variations in *VDR* and *CASR* genes with colorectal cancer all-cause and disease-free survival;
4) Assess potential interactions of *VDR* and *CASR* with pre-diagnostic dietary vitamin D and calcium intakes on survival among colorectal cancer patients.
5) Investigate whether pre-diagnostic consumption of vitamin D, calcium, and dairy products (total, milk, yogurt, and cheese) is associated with colorectal cancer overall and disease-free survival;
6) Explore if these associations vary by sex, physical activity, alcohol drinking and cigarette smoking status.

1.3 Hypotheses

We hypothesize that prediagnostic consumption of vitamin D, calcium, and dairy products and genes involved in their metabolic pathways are related to colorectal cancer risk and survival. Specifically,

1) the C allele of the *GC* SNP rs2282679 is associated with increased risk of colorectal cancer incidence and mortality;
2) the *VDR* and *CASR* genes are related to colorectal cancer survival at the gene level with effect modification by prediagnostic dietary vitamin D and calcium intakes;
3) higher vitamin D, calcium, and dairy intakes prediagnosis are associated with better overall and disease-free survival in colorectal cancer patients;

1.4 Organization of thesis

This thesis will be organized into four chapters. Chapter 1 provides an overall introduction to this study. Chapter 2 is a review of the epidemiological literature to summarize the epidemiology of colorectal cancer, molecular mechanisms and pathology of colorectal cancer as well as colorectal cancer risk and prognostic factors that are closely related to this dissertation. Chapter 3 presents three related, yet standalone subprojects in manuscript format; each manuscript includes its own *Introduction, Methods, Results, Discussion,* and *Conclusion* sections. Finally, the last chapter in this thesis (Chapter 4) presents a summary of the findings from the previous chapters, a general discussion, and recommendations for further research directions.

Chapter 2 – Literature Review

2.1 Epidemiology of colorectal cancer

2.1.1 Colorectal cancer incidence and trends

The global distribution of colorectal cancer varies widely, with over 60% of all cases occurring in developed countries with a Western culture, such as Australia, New Zealand, parts of Europe, and North America. Many less developed countries such as India, China, and most of Latin America have the lowest incidence of colorectal cancer [2]. Colorectal cancer incidence rates worldwide change over time, with a rapid increase in countries undergoing societal and economic transition, including Philippines, China, Colombia, Bulgaria, Brazil, Russia, and many others [36]. Rapid lifestyle changes due to progressive 'westernization' may be responsible for the geographic variation in colorectal cancer incidence as well as the substantial increasing trends of colorectal cancer incidence in countries in transition [36]. These lifestyle risk factors include smoking, high consumption of red/processed meats, and physical inactivity [37]. Stabilizing or decreasing trends of colorectal cancer incidence is observed in highly developed countries where incidence rates remain higher than for most other countries, including United States, Austria, New Zealand, Australia, and Japan. The reduced cancer incidence is most likely due to an increase in screening tests and polypectomy, as precancerous polyps can be detected and removed during colonoscopy [2].

In Canada, colorectal cancer accounts for 13% of all cancers [38]. The age-standardized incidence rate of the disease is highest in NL and Quebec and lowest in British Columbia [38]. According to the 2016 Canadian Cancer Statistics [38], colorectal cancer incidence rate declined for both sexes from the mid-1980s to the mid-1990s followed by a rapid rise till 2000. Over the last decade, national incidence rates have again decreased. As of 2016, 10

Canadian provinces have introduced organized colorectal cancer screening program [37, 39]. The implementation of screening programs may initially increase colorectal cancer incidence rate due to improved reporting but may reduce the incidence long term because of the removal of colonic polyps at a colonoscopy [36].

2.1.2 Colorectal cancer mortality and trends

Globally, approximately 700,000 deaths attributable to colorectal cancer were recorded in 2012, which is, nearly 8% of all cancer deaths [40]. On the list of lethal cancers, colorectal cancer was the fourth-leading cause of death among cancers that affect both sexes [2]. In recent years, declining mortality rates were seen in regions with high development index such as North America, Australia, New Zealand, and Western Europe while increases in mortality over the most recent 10 years were reported in some parts of Eastern Europe, Latin America, the Caribbean, and Asia at a crude rate of 5-15% per year, and the rates were quite similar for men and women [40]. The increases in colorectal cancer mortality in these countries may reflect the lack of basic heath infrastructure or limited access to early diagnosis and therapies [36].

In Canada, colorectal cancer is the second leading cause of cancer death in men and the third leading cause of cancer death in women and is responsible for 12% of all cancer deaths [38]. The age-standardized mortality rate was 28.4/100,000 among men and 19.2/100,000 among women in 2016, with the highest rate of colorectal cancer deaths in the country observed in Newfoundland and Labrador, 26.4/100,000 for women and 46.1/100,000 for men, nearly twice as high as the rates in Alberta (16.5 per 100,000 population for women) and British Columbia (26.4 per 100,000 population for men) [38]. Multiple lifestyle factors such as diet and physical activity, differential participation of screening as well as access to quality health care and diagnostic services may account for this interprovincial variation in

the rates of colorectal cancer mortality [38]. The current trends in colorectal cancer mortality rate from Canada is encouraging. Specifically, deaths from colorectal cancer have significantly decreased from 2003 to 2016 for both men (2.3% per year) and women (2.0% per year) [38]. A part of this decline may be driven by a decrease in the overall incidence rate reported over the past years as well as improved diagnosis and treatment of the disease.

2.2 Molecular mechanisms and pathology of colorectal cancer

Colorectal cancer usually presents in one of three major forms: hereditary non-polyposis, familial adenomatous polyposis, or sporadic. Hereditary and familial colorectal cancer has been classically associated with germline mutations. Hereditary non-polyposis comprises 10% of all colorectal cancer and presents as well-defined colorectal cancer predisposition syndromes, such as Lynch syndrome and familial polyposis coli, which involve highly penetrant genetic mutations in the DNA mismatch repair genes. Familial colorectal cancer accounts for approximately 25% of cases and does not follow a precise Mendelian inheritance pattern. Sporadic colorectal cancer comprises the majority with approximately 75% of the cases with no apparent hereditary predisposition.

Some well-described colorectal cancer predisposition syndromes in inherited forms of colorectal cancer are as follows:

1) Lynch syndrome (often referred to as Hereditary Non-polyposis Colorectal Cancer (HNPCC), which results from germline mutations in the mismatch repair genes, including MLH1, MSH2, MSH6 and PMS2. The increased risk is due to diminished self-repairing capabilities of DNA. More than 90% of colorectal cancers arising in HNPCC patients demonstrate the microsatellite instability (MSI) phenotype[41].
2) Familial adenomatous polyposis (FAP), which is caused by a germline mutation that inactivates the adenomatous polyposis coli (APC) tumor suppressor gene. Individuals

affected by FAP usually develop numerous adenomatous polyps in the colorectum during young adulthood. If left untreated, the hamartomatous polyps will inevitably transform into colorectal cancer.

3) Other forms of colorectal cancer syndromes include: MUTYH-Associated Polyposis, Peutz-Jeghers Syndrome, Juvenile Polyposis Syndrome, Bannayan-Ruvalcaba-Riley Syndrome, and Mixed Polyposis Syndrome [42].

Three distinct molecular pathways involved in sporadic colorectal cancer have been recognized. They are not mutually exclusive and some tumors may involve multiple driver pathways.

1) Chromosomal instability (CIN) pathway: CIN is a major cause of genomic instability accounting for approximately 65-70% of sporadic colorectal cancer [43]. It is characterized by gain or loss of whole or portions of chromosomes that harbor certain tumor suppressor genes or oncogenes integral for colorectal cancer carcinogenesis, e.g., adenomatous polyposis coli (*APC*) gene, *K-ras*, *p53* and *SMAD* genes [43, 44].
2) Mismatch repair (MMR) deficient, microsatellite instability pathway: Instability of microsatellites is recognized by frameshift mutations in the microsatellite repeats. It results from impaired DNA MMR function that fails to correct mismatch errors generated during DNA replication. Germline mutations in MMR genes may result in hereditary non-polyposis colorectal cancer, while MSI caused by somatic mutation or promoter region hypermethylation of MMR genes is found in 15% of sporadic colorectal cancer. MSI-High in sporadic colorectal cancer is usually associated with promoter hypermethylation of the MLH1 gene.
3) CpG island methylator phenotype (CIMP) pathway: In CIMP, transcriptional regulation of gene expression is influenced by aggregations of methylation-sensitive CpG dinucleotides (CpG islands) upstream of promoter regions. DNA aberrant methylation on

CpG dinucleotides results in transcriptional silencing of cancer-related genes, including tumor-suppressor genes, with effects similar to those of loss-of-function mutations in tumorigenesis [45]. Genes involved in colorectal cancer tumorigenesis that are found to be silenced by promoter hypermethylation include APC, MLH1, MCC, MGMT, etc. [43].

2.3 Colorectal cancer risk factors

2.3.1 Lifestyle-related factors

To date, a variety of lifestyle and environmental factors have been associated with increased risk of colorectal cancer, these including consumption of red/processed meat, cigarette smoking, alcohol drinking, and physical inactivity; however; the results have often been conflicting.

2.3.1.1 Diet

Diet has been implicated in colorectal cancer etiology not only because of its physical interaction with the colorectal mucosa but also because the relationship between the "Western dietary pattern" and a high incidence of colorectal cancer [46]. Although numerous dietary risk factors have been reported, the findings have not yet been convergent. For example, a diet rich in fruits and vegetables has been associated with a reduced risk of colorectal cancer [48]; however, a pooled analysis of nearly 6,000 colon cancer patients among 756,217 subjects from 14 cohorts found non-significant association for individuals who consumed the most amount of fruits and vegetables [49]. The consumption of dietary vitamin D or serum vitamin D has also been linked to colorectal cancer risk [50], but neither a published meta-analysis of 2813 colorectal cancer patients from 10 cohorts [51] nor the World Cancer

Research Fund/American Institute of Cancer Research (WCRF/AICR) Continuous Update Project (CUP) updated meta-analysis for dietary vitamin D showed statistically significant associations with colorectal cancer risk [37]. In addition, dairy products have been hypothesized to be inversely associated with colorectal cancer risk due to their high content of calcium [37]. The CUP meta-analysis [52] reported a 17% decreased risk per 400 g/day of total dairy products and a 9% decreased risk per 200 g/day of milk intake, but indicated that there was limited evidence for individual products. To date, only intakes of alcoholic drinks and red and processed meat are regarded as convincing dietary risk factors for colorectal cancer based on the 2011 expert report from CUP [37].

2.3.1.2 Smoking

Smoking is clearly associated with malignancies in lung and larynx [53]. The IARC 2009 monograph on smoking and cancer added colorectal cancer as causally associated with smoking [54]. A large prospective cohort study from the United States [55], which followed 184,187 people from 1992 to 2005, found that current smokers (HR, 1.27; 95% CI, 1.06-1.52) and former smokers (HR, 1.23; 95% CI, 1.11-1.36) were significantly more likely to develop colorectal cancer when compared with lifelong nonsmokers. Recent data suggest that the association of smoking and colorectal cancer incidence may differ by tumor molecular phenotype, such as tumors that harbor *BRAF* V600E mutation, microsatellite instability (MSI), or the CpG island methylator phenotype [56, 57] although this evidence base is still emerging.

The mechanisms whereby smoking increases the risk of colorectal cancer have not been fully delineated. Carcinogens in cigarettes, such as polycyclic aromatic hydrocarbons and aromatic amines [58-60] may reach the bowel mucosa through direct ingestion [61] and through the circulatory system [62] thus exerting growth promoting effects

on cancer cells in the colorectum and increasing colorectal cancer incidence. More recently, smoking has been associated with elevated expression of DNA methyltransferase, suggesting that it may promote tumor development through epigenetic mechanisms [63].

2.3.1.3 Physical activity

Physical inactivity is a well-recognized risk factor for colorectal cancer independent of other potential confounders. Abundant evidence from case-control and cohort studies showed a reduced risk of colorectal cancer with increased levels of physical activity. The results of a published meta-analysis on high versus low levels of leisure time physical activity in relation to colorectal cancer suggested a reduced risk by 20% in men and by 14% in women for colon cancer [64]. The 2011 WCRF/AICR expert panel concluded that physical inactivity as a cause of colon cancer was convincing [37].

2.3.2 Genetic factors

A family history or adenomatous polyps is a strong risk factor of colorectal cancer. Inherited forms of colorectal cancer, such as familial adenomatous polyposis and Lynch syndrome, involve rare germline mutations in highly penetrant, autosomal dominant genes, especially in the APC gene and DNA mismatch repair gene. However, these high-penetrance mutations only account for less than 5% of the genetic risk [65]. In sporadic forms of colorectal cancer, recent genome-wide association studies (GWAS) have identified over 20 polymorphic genetic loci that are associated with susceptibility to colorectal cancer [66]. However, all the identified variants (high-penetrance variants plus GWAS-identified variants) explain only a small fraction of colorectal cancer heritability. To date, a large number of colorectal cancer susceptibility genes have been identified on chromosome segments 8q23.3,

8q24, 9p24, 10p14, 11q23, 15q13, and 18q21 *et al* [63, 67, 68].

2.3.3 Genetic polymorphisms investigated in this thesis

Evidence from both epidemiological [69, 70] and experimental studies [71, 72] supports a reduced risk of colorectal cancer (CRC) by higher intake or blood levels of vitamin D. The anti-carcinogenic effects of vitamin D might vary by genes involved in its metabolism-related pathways, including the vitamin D-binding protein gene (GC), vitamin D receptor (VDR) and calcium Sensing Receptor (CASR).

2.3.3.1 Vitamin D binding protein gene polymorphisms and colorectal cancer risk

As the major carrier protein in systemic circulation, vitamin D biding protein reversibly binds and transports vitamin D metabolites to different target organs, including the colorectum, thereby influencing the bioavailability of active 25-hydroxyvitamin D (25(OH)D) [16, 17] . Additionally, vitamin D biding protein is the precursor molecule of a potent macrophage-activating factor (GcMAF) [73], which is highly tumoricidal against various malignancies through its ability to inhibit endothelial angiogenesis [74, 75] and stimulate the inflammation-primed phagocytic activity of tumoricidal macrophages [76]. Therefore, vitamin D biding protein would be hypothesized to play an important role in CRC initiation and progression, either alone or in combination with vitamin D [77].

The gene encoding vitamin D binding protein, GC gene, is highly polymorphic. The single nucleotide polymorphism (SNP) rs2282679 A>C is one of the most commonly studied variants in this gene, which has been shown to be robustly correlated with serum levels of 25(OH)D in recent genome-wide association studies (GWAS) (P=2.0×10^{-30}) [78, 79]; more specifically, per copy of the risk C allele was associated with an approximately 50% elevated

risk for hypovitaminosis among Caucasians [79]. Prior studies on GC variants have been performed on melanoma [80], prostate [81] and breast cancers [82, 83]; however, we found only two studies that evaluated the specific association of the GC rs2282679 polymorphism with CRC risk [84, 85]. A multicenter case-control study of 10,061 CRC cases and 12,768 controls of European ancestry found no evidence for associations between *GC* rs2282679 and the risk of CRC overall or for colon or rectal tumor separately [85]. In a Mendelian Randomization study in Scotland, Theodoratou *et al.* [84] reported a similarly nonsignificant association of rs2282679 wild-type A allele with CRC risk (Per A allele: OR, 0.97; 95% CI, 0.90-1.06). No prior studies were found that specifically examined the interaction between rs2282679 polymorphism and vitamin D on CRC; yet, two other *GC* SNPs, rs17467825 and rs7041, which are in strong linkage disequilibrium with *GC* rs2282679 (γ^2=1.0 and 0.6 respectively) [79], have been associated with a slightly greater risk of CRC among individuals who consumed total vitamin D above the median in a multicenter case–unaffected sibling control study, though the interactions were not significant [86].

2.3.3.2 Vitamin D receptor gene polymorphisms and colorectal cancer risk

VDR, a member of the nuclear receptor superfamily, could bind to vitamin D, enabling the transactivation of target genes that promote cellular differentiation [18], induce apoptosis [19], and inhibit angiogenesis and proliferation [20]. Thus, *VDR* has been implicated in colorectal carcinogenesis.

The *VDR* gene is located at chromosome 12 (12q13.1). It is highly polymorphic with over 100 identified SNPs. Some functional polymorphisms have been evaluated in previous association studies on colorectal cancer, these including FokI (rs2228570; exon 2), BsmI (rs1544410; intron 8), TaqI (rs731236; exon 9), ApaI (rs7975232; intron 8), as well as the promoter Cdx2 (rs11568820; exon 1e). The FokI translational start codon polymorphism alters the VDR structurally, with the F-variant VDR being three amino acids shorter and

functionally more effective than the protein produced from the f allele [87, 88]. BsmI, ApaI and TaqI polymorphisms have been reported to influence *VDR* expression and thus serum levels of $1,25(OH)_2D_3$, but they are speculated to affect *VDR* function through linkage disequilibrium with other mutations in the 3-UTR region that alter mRNA transcriptional activity and stability [89, 90]. Although not a universal finding, the B allele of the *VDR* BsmI polymorphism has been found to be linked with a reduced risk of colorectal cancer [22, 28]. Carriage of other polymorphic alleles in the *VDR* gene revealed either null [91, 92] or conflicting associations [88, 93, 94]. In a systematic meta-analysis of 23 population-based studies on *VDR* gene polymorphisms and colorectal cancer risk, only the *VDR* BsmI polymorphism was correlated with an elevated risk of colon cancer (BB vs bb: odds ratio, 0.87; 95% CI, 0.80-0.94; $P = 3 \times 10^{-4}$) [28].

2.3.3.3 Calcium sensing receptor gene polymorphisms and colorectal cancer risk

The *CASR* is crucial for the maintenance of extracellular calcium homeostasis by affecting parathyroid hormone secretion and calcium reabsorption [21, 22]. It may also influence vitamin D metabolism. Indeed, *CASR* polymorphisms have been related to the risk of colorectal adenomas [22, 24, 30, 95] and advanced stage of rectal cancer in some studies [96]. In a comprehensive analysis of various *CASR* polymorphisms and colon cancer risk, Dong et al. [24] reported that several SNPs in *CASR* (rs10934578, rs12485716, rs2270916 and rs4678174) were significantly related to proximal colon cancer risk, whereas these SNPs were not associated with colorectal cancer risk in others [97]. In the work by Kim et al [90], these SNPs were significant only under low calcium intake. Similarly, prior findings for polymorphisms of other two high-interest SNPs in *CASR*, rs1801725 and rs1042636, are either null [22, 24, 30] or conflicting [31, 96, 98]. A recent large population meta-analyses

on *CASR* and colorectal cancer risk found that individuals with GG genotype of rs1042636 had a risk reduction of 25% for distal colon cancer and 32% for proximal colon cancer compared to those with the wild type genotype [99].

2.4 Determinants of survival of patients with colorectal cancer

2.4.1 Clinicopathologic Factors

The prognosis of colorectal cancer varies widely across patients, and depends upon various factors, including tumor stage, grade, and molecular phenotype.

2.4.1.1 Tumor stage

Tumor stage is the most crucial prognostic factor for colorectal cancer. Currently, the tumor-node-metastasis (TNM) stage system published by American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) remains the gold standard of prognostication in colorectal cancer patients, which is routinely used for long-term survival and treatment stratification [100]. T: local extent of the primary tumor; N: the status of the lymph nodes near to the colorectum; and M: distant metastases [100]. In general, the lower the stage at diagnosis, the better the prognosis. The 5-year survival rates were estimated at 90%, 71%, and 14% for those with localized, regional, and distant metastatic colorectal cancer, respectively [101].

2.4.1.2 Tumor differentiation grade

Based on the appearance differentiation of cancer cells, tumor grade is described by four degrees of severity: G1 for a well-differentiated tumor (low grade) to G4 for an

undifferentiated tumor (high grade). High-grade cancers generally have a higher metastatic potential and thus a poorer prognosis than low-grade cancers.

2.4.1.3 MSI status

MSI, a hallmark of Lynch syndrome, is driven by dysfunction of DNA mismatch repair. Tumors that have cells with MSI-high (MSI-H) status have a significantly longer survival when compared with tumors without MSI (microsatellite stable) [102]. The 2010 AJCC cancer staging manual recommended MSI testing for cancer patients for prognostic purposes [100].

2.4.1.4 BRAF V600E mutation status

The BRAF V600E missense mutation makes it oncogenic. Patients with BRAF mutant colorectal cancer have worse overall survival due to low response rates to conventional therapies. The 5-year overall survival rates were 47.5% for BRAF mutant colorectal cancer compared to 69.7% for wild-type tumors [103].

2.4.2 Lifestyle related factors

2.4.2.1 Diet

Many studies have investigated the role of dietary factors in colorectal cancer survival, but most have produced inconclusive results. Our previous research found that high adherence to the processed meat pattern, characterized by high consumption of processed red meat and cured fish prior to diagnosis was correlated with worse overall survival among colon cancer patients in Newfoundland [104]; a similar conclusion was drawn from a

prospective cohort study in the United States [105]. Another U.S. study, the California Teachers Study, however, did not confirm an association between meat intake and colorectal cancer-specific mortality [106]. As for dairy products, yogurt intake was negatively associated with rectal cancer mortality in men in a large Japanese cohort study of 45,181 men and 62,643 women [11]. In the Cancer Prevention Study-II Nutrition Cohort Study, a significant inverse association was observed for post-diagnosis consumption of calcium and milk in relation to all-cause death amongst colorectal cancer patients [12]. However, the results of three other studies [10, 12, 13], conducted in the United States, Japan, and western Europe, showed however no association between pre-diagnostic intake of diary and calcium and colorectal cancer survival. A high fruit and vegetable consumption was not associated with colorectal cancer mortality in two prospective cohort studies [107, 108] but was counterintuitively related to an increased colon cancer mortality rate among women in another prospective cohort study conducted by Kojima et al. [11].

2.4.2.2 Smoking

Findings on the association between cigarette smoking and survival of colorectal cancer have been mixed. Some studies [57, 109, 110] showed that cigarette use may have a negative influence on colorectal cancer survival while others [111-113] reported that there is no remarkable difference in prognosis of colorectal cancer between smokers and never smokers. A published meta-analysis of 62,278 colorectal cancer patients from 16 studies demonstrated that current smokers, assessed both before and after diagnosis, had a higher risk of all-cause mortality (HR, 1.26; 95% CI, 1.15-1.37) compared with never smokers [114].

2.4.2.3 Physical activity

Reports on physical activity and the risk of colorectal cancer recurrence and mortality have been fairly consistent. Increasing level of physical activity both before and after diagnosis trended to reduce the risk of all-cause death or cancer-specific death among colorectal cancer patients [115-117]. Haydon *et al* [118] analyzed 526 colorectal cancer patients who participated in the Melbourne Collaborative Cohort Study and found a favorable association between pre-diagnosis physical activity level and cancer-specific survival. A recent meta-analysis of patients with any post-diagnostic physical activity versus those with no physical activity demonstrated a 26% risk reduction in colorectal cancer-specific mortality [119]. While efforts have been made in these studies to account for reverse causality, one cannot rule out the possibility that the worse prognosis in patients with less physical activity is because less physical activity is well related with advanced disease stage or progression [120].

2.4.3 Genetic polymorphisms investigated in this thesis

2.4.3.1 Vitamin D binding protein gene polymorphisms and colorectal cancer survival

To date, only one study has explored the association between the functional *GC* rs2282679 variant and CRC prognosis [121]. In this retrospective series of 264 stages II and III colon cancer patients with surgery alone, the GG genotype of the *GC* SNP rs2282679, which reflected lower 25(OH)D levels, was significantly associated with a reduced time to colon cancer recurrence (HR, 3.30; 95% CI, 1.09–9.97, *P*=0.034) [121].

2.4.3.2 Vitamin D receptor gene polymorphisms and colorectal cancer survival

Several functional polymorphisms in *VDR* (e.g., BsmI) and *CASR* (e.g., rs1801725 and rs4678174) have been related to the risk of colorectal cancer, but their potential relevance to

colorectal cancer survival has been inadequately examined. In addition, previous association studies on *VDR* polymorphisms and cancer survival have primarily concentrated on cancers of the breast [122-124] and prostate [125, 126], but data for colorectal cancer prognosis are comparatively sparse. Only minimal studies have been published on colorectal cancer outcome and all revealed no significant relationship between any SNP in *VDR* and the risk of colorectal cancer recurrence or mortality [15, 32-34, 127]. A recent meta-analysis of VDR genetic variants and cancer outcome supported an association of rs1544410 (Bsml) TT/TC genotypes with a worse OS compared to the CC genotype for all cancers combined (HR, 1.40; 95% CI, 1.05–1.75), but the association with colorectal cancer outcome was not significant (HR, 1.13; 95% CI, 0.92-1.34).

2.4.3.3 Calcium sensing receptor gene polymorphisms and colorectal cancer survival

Although there are several published papers investigating polymorphisms in *CASR* and colorectal cancer risk, only two studies [15, 31] have evaluated the association between *CASR* and colorectal cancer survival. Bácsi and colleagues [31] identified 538 Hungarian subjects (278 colorectal cancer patients and 260 healthy controls) between 2005 and 2007 and followed them up for colorectal cancer recurrence and mortality for a median observation period of 17 months. Based on their analysis, the CASR rs1801725 polymorphism was not associated with colorectal cancer DFS or OS. Fedirko *et al* [15] studied 1,202 colorectal cancer patients in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort and found CASR SNP rs1801725 not associated with survival in colorectal cancer either. Nevertheless, only one SNP (rs1801725) was included in both studies.

2.5 Methods designed to assess dietary intakes

Accurate assessment of dietary intake is a key element of nutrition and health research.

The three most often applied methods of dietary assessment in epidemiological research are the following:

2.5.1 Diet record

A diet record is a self-reported account of all foods consumed by an individual over one or several consecutive days. It is an open-ended survey and therefore there is no limit on the number of foods that the respondents can reported. Participants are required to record details such as brand name, cooking method, and where consumed for all foods, beverages and possibly, dietary supplements as they are consumed during the reporting period. The amount of each food item consumed is either estimated using photographs or food models, or measured using a scale or other standard measuring containers. It typically takes at least 15 minutes to complete a single day food record and the quality of the report can be increased with the help from a trained research staff. This method has many strengths. First, it uses open-ended questions so that abundant and detailed intake data can be collected and analyzed for various purposes. Second, no interviewer is required and recall bias is unlikely. Limitations to this method include a relatively large burden that is imposed on respondents and possible dietary changes if measures are repeated among respondents. In addition, this method is expensive, time-consuming, and focused on short-term dietary exposure only; however, long-term intake is of particular interest when examining chronic diseases.

2.5.2 24-hour recall

This method requires a well-trained interviewer to ask the respondents to provide a comprehensive and detailed report of all foods and drinks consumed in the past 24 hours prior to the interview. It can be conducted via telephone or face-to-face interview. Details such as food preparation methods and portion size of each food are also captured. This structured

interview usually takes 20-60 minutes to complete. Advantages of the 24-hour recall are the detailed dietary intake data and minimal respondent burden (literacy not required) during data collection. However, recall bias is inevitable as all information relies on participants' past memories.

2.5.3 Food frequency questionnaire (FFQ)

An FFQ consists of a finite list of foods and beverages and a selection of options regarding the frequency of consumption of each food over a specific period (typically 1 year). The number of foods queried may range from 80 to 150 depending on the foods of interest. Many FFQs ask questions on common preparation methods and usual portion size in addition to frequency of consumption of each food item. Nutrient intakes can be calculated by multiplying the frequency of each food by the nutrient content in a serving of that food via computerized software programs. This questionnaire normally requires 20-40 minutes to complete and can be self-administered or gathered via interview. This tool enables researchers to estimate the long-term dietary intakes in a relatively easy, inexpensive, and time-efficient manner. Therefore, FFQs are the most commonly used instrument to assess dietary intake in large-scale epidemiologic studies. Limitations to this tool include its relatively low accuracy due to recall bias and estimation of portion sizes and the requirement for validation of developed questionnaires.

In summary, although vitamin D, calcium, and dairy products are inversely related to colorectal cancer (CRC) incidence in the literature, little is known of their influence on CRC survival. Additionally, the inverse association between these foods/nutrients and CRC incidence may be mediated by *GC*, *VDR*, and *CASR*, but the influence of variants in these

genes on patients' survival after a diagnosis of CRC has been inadequately examined. These associations, if any, warrant further investigation.

Chapter 3 – Co-authorship Statement

This project was part of a multidisciplinary colorectal cancer team project and would not have been possible without the substantial input from many people. Specifically, the CIHR Colorectal Cancer Research Team members contributed to this study by recruiting eligible study participants; collecting survey questionnaires; and confirming medical records. Germline DNA samples were genotyped by professionals in the Centrillion Biosciences (USA) and the USC Norris Comprehensive Cancer Center (Los Angeles, USA). Dr. Sevtap Savas obtained information on survival outcome of colorectal cancer patients. The role of this author included the conceptualization and design of the three specific research subprojects presented in this dissertation, secondary analysis and interpretation of the data, and drafting of the thesis.

Specifically, for paper 1, Yun Zhu designed the subproject proposal, led the data analysis and findings interpretation, and wrote the first version of the manuscript. Jennifer R. Woodrow, Sevtap Savas, Yuming Li, Peter T. Campbell, Xin Zhou, and Ning Yang consequently revised the manuscript. Peizhong Peter Wang, Guangju Zhai, Bharati Bapat devised and commented on the overall research design and the results interpretation. Elizabeth Dicks, John R. Mclaughlin, Patrick S. Parfrey critically commented, and oversaw the scientific implementation of this study.

For paper 2, Yun Zhu wrote the subproject proposal, analyzed the data and drafted the manuscript. Peizhong Peter Wang, Guangju Zhai, Patrick S. Parfrey, and John R. Mclaughlin conceived and designed this study. Jennifer R. Woodrow, Peter T. Campbell, Ishor Sharma, Yuming Li, Xin Zhou, Ning Yang, and Bharati Bapat revised the paper. Elizabeth Dicks, Patrick S. Parfrey, Guangju Zhai, Sevtap Savas, and John R. Mclaughlin contributed to sample and data collection.

For paper 3, Yun Zhu led the data analysis and findings interpretation, and wrote the first

version of the manuscript. Jennifer R. Woodrow, Sevtap Savas, Yuming Li, Peter T. Campbell, Xin Zhou, and Ning Yang consequently revised the manuscript. Peizhong Peter Wang, Guangju Zhai, Bharati Bapat conceptualized, devised, and commented on the overall research design and the results interpretation. Elizabeth Dicks, John R. Mclaughlin, Patrick S. Parfrey critically commented, and oversaw the scientific implementation of this study.

Chapter 4 – Research Papers

4.1 Paper 1. Association of rs2282679 A>C Polymorphism in Vitamin D Binding Protein Gene with Colorectal Cancer Risk and Survival: Effect Modification by Dietary Vitamin D Intake

This paper [128] has been submitted to BMC Cancer for the consideration of publication. Yun Zhu is the first author of the paper.

Authors

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Abstract

Background

The rs2282679 A>C polymorphism in the vitamin D binding protein gene is associated with lower circulating levels of vitamin D. We investigated associations of this SNP with colorectal cancer risk and survival and whether the associations vary by dietary vitamin D intake and tumor molecular phenotype.

Methods

A population-based case-control study identified 637 incident colorectal cancer cases (including 489 participants with follow-up data on mortality end-points) and 489 matched controls. Germline DNA samples were genotyped with the Illumina Omni-Quad 1 Million chip in cases and the Affymetrix Axiom® myDesign™ Array in controls. Logistic regression

examined the association between the rs2282679 polymorphism and colorectal cancer risk with inclusion of potential confounders. Kaplan-Meier curves and multivariable Cox models assessed the polymorphism relative to overall survival (OS) and disease-free survival (DFS).

Results

The rs2282679 polymorphism was not associated with overall colorectal cancer risk; there was evidence, however, of effect modification by total vitamin D intake ($P_{interaction}$=0.019). Survival analyses showed that the C allele was correlated with poor DFS (per-allele HR, 1.36; 95%CI, 1.05-1.77). The association of rs2282679 on DFS was limited to BRAF wild-type tumors (HR, 1.58; 95%CI, 1.12-2.23). For OS, the C allele was associated with higher all-cause mortality among patients with higher levels of dietary vitamin D (HR, 2.11; 95%CI, 1.29-3.74), calcium (HR, 1.93; 95%CI, 1.08-3.46), milk (HR, 2.36; 95%CI, 1.26-4.44), and total dairy product intakes (HR, 2.03; 95%CI, 1.11-3.72).

Conclusion

The rs2282679 SNP was not associated with overall colorectal cancer risk, but may be associated with survival after cancer diagnosis. The association of this SNP on survival among colorectal cancer patients may differ according to dietary vitamin D and calcium intakes and according to tumor *BRAF* mutation status.

Key Words: Vitamin D binding protein, Genetic polymorphism, Colorectal cancer, Dietary vitamin D, Microsatellite instability, *BRAF*

Background

Colorectal cancer is a complex, multifactorial disease resulting from multiple genetic

and environmental factors [129]. Vitamin D from diet, supplements, and cutaneous synthesis from sunlight, is associated with lower risks of colorectal cancer incidence [130-133] and mortality [14, 127, 134-137]. The anti-carcinogenic effects of vitamin D might vary by the vitamin D-binding protein (DBP) [16]. As the major carrier protein in systemic circulation, DBP reversibly binds and transports vitamin D metabolites to different target organs, including the colorectum, thereby influencing the bioavailability of active 25-hydroxyvitamin D (25(OH)D) [16, 17] . Additionally, DBP is the precursor molecule of a potent macrophage-activating factor (GcMAF) [73], which is highly tumoricidal against various malignancies through its ability to inhibit endothelial angiogenesis [74, 75] and stimulate the inflammation-primed phagocytic activity of tumoricidal macrophages [76]. Therefore, DBP would be hypothesized to play an important role in colorectal cancer initiation and progression, either alone or in combination with vitamin D [77].

The gene encoding DBP, *GC* gene, is highly polymorphic. The single nucleotide polymorphism (SNP) rs2282679 A>C is one of the most commonly studied variants in this gene, which has been shown to be robustly correlated with serum levels of 25(OH)D in recent genome-wide association studies (GWAS) [78, 79]; specifically, the C allele of this SNP is associated with lower levels of 25(OH)D [79]. Prior studies on *GC* variants have been performed on melanoma [80], prostate [81] and breast cancers [82, 83]; however, we found only two studies that evaluated the specific association of the *GC* rs2282679 polymorphism with colorectal cancer risk, with both studies reporting no evidence of association [84, 85]. Another study reported that the *GC* rs2282679 SNP was associated with prognosis for patients diagnosed with stages II and III colon cancer [121].

Microsatellite instability (MSI) and BRAF V600E hotspot mutation are important molecular classifiers in colorectal cancer, which define distinct colorectal cancer subgroups arising from different oncogenic pathways. Microsatellite unstable (MSI-H) tumors are generally associated with superior prognosis [138] whereas BRAF-mutated cancers are

related to inferior survival rate [139, 140]. Therefore, it is plausible that factors associated with colorectal cancer risk and survival differ across tumor molecular subtypes defined by MSI and BRAF mutation status. Prior studies have reported that the associations between genetic variations in vitamin D and calcium metabolic pathway and colorectal cancer vary according to MSI status, with significant associations for microsatellite unstable colorectal cancer only [86, 97]. However, no study has yet evaluated the relationship between the GC rs2282679 polymorphism and colorectal cancer by these tumor molecular alternations.

In this analysis, we assessed the associations of the *GC* rs2282679 variant with colorectal cancer risk and survival. We additionally evaluated the potential influence of this SNP according to dietary vitamin D, calcium, milk, and total dairy product intake and whether associations varied by tumor microsatellite instability (MSI) or *BRAF* Val600Glu mutation status.

Materials and methods

Study participants

Study data and biologic specimens were drawn from the Newfoundland and Ontario Colorectal Cancer Study, a large population-based case-control study designed to identify genetic and environmental risk and prognostic factors for colorectal cancer [4, 141]. For the current study, only the participants from the NL portion were analyzed. Colorectal cancer patients in this study were identified through the Newfoundland Familial Colorectal Cancer Registry (NFCCR), a provincial-wide electronic reporting system of both familial and sporadic forms of colorectal cancer. Patients were eligible for inclusion if they were:

1) Newly diagnosed with colorectal cancer with pathological confirmation (International Classification of Diseases (ICD)-9 codes: 153.0-153.9, 154.1-154.3 and 154.8; or ICD-10 codes: C18.0-C18.9, C19.9, and C20.9);

2) Diagnosed between January 1999 and December 2003;

3) Aged 20 to 75 years at the time of diagnosis;

4) Residents of NL who had lived in NL for at least two years at the time of diagnosis.

Controls were selected by random digit dialing and matched on age (±5 years) and sex with cases at baseline [142]. Briefly, a list of 192,000 possible residential telephone numbers were generated and arranged in random order. Initial contacts were made by well-trained interviewers through dialing those numbers sequentially until a desired number of controls was finally reached [142].

Eligible patients were inquired regarding their willingness to participate. If patients died before enrollment, a close relative who had lived with the deceased patient was invited to participate. All consenting participants were sent self-administered risk factor questionnaires (a Food Frequency Questionnaire (FFQ, Appendix 2), a Family History Questionnaire (FHQ), and a Personal History Questionnaire (PHQ, Appendix 1)), and were asked to provide blood samples and for permission to access their tumor specimens and medical records (for cases). A total of 656 cases and 696 controls completed detailed questionnaires and donated a blood sample. Of the 656 cases, 490 were followed for mortality and recurrence from the date of cancer diagnosis to April 2010. Vital status (i.e., death, recurrence, and metastasis) was ascertained through periodic follow-up questionnaires (e.g., FHQ), local newspapers, death certificates, pathology records, autopsy records, physicians' notes, surgical reports, and from records at the Dr. H. Bliss Murphy Cancer Care Foundation. The main study survival outcomes were death from all-causes (i.e., overall survival (OS)) and disease-free survival (DFS), defined as death, recurrence, or metastasis (whichever came first). Follow-up time began at colorectal cancer diagnosis, and individuals who were lost to follow-up or did not die, had a recurrence, or had a metastasis were censored at the time of their last contact.

Exclusions were made if patients had equivocal genotype or clinical outcome, or failed to provide sufficient information on other critical predictors. Thus, 637 cases and 489

controls for risk analyses and 489 patients for survival analysis were included in the final study.

Diet assessment and baseline information collection

Information on diet and other lifestyle, medical and demographic characteristics was gathered with self-administered questionnaires. The dietary questionnaire was an adaptation of the Hawaii semi-quantitative FFQ to assess the dietary habits of participants from a year prior to disease diagnosis (cases) or interview (controls), which has been validated in a prior study [143]. The FFQ contained questions regarding the brand and frequency of consumption of 170 foods and beverages plus multivitamin and individual vitamin supplements [144]. The nutrient intakes from diet were calculated by multiplying the frequency of consumption of each food item by the nutrient content per average unit [132]. Total daily nutrient intakes were computed by incorporating supplement use in addition to intakes from diet. The PHQ collected information from each participant on socio-demographics (e.g., age, gender, ethnicity, and education attainment), medical conditions, bowel screening history, aspirin use, physical activity, and recent or prior alcohol and tobacco use. The FHQ gathered baseline and follow-up family history data from the participants.

Genotyping

Genotyping for the *GC* rs2282679 allele was conducted using the Illumina Human Omni-Quad Beadchip that contains about 1.1 million SNPs at Centrillion Biosciences (USA). Control individuals were genotyped in the Laboratory of Dr. Stephen Gruber (Director, USC Norris Comprehensive Cancer Center, Los Angeles) using the Affymetrix Axiom® myDesign™ GW Array Plate, which contains 1.3 million probes. To monitor quality and consistency between the two platforms, DNA samples from 200 colorectal cancer patients were typed on both platforms. As the DNA from cases and controls were genotyped on

different platforms, a genotype imputation strategy was implemented to integrate the two datasets using IMPUTE2 [145] with multi-population reference panels from 1000 Genomes (Phase 1). The imputation approach was validated based on the overlapping SNPs between the two platforms and the genotypes from 200 colorectal cancer samples that were typed on both platforms. SNPs with genotype concordance <97% across the two platforms were removed from further analysis. For the purpose of the current study, directly measured data from both arrays on rs2282679 were retrieved from the genome-wide SNP genotype database of the NFCCR.

Our protocol for MSI and *BRAF* V600E mutation analyses in tumor DNA has been described previously [146-148]. MSI status was evaluated with 5 to 10 microsatellite markers. Tumors were deemed MSI-high if ≥30% of the repeats were unstable and MS-stable/MSI-low if <30% of the repeats were unstable. The c.1799 T>A variant (Val600Glu mutation) region of the *BRAF* gene was amplified by *BRAF* allele-specific polymerase chain reaction (PCR) technique.

Statistical analysis

Group comparisons between cases and controls were performed with two-sample t test for continuous variables and Chi-square (χ^2) test for categorical variables. The Hardy-Weinberg Equilibrium for rs2282679 genotype was evaluated using χ^2 goodness-of-fit test. Unconditional logistic regression was used to estimate the association between the rs2282679 *GC* SNP and risk for colorectal cancer as odds ratio (OR) with 95% confidence interval (CI). Initially, logistic regression models only included genotype, age, and sex. More complex models also included family history of colorectal cancer, screening procedure, multivitamin use, folic acid intake, smoking history, and education attainment. These covariates were retained in the final models because they entered the model at P<0.1, altered the parameter estimates by >10%, and/or improved the model fit.

In survival analysis, survival curves were constructed with the Kaplan–Meier method. The log-rank test and the Cox regression models were used for univariable and multivariable survival analyses to assess the association between the SNP of interest and OS and DFS of colorectal cancer. The assumption of proportional hazards for each Cox model was verified by testing the statistical significance of time-dependent covariates in the model. The hazard rate ratio (HR) and 95% CI were calculated from the Cox models. As the true inheritance mode of the rs2282679 polymorphism has not yet been established, the SNP was analyzed for risk and survival under dominant, additive, and recessive models. Given the limited sample size in some subgroups, we combined those who carried at least one of the minor C alleles in stratified analysis by selected tumor molecular phenotype. Linear trend for gene dose effect was tested by modeling ordinal variables of allele dose (0, 1, and 2) as a continuous variable. Gene-environment interactions were tested by introducing a multiplicative interaction term into the model and assessing its significance with the Wald method. Two-sided exact $P<0.05$ was considered statistically significant. We did not adjust for multiple comparisons because the sub-tests in the current study are not independent of each other since the stratified variables are highly correlated (i.e., vitamin D, calcium, and dairy products). Although adjustment for multiple testing reduces type I error, it increases type II error and errors of interpretation [149]. All data management and analyses were performed using SAS software, Version 9.3.

Results

The rs2282679 polymorphism was in Hardy-Weinberg equilibrium ($P>0.05$). Among controls, the genotype frequency was 7.4% homozygous (CC), 42.5% heterozygous (AC) and 50.1% wild-type homozygous (AA); the observed minor allele frequencies in the controls were comparable to that previously reported [84]. During a maximum follow-up of 10.9 years

(mean: 6.3 years), 150 deaths occurred among the 489 patients included in the survival analysis. The cause of death defined by ICD codes was obtained for 105 of 150 deceased patients; thereof the majority (90.5%) was due to colorectal cancer.

Baseline characteristics of cases and controls

Cases and controls had similar sex and ethnicity distributions, and the majority reported their race as White (Table 4.1.1). Relative to cases, controls were slightly younger, leaner (lower body mass index), better educated, less likely to smoke, and more likely to have had a colorectal cancer screening/early detection procedure. Among those who completed the FFQ, total vitamin D and calcium intakes were significantly higher in controls than in cases (*P*=0.001). Family history of colorectal cancer (first-degree relatives affected only) was reported by 9.8% of the patients and 7.5% of the controls. MSI-high was identified in 61 of 507 (12.0%) tumors, and *BRAF* Val600Glu mutation was detected in 10.8% of tumors.

Table 4.1.1 Selected demographical and clinicopathological characteristics of study population (cases and controls)

Characteristics	Cases	Controls	*P*-value [a]
	No. (%)	No. (%)	
Age (year) [b]	63.1±8.6	61.2±9.0	0.001
BMI (kg/m^2) [b]	28.1±5.0	27.3±4.5	0.003
Sex			
Men	399(62.9)	272(58.5)	
Women	235(37.1)	193(41.5)	0.136
Race			
White	615(97.0)	444(95.5)	
Other	19(3.0)	21(5.0)	0.184
Family history of CRC			
Yes	62(9.8)	35(7.5)	
No	572(90.2)	430(92.5)	0.194
Level of education			
Lower than high school	302(47.8)	140(30.2)	
High-school graduate	100(15.8)	74(16.0)	
College	177(28.0)	175(37.7)	
Bachelor or higher	53(8.4)	75(16.1)	<0.001
Smoking history			
Current	129(20.3)	58(12.5)	
Former	325(51.3)	228(49.4)	
Never	180(28.4)	176(38.1)	<0.001
Reported screening procedure			
Yes	66(10.4)	107(23.0)	
No	568(89.6)	358(77.0)	<0.001

Fruit intake (servings [c]/wk) [b]	9.7±8.3	10.9±8.1	0.027
Total vitamin D intake (μg/d) [b]	8.7±6.4	10.2±7.9	0.001
Total calcium intake (mg/d) [b]	1019.8±488.8	1097.1±567.7	0.018
Milk (g/d) [b]	287.1±278.0	299.9±295.5	0.465
Total dairy products (g/d) [b]	364.5±303.3	389.9±340.0	0.200
Tumor location			
Colon	417(65.8)	–	–
Rectum	217(34.2)	–	–
MSI status			
MSS/MSI-L	446(88.0)	–	–
MSI-H	61(12.0)	–	–
BRAF mutation status			
Wild type	445(89.2)	–	–
BRAF mutant	54(10.8)	–	–

Abbreviations: BMI: body mass index; CRC: colorectal cancer; MSI: microsatellite instability; MSI-H: microsatellite instability-high; MSS/MSI-L: microsatellite stable/microsatellite instability-low.

[a] *P*-values are for the significance of the two-sample t test for continuous variables and of the chi-square test for categorical variables.

[b] Continuous variables presented as mean±s.d. (standard deviation).

[c] A serving of fruit is: 1 medium-sized fresh fruit; ½ cup of chopped, cooked, or canned fruit; ¼ cup of dried fruit; 6 ounces of fruit juice (50%-100% pure juice).

Association of rs2282679 genotype with colorectal cancer risk

The rs2282679 SNP was not associated with risk of colorectal cancer overall or when stratified by MSI or BRAF-mutation subtypes (Table 4.1.2). Specifically, the odds ratio was 1.10 (95% CI, 0.88-1.37) per variant C allele and 1.21 (95% CI, 0.70-2.09) in CC homozygotes compared with AA homozygotes. The ORs were similar for men and women and did not differ according to tumor anatomical sub-site (data available upon request).

Table 4.1.2 Frequency distribution and associations of *GC* SNP rs2282679 with colorectal cancer risk (overall and by molecularly defined subtypes)

rs2282679 Genotype/Allele	Cases (N=637) N (%)	Controls (N=489) N (%)	OR (95% CI) [a]	OR (95% CI) [b]
All cases *vs.* controls				
AA	309(48.5)	245(50.1)	1.00	1.00
AC	282(44.3)	208(42.5)	1.08(0.82-1.41)	1.10(0.83-1.47)
CC	46(7.2)	36(7.4)	1.06(0.63-1.77)	1.21(0.70-2.09)
P_{trend} [c]			0.647	0.403
CC+AC (*vs.* AA)	328(51.5)	244(49.9)	1.07(0.83-1.39)	1.12(0.85-1.47)
CC (*vs.* AC+AA)	46(7.2)	36(7.4)	1.02(0.62-1.68)	1.15(0.68-1.97)
Per C allele			1.05(0.85-1.29)	1.10(0.88-1.37)
BRAF V600E mutant cases *vs.* controls				
AA	25(46.3)	245(50.1)	1.00	1.00
CC+AC [d]	29(53.7)	244(49.9)	1.03(0.54-1.96)	1.10(0.56-2.16)
BRAF wild-type cases *vs.* controls				
AA	223(50.1)	245(50.1)	1.00	1.00
CC+AC [d]	222(49.9)	244(49.9)	1.08(0.82-1.42)	1.10(0.82-1.47)
MSI-H cases *vs.* controls				

AA	31(50.8)	245(50.1)	1.00	1.00
CC+AC [d]	30(49.2)	244(49.9)	0.99(0.56-1.80)	1.03(0.56-1.90)
MSS/MSI-Low cases *vs.* controls				
AA	223(50.0)	245(50.1)	1.00	1.00
CC+AC [d]	223(50.0)	244(49.9)	1.09(0.83-1.44)	1.15(0.86-1.53)

Abbreviations: OR: odds ratio; MSI-H: microsatellite instability-high; MSS/MSI-L: microsatellite stable/microsatellite instability-low;

[a] Crude model adjusted for age and sex.

[b] Multivariate model additionally adjusted for family history of colorectal cancer, screening procedure, multivitamin use, folic acid intake, smoking history, and education attainment where applicable.

[c] Linear trend tested by modeling the ordinal variables of genotype dose as a continuous variable.

[d] CC and AC genotypes were analyzed jointly because of limited sample size in some subgroups.

Interactions of rs2282679 genotype with dietary characteristics in relation to colorectal cancer risk

A previous NFCCS study demonstrated that total vitamin D intake was inversely associated with colorectal cancer incidence [132]. We therefore cross classified subjects on total vitamin D intake and other related dietary factors with rs2282679 genotype for colorectal cancer risk (Table 4.1.3). Among participants with the more common AA genotype, we confirmed the association, observing a 2.5-fold increased risk of colorectal cancer in individuals consuming total vitamin D in the lowest tertile than in those in the highest tertile (95% CI, 1.52-4.09). Among carriers of the C allele, however, no appreciable difference in ORs between strata of total vitamin D intake was observed. Intriguingly, carrying the risk allele (i.e. the heterozygous/homozygous genotypes) conferred an enhanced risk for this malignancy in the presence of high level of vitamin D intake (OR, 1.65; 95% CI, 1.01-2.71; $P_{\text{interaction}}$=0.019). Stratification on consumption of milk and total dairy products suggested borderline significant effect modifications ($P_{\text{interaction}}$=0.056 for milk and 0.079 for total dairy products). Additionally, there was no evidence that the associations of this SNP with colorectal cancer risk were modified by body mass index (obese *vs.* not obese), drinking status (non-drinkers *vs.* drinkers) or smoking history (non-smokers *vs.* smokers) (data available upon request).

Table 4.1.3 Risk estimates for interactions between *GC* rs2282679 genotypes and non-genetic dietary factors

	GC rs2282679 genotype		
	AA OR (95% CI)[a]	AC+CC OR (95% CI) [a]	$P_{interaction}$ [b]
No. of cases/controls	309/245	328/244	
Total vitamin D intake (μg/d)			
Highest tertile (≥10.1)	1.00	**1.65(1.01-2.71)**	
Lowest tertile (<5.0)	**2.50(1.52-4.09)**	**1.72(1.03-2.90)**	0.019
Total calcium intake (mg/d)			
Highest tertile (≥1195.4)	1.00	1.45(0.89-2.36)	
Lowest tertile (<761.4)	1.21(0.73-2.00)	1.63(0.95-2.81)	0.827
Milk (g/d)			
Highest tertile (≥321.3)	1.00	1.61(0.99-2.61)	
Lowest tertile (<146.5)	1.46(0.90-2.38)	1.21(0.72-2.04)	0.056
Total dairy products (g/d)			
Highest tertile (≥432.0)	1.00	1.61(0.99-2.61)	
Lowest tertile (<210.6)	1.44(0.90-2.32)	1.27(0.75-2.14)	0.079

Abbreviations: OR: odds ratio.
[a] Adjusted for age, sex, family history of colorectal cancer, screening procedure, folic acid intake, smoking history, and education attainment where applicable.
[b] *P* for interaction is computed with Wald method testing significance of multiplicative interaction term between GC SNP rs2282679 genotype and respective stratified variable.
Those with *P*<0.05 are in bold.

Association of rs2282679 genotype and survival outcome

Survival analysis showed a positive association between rs2282679 polymorphism and reduced DFS of colorectal cancer, with the co-dominant CC and the dominant CC+AC *vs.* AA model exhibiting 1.93- and 1.40- fold increases in the risk for DFS, respectively (Table 4.1.4, Figure 4.1.1). The per-allele HR was 1.36 (95% CI, 1.05-1.77; P_{trend}=0.020). The adverse prognosis in relation to the risk allele was limited to *BRAF* wild-type tumors (HR, 1.58; 95% CI, 1.12-2.23). Among patients harboring the *BRAF* Val600Glu mutation, the DFS was essentially the same for individuals with and without the C allele (HR, 0.95; 95% CI, 0.25-3.62; $P_{interaction}$=0.043). Although no evidence existed for a differential prognostic role of rs2282679 according to MSI status, patients with the AA genotype/MSI-high tumors experienced the most favorable DFS whereas patients with AC+CC genotypes/MSS+MSI-low tumors had the worst DFS (Figure 4.1.2). The 5-year DFS of colorectal cancer was 92% for AA/MSI-high, 82% for AC+CC genotypes/MSI-high, 68% for AA genotype/MSS+MSI-low, and 65% for AC+CC genotypes/MSS+MSI-low tumors (Log-rank *P*=0.0013, Figure

4.1.2). Our results did not confirm a prognostic relevance of rs2282679 in OS of colorectal cancer.

Table 4.1.4 Association between *GC* SNP rs2282679 genotypes and overall and disease free colorectal cancer survival (overall and stratified by tumor molecular phenotype)

rs2282679 Genotype/Allele	Disease-Free Survival		$P_{interaction}$ [d]	Overall Survival		$P_{interaction}$ [d]
	No. of Events [a] /At Risk	HR (95% CI) [b]		No. of Events [a] /At Risk	HR (95% CI) [b]	
Total colorectal cancer						
AA	83/245	1.00		69/245	1.00	
AC	80/207	1.33(0.94-1.88)		68/208	1.24(0.85-1.81)	
CC	16/36	**1.93(1.06-3.52)**		13/36	1.60(0.85-3.02)	
P_{trend} [e]		0.020			0.107	
CC+AC (*vs.* AA)	96/243	**1.40(1.00-1.95)**		81/244	1.29(0.90-1.85)	
CC (*vs.* AC+AA)	16/36	1.69(0.95-2.99)		13/36	1.44(0.79-2.65)	
Per C allele		**1.36(1.05-1.77)**			1.26(0.95-1.66)	
BRAF Val600Glu mutant						
AA	8/20	1.00		5/20	1.00	
CC+AC [f]	8/26	0.95(0.25-3.62)		7/26	1.53(0.47-4.95)	
BRAF wild-type						
AA	65/200	1.00		55/200	1.00	
CC+AC [f]	83/195	**1.58(1.12-2.23)**	0.043	70/196	1.33(0.93-1.91)	0.892
MSI-H						
AA	3/25	1.00		0/26	1.00	
CC+AC [f]	5/28	1.26(0.18-8.96)		3/28	NC [g]	
MSS/MSI-L						
AA	76/204	1.00		65/204	1.00	
CC+AC [f]	87/203	1.34(0.97-1.87)	0.702	74/204	1.26(0.89-1.77)	0.210

Abbreviations: HR, hazard rate ratios; CI, confidence interval; MSI-H: microsatellite instability-high; MSS/MSI-L: microsatellite stable/microsatellite instability-low;

[a] Events are defined as deaths for overall survival and death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival.

[b] Crude model adjusted for sex, age at diagnosis, and tumor stage at diagnosis.

[c] Multivariate Cox model additionally adjusted for marital status, race, reported chemoradiotherapy, MSI status, *BRAF* mutation status, tumor location, fruit intake, body mass index where applicable.

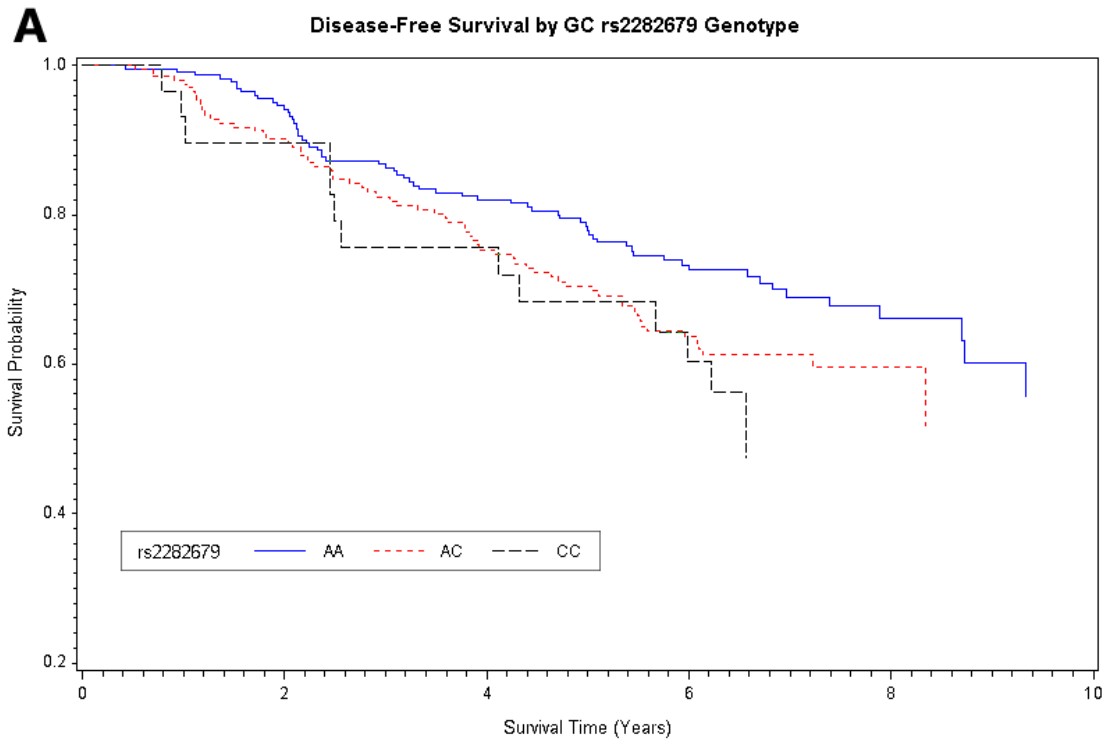
[d] *P* for interaction is computed with Wald method testing significance of multiplicative interaction term between *GC* SNP rs2282679 genotype and molecular subtype

[e] Linear trend tested by modeling the ordinal variables of genotype dose as a continuous variable.

[f] CC and AC genotypes were analyzed jointly because of limited sample size in some subgroups.

[g] NC: not calculated.

Those with $P<0.05$ are in bold.



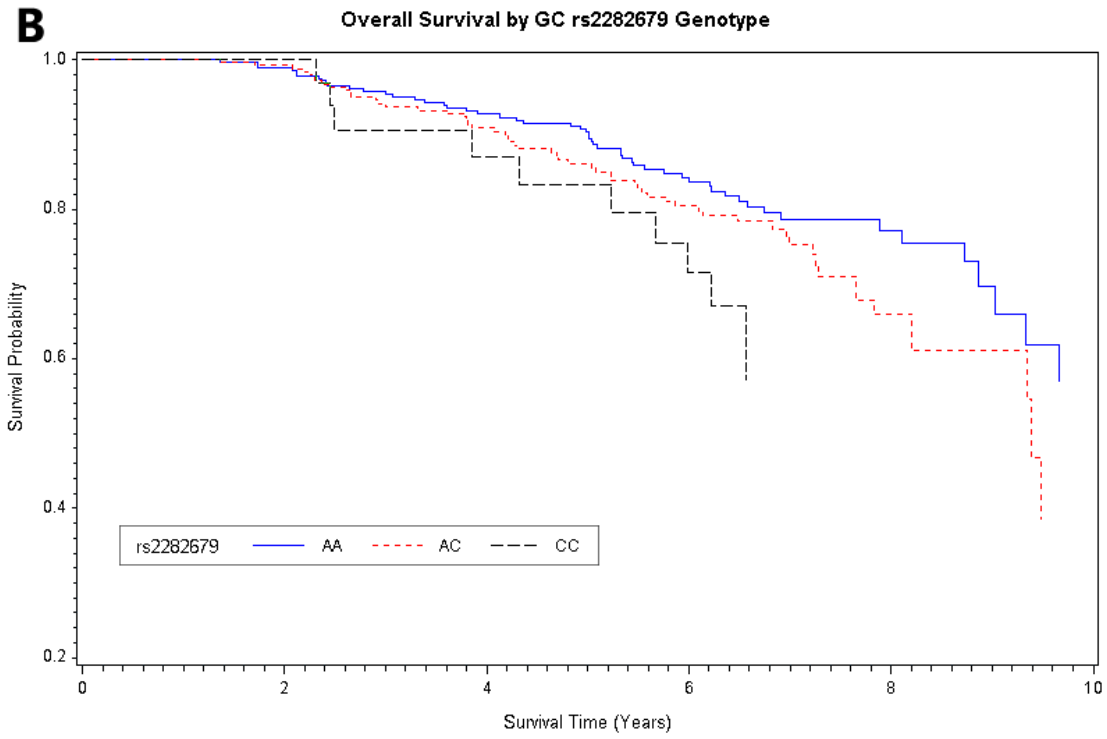


Figure 4.1.1 Survival curves for (A) disease-free survival and (B) overall survival by *GC* rs2282679 genotypes. Adjusted for sex, age at diagnosis, tumor stage at diagnosis, marital status, race, reported chemoradiotherapy, MSI status, *BRAF* mutation status, tumor location, fruit intake, and body mass index.

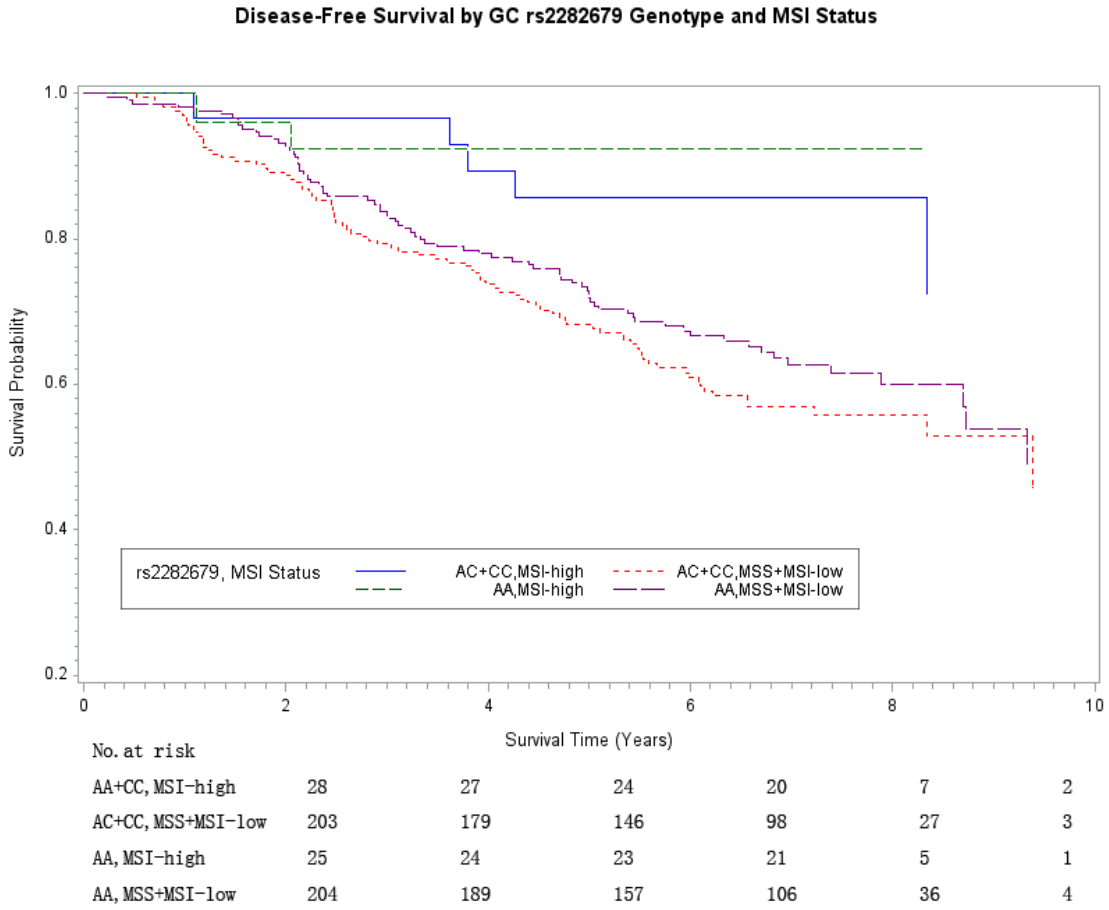


Figure 4.1.2 Kaplan-Meier survival curves for disease-free survival according to *GC* rs2282679 genotypes and MSI status.

Interactions of rs2282679 genotype with dietary characteristics in relation to colorectal cancer survival

The *GC* rs2282679 genotype interacted with dietary factors to influence OS after colorectal cancer diagnosis (Table 4.1.5). Specifically, the positive association between carriage of the C allele and poor OS seemed limited to patients in higher categories of dietary vitamin D, calcium, milk, and total dairy product intakes; the HRs associated with the AC+CC genotypes were 2.11 (95% CI, 1.29-3.74; $P_{interaction}$=0.040), 1.93 (95% CI, 1.08-3.46; $P_{interaction}$=0.043), 2.36 (95% CI, 1.26-4.44; $P_{interaction}$=0.004), and 2.03 (95% CI, 1.11-3.72; $P_{interaction}$=0.024), respectively. Low intake of pre-diagnostic milk was associated with worse

OS among wild-type homozygotes (AA) only, with the HR equaled to 2.09 (95% CI, 1.09-4.02). For DFS, effect-modification analyses yielded comparable but nonsignificant results. Analyses using total vitamin D or total calcium intakes (diet+supplements) did not show any associations (patterns) more significant or different. Therefore, we only present results stratified by dietary intakes. Stratification on tumor subsite (colon *vs.* rectum), reported chemoradiation therapy (yes *vs.* no) or smoking history (non-smokers vs. smokers) detected no significant interaction (data available upon request).

Table 4.1.5 Association between *GC* rs2282679 genotypes and overall and disease free colorectal cancer survival stratified by non-genetic dietary factors

	GC rs2282679 genotype		
	AA HR (95% CI) [a]	AC+CC HR (95% CI) [a]	$P_{interaction}$ [b]
Disease-Free Survival			
Dietary vitamin D (μg/d)			
Highest tertile (≥7.1)	1.00	1.65(0.97-2.82)	
Lowest tertile (<4.6)	1.03(0.58-1.83)	1.08(0.56-2.08)	0.191
Dietary calcium (mg/d)			
Highest tertile (≥1120.2)	1.00	1.58(0.92-2.71)	
Lowest tertile (<702.1)	1.06(0.59-1.91)	1.14(0.62-2.11)	0.355
Milk (g/d)			
Highest tertile (≥274.6)	1.00	1.63(0.92-1.84)	
Lowest tertile (<128.1)	1.21(0.68-2.16)	0.97(0.51-1.84)	0.083
Total dairy products (g/d)			
Highest tertile (≥411.8)	1.00	1.51(0.87-2.60)	
Lowest tertile (<199.2)	1.00(0.57-1.77)	0.99(0.53-1.83)	0.289
Overall Survival			
Dietary vitamin D (μg/d)			
Highest tertile (≥7.1)	1.00	**2.11(1.29-3.74)**	
Lowest tertile (<4.6)	1.28(0.68-2.42)	1.11(0.54-2.27)	0.040
Dietary calcium (mg/d)			
Highest tertile (≥1120.2)	1.00	**1.93(1.08-3.46)**	
Lowest tertile (<702.1)	1.55(0.82-2.91)	1.18(0.59-2.35)	0.043
Milk (g/d)			
Highest tertile (≥274.6)	1.00	**2.36(1.26-4.44)**	
Lowest tertile (<128.1)	**2.09(1.09-4.02)**	1.26(0.61-2.62)	0.004
Total dairy products (g/d)			
Highest tertile (≥411.8)	1.00	**2.03(1.11-3.72)**	
Lowest tertile (<199.2)	1.77(0.94-3.33)	1.27(0.63-2.58)	0.024

Abbreviations: HR, hazard rate ratios; CI, confidence interval;

[a] Adjusted for sex, age at diagnosis, stage at diagnosis, marital status, race, reported chemoradiotherapy, MSI status, *BRAF* mutation status, tumor location, body mass index where applicable.

[b] *P* for interaction is computed with Wald method testing significance of multiplicative interaction term between GC SNP rs2282679 genotype and respective stratified variable.

Those with *P*<0.05 are in bold.

Discussion

In this study, we observed no clear association of rs2282679 SNP with overall colorectal cancer risk, but noted a suggestive association of the CC genotype with DFS. A previous multicenter case-control study of 10,061 colorectal cancer cases and 12,768 controls of European ancestry found no evidence for associations between *GC* rs2282679 and the risk of colorectal cancer overall or for colon or rectal tumor separately [85]. In a Mendelian Randomization study in Scotland, Theodoratou *et al.* [84] reported a nonsignificant association of rs2282679 wild-type A allele with colorectal cancer risk (Per A allele: OR, 0.97; 95% CI, 0.90-1.06). The only available previous study investigating the prognostic effect of the SNP on colorectal cancer reported consistent results that *GC* rs2282679 polymorphism was significantly associated with reduced time to recurrence (HR, 3.30; 95% CI, 1.09–9.97, P=0.034) in stages II and III colon cancer patients treated with surgery alone [121].

Referring to two recent GWAS studies [78, 79], the *GC* rs2282679 has been identified as the strongest genomic predictor of serum vitamin D level ($P=2.0\times10^{-30}$). Per copy of the risk C allele was associated with an approximately 50% elevated risk for hypovitaminosis among Caucasians [79]. In another study by Zhang *et al.* [150], the C allele of this SNP was associated with lower circulating DBP concentrations and thus lower 25(OH)D bioavailability to target organs. Together with the fact that vitamin D has been shown to reduce the growth of colorectal cancer xenografts by influencing cell growth, differentiation, apoptosis, as well as immune-modulation, the elevated risk of C allele carriers may be attributed to their low circulating DBP and 25(OH)D concentrations relative to noncarriers [151-153]. Alternatively, DBP can be converted to GcMAF, an activator of macrophages, by stepwise incubation of β-galactosidase and sialidase [76]. GcMAF could activate phagocytosis of macrophages during inflammation, reduce tumor growth and stimulate cell

apoptosis [74, 75]. In addition, GcMAF has been demonstrated to have the potential utility as an antitumorigenic drug for metastatic breast cancer [154]. Therefore, genetic variation in *GC* may alternatively influence cancer outcome via GcMAF, a biological mechanism independent of vitamin D levels.

We found that carriage of the risk C allele was associated with an increased likelihood of colorectal cancer incidence in patients with high vitamin D intake ($P_{\text{interaction}}$=0.019). No prior studies were found that specifically examined the interaction between rs2282679 polymorphism and vitamin D on colorectal cancer; yet, two other *GC* SNPs, rs17467825 and rs7041, which are in strong linkage disequilibrium with *GC* rs2282679 (γ^2=1.0 and 0.6 respectively) [79], have been associated with a slightly greater risk of colorectal cancer among individuals who consumed total vitamin D above the median in a multicenter case–unaffected sibling control study, though the interactions were not significant [86]. In addition, low vitamin D intake conferred higher risk of colorectal cancer among wild-type AA carriers but less obvious effect among AC or CC carriers; therefore, subjects with the AC/CC genotype might derive little benefit from high vitamin D intake, which may be due to their low affinity and abundance of DBP that might influence the function of vitamin D. Previous research [92] found that serum 25(OH)D level had greater effect on colorectal adenoma among patients with high total calcium intake; it is therefore unsurprising that we also observed a particularly strong association between the variation and all-cause mortality in patients at the higher calcium category. Based on these observations, we may speculate that the influence of rs2282679 polymorphism on either carcinogenesis or progression of colorectal cancer was strengthened by a metabolically permissive environmental condition characterized by high levels of dietary vitamin D, calcium, or foods rich in vitamin D and calcium [155].

In this study, the associations between rs2282679 SNP and DFS and OS were of similar patterns but stronger with DFS than OS. The difference in results may be explained by several

reasons. It is plausible that many deaths among colorectal cancer patients are preceded by tumor metastasis or recurrence. Thus, the DFS end point may be dominated by metastasis and recurrence rather than deaths from all causes [156]; the difference in outcomes may have affected the results. A second possible explanation is that the power to detect an association for OS is less than that for DFS as the OS end point requires extended follow-up [156]. Therefore, the non-significant P values for OS might reflect inadequate power rather than a true lack of effect.

Our data suggest that the *GC* rs2282679 variation may be associated with poor DFS among patients with *BRAF* wild-type tumors, but not among *BRAF* mutant tumors ($P_{interaction}$=0.043). Although intriguing, the interaction of the SNP with tumor *BRAF* mutation status should be interpreted with caution because of a limited statistical power caused by low number of patients with *BRAF* mutant tumors, as well as the lack (at least to date) of exact mechanism of action underlying the prognostic value of this gene only in *BRAF* mutated colorectal cancer. Additionally, we observed an additive effect of the rs2282679 genotype combined with MSI status; unsurprisingly, the most favorable prognosis as determined by DFS was seen among patients with AA/MSI-high tumors (*vs.* AC+CC/MSS+MSI-low). MSI has been established as a prognostic biomarker that confers survival advantage to colorectal cancer due to increased apoptosis rate and high lymphocytic infiltration [157-160]. Our observations suggest that the prediction model of colorectal cancer outcome should additionally integrate the rs2282679 genotype. These results may provide relevant information for identification of patients with increased susceptibility to colorectal cancer incidence and mortality and for patient assignment to interventions that are tailored to the individual. Additional studies should be addressed to investigate the role of rs2282679/MSI classification in predicting the response to therapeutic lifestyle interventions.

One limitation of our study is that only one genetic variant of the *GC* gene was evaluated, thereby providing incomplete coverage of this gene; and we cannot exclude that genetic

polymorphisms in other genes in the vitamin D metabolism pathway may also influence overall colorectal cancer initiation and progression. It is also possible that rs2282679 is not the true causal variant in itself but acts as a proxy through linkage disequilibrium (LD). Moreover, plasma 25(OH)D levels were not measured in this study. The lack of 25(OH)D measurements impeded us to test the relations of *GC* rs2282679 polymorphisms with plasma vitamin D concentration and to evaluate the extent to which the high risk of colorectal cancer mortality associated with the C allele is mediated through low 25(OH)D levels. Furthermore, dietary vitamin D intake may not accurately reflect each participant's vitamin D status since dietary history as measured by the FFQ is imprecise, and neither dermatic synthesis of vitamin D from sun exposure nor long-term dietary vitamin D intake was taken into account. Additionally, individuals were asked to report dietary exposures from one year prior to diagnosis for cases and one year prior to recruitment for controls; therefore, cases recalled dietary intakes from years earlier than controls. The longer recall period increases the rate of recall error resulting in higher likelihood of exposure misclassification in the case group.

Among the strengths of the study is the careful data collection, with a combination of results from genotyping and epidemiologic questionnaires. The availability of information on known environmental and genetic risk factors of colorectal cancer allowed us to investigate potential gene-gene or gene-environment interactions. The relatively large sample size with up to 10 years of follow-up permitted enough power to discern the significant gene-gene and gene-environment interactions in modifying colorectal cancer risk and survival using stratified analyses, which could be missed in smaller investigations. Finally, we were able to link the *GC* rs2282679 genotype to both risk and survival of colorectal cancer to recapitulate the entire spectrum of the disease from initiation through progression [161].

Conclusions

Our data demonstrate that the *GC* rs2282679 polymorphism is not associated with colorectal cancer risk overall, but suggest a possible reduced DFS after colorectal cancer diagnosis. These results identified an association between the *GC* SNP rs2282679 and DFS of colorectal cancer and effect modifications by vitamin D intake and *BRAF* mutation status. The genotype at the *CG* rs2282679 locus, along with vitamin D and *BRAF* mutation status, has potential utility as a susceptibility and prognostic biomarker of colorectal cancer. Future studies should verify these findings in other populations as well as clarify the molecular mechanisms behind the differential effects of the SNP on colorectal cancer outcomes according to vitamin D and *BRAF* mutation status.

4.2 Paper 2. Vitamin D Receptor and Calcium Sensing Receptor Polymorphisms and Colorectal Cancer Survival in the Newfoundland and Labrador population

This paper [162] has been published in British Journal of Cancer. Yun Zhu is the first author of the paper.

Authors

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Abstract

Background

Increased serum levels of vitamin D and calcium have been associated with lower risks of colorectal cancer incidence and mortality. These inverse associations may be mediated by the vitamin D receptor (VDR) and the calcium sensing receptor (CASR). We investigated genetic variants in *VDR* and *CASR* for their relevance to colorectal cancer prognosis.

Methods

A population-based cohort of 531 colorectal cancer patients diagnosed from 1999 to 2003 in Newfoundland and Labrador, Canada, was followed for mortality and cancer recurrence until April 2010. Germline DNA samples were genotyped with the Illumina Omni-Quad 1 Million chip. Multivariate Cox models assessed 41 tag SNPs and relative haplotypes on *VDR* and *CASR* in relation to all-cause mortality (overall survival, OS) and disease-free survival (DFS).

Results

Associations at the gene-level were observed between polymorphic variations in the *VDR* gene and the DFS of rectal cancer patients (P=0.037) as well as between the *CASR* and the OS of colon cancer patients (P=0.014). Haplotype analysis within linkage blocks of CASR revealed the G-G-G-G-G-A-C haplotype (rs10222633-rs10934578-rs3804592-rs17250717-A986S-R990G-rs1802757) to be associated with a decreased OS of colon cancer (HR, 3.15; 95%CI, 1.66-5.96). Potential interactions were seen among prediagnostic dietary calcium intake with the *CASR* R990G (P_{int}=0.040) and the *CASR* G-T-G-G-G-G-C haplotype for rs10222633-rs10934578-rs3804592-rs17250717-A986S-R990G-rs1802757 (P_{int}=0.017), with decreased OS time associated with these variants limited to patients consuming dietary calcium below the median, although the stratified results were not statistically significant after correction for multiple testing.

Conclusions

Polymorphic variations in *VDR* and *CASR* may be associated with survival after a diagnosis of colorectal cancer.

Keywords: Vitamin D receptor, calcium sensing receptor, polymorphism, gene-environment interaction, colorectal cancer survival

Background

Evidence from both epidemiological [69, 70] and experimental studies [71, 72] supports a reduced risk of colorectal cancer by higher intake or blood levels of vitamin D. Vitamin D mediates its action through binding to the vitamin D receptor (*VDR*), a member of the nuclear receptor superfamily that is expressed in various cell types, including colorectal epithelial cells. This binding enables the transactivation of target genes that promote cellular

differentiation [18], induce apoptosis [19], and inhibit angiogenesis and proliferation [20]. Thus, *VDR* has been implicated in colorectal carcinogenesis. Another gene that influences vitamin D metabolism is the calcium sensing receptor (*CASR*), which is essential for calcium homeostasis and cellular growth kinetics [21, 22]. In the *CASR* promoter region, vitamin D response elements have been discovered, providing evidence at the molecular level for a potential interaction between vitamin D and calcium in colorectal cancer [23, 24]. Recent studies have also demonstrated a critical role of *CASR* as a tumor suppressor in the large intestine [25]. Expression of this receptor has been shown to be reduced in colon cancer cells as compared to normal colonic epithelial cells [26].

Current molecular studies have identified numerous single-nucleotide polymorphisms (SNPs) in the human *VDR* and *CASR* genes, but only a handful that are considered potentially functional have been examined in relation to colorectal cancer risk, including FokI (rs10735810) [22, 27], BsmI (rs1544410) [22, 28], ApaI (rs7975232) [27, 29], and TaqI (rs731236) [29] in *VDR*, and A986S (rs1801725) [24, 30] and R990G (rs1042636) [24] in *CASR*. Several studies have linked one or more of these variants to colorectal cancer and, particularly, the VDR BsmI bb (GG) [22, 28] and CASR A986S (TT) genotypes [31] were related to an increased risk of colorectal cancer. However, very few relevant studies on colorectal cancer survival have been published and none of these were from the Canadian population [15, 31-34]. Limited evidence shows no association of polymorphisms in *VDR* and *CASR* with survival after colorectal cancer diagnosis, but can be criticized for limited power or incomplete coverage of the variation within the gene [15, 31]. In addition, little is known regarding how pre- or postdiagnostic dietary factors could interact with *VDR* and *CASR* genotypes to influence colorectal cancer prognosis [15]. This is important especially because long-term eating habits prior to diagnosis may affect postdiagnostic diet, and because cancer patients may have a strong desire to make positive changes, and may benefit from

recommendations on a healthy diet and supplement use as a complement to their therapy [15, 35].

Therefore, in this analysis, we examined the hypothesis that genetic variations in *VDR* or *CASR* influence survival among colorectal cancer patients with possible effect modification by prediagnostic dietary vitamin D and calcium intakes within the context of a population-based cohort study in Newfoundland.

Materials and methods

Study population

The study was performed as part of the Newfoundland Familial Colorectal Cancer Study (NFCCS) effort to investigate environmental and genetic influences on colorectal cancer risk and survival outcome. The detailed rationale and methodology of the NFCCS has been described elsewhere [4, 146, 163]. Briefly, histologically confirmed cases of colorectal cancer diagnosed under age 75 between 1997 and 2003 were recruited in the province of Newfoundland & Labrador. The 531 patients (201 women and 330 men) included in the current study represented a subset of patients enrolled in the NFCCS (n=737) who had both disease-outcome data and a germline DNA sample available. Informed consent was obtained for all participants, and the study was carried out with the approval by the Health Research Ethics Authority of Memorial University of Newfoundland in accordance with the tenets of the declaration of Helsinki.

Diet assessment and baseline information collection

Detailed information about demographics, race and ethnicity, individual behaviors, medical history, detailed cancer family history, bowel screening history, and use of alcohol and tobacco was gathered via self-administered Family History Questionnaires (FHQ) and

Personal History Questionnaires (PHQ). Participants also completed a 169-item Food Frequency Questionnaire (FFQ) at the time of recruitment that addressed their dietary intake in the one year prior to their diagnosis. The FFQ was self-administered and semi-quantitative that had previously been validated in the Newfoundland and Labrador population [143]. The nutrient intakes from diet were calculated by multiplying the frequency of consumption of each food item by the nutrient content per average unit [132].

Study outcomes

Study participants were followed for recurrence and mortality from the date of cancer diagnosis until April 2010, with a combination of active follow-up (periodic follow-up questionnaires, e.g., FHQ) and record linkage to death certificates, pathology reports, autopsy records, physicians' notes, and surgical reports. Additional data were obtained from the Dr. H. Bliss Murphy Cancer Care Foundation. The main outcomes used for this study were overall survival (OS) and disease-free survival (DFS). The end-point event for the overall survival analysis was death from all-causes and for the disease-free survival analysis was death from any cause, colorectal cancer recurrence, or metastasis, whichever came first.

Genotyping and SNP selection

Genotyping of peripheral blood DNA samples was performed using the Illumina Human Omni-Quad Bead chip that contains about 1.1 million SNPs at Centrillion Biosciences (USA). For quality control purposes, genomic DNA from 200 duplicate samples were sent to the Laboratory of Dr. Stephen Gruber (Director, USC Norris Comprehensive Cancer Center, Los Angeles) for genotyping using the Affymetrix Axiom® myDesign™ GW Array Plate, which contains 1.3 million probes. SNPs with genotype concordance <97% between the two platforms were dropped from all analyses.

We used an aggressive tagging approach to limit the number of SNPs examined to the

most relevant. Tagging SNPs capturing most of the common variation in the candidate gene regions were identified using Plink v1.07 based on the following criteria: the minor allele frequency (MAF) of the SNP≥5%; pairwise r^2>0.9; and at least 50 base pairs from any adjacent SNPs [164]. The regions analyzed included about 103 kb of the *CASR* gene and 65 kb of the *VDR* gene. This process identified 24 SNPs for *VDR* and 14 SNPs for *CASR*. Additionally, a priori, we also selected 4 high interest SNPs reported in previous colorectal cancer studies, including *VDR* BsmI, *CASR* R990G, *CASR* rs1802757, and *CASR* A986S. For all genotypes, the call frequency was >99.5% except for one SNP (VDR rs2238135, 99.2%). The distribution of the genotypes of all SNPs examined in this study fitted Hardy-Weinberg proportions, with the exception of VDR rs3847987 ($P < 0.001$), which was excluded from analysis.

Our protocol for MSI testing and mutation detection on *BRAF* V600E in tumor DNA has been described elsewhere [146]. MSI status was determined using 5 to 10 microsatellite markers. Mutant alleles in the *BRAF* gene were detected using the allele-specific polymerase chain reaction (AS-PCR) technique [146].

Statistical analysis

The log-rank test compared the survival distributions across groups of baseline factors. We utilized a principal component (PC) analysis that accounts for linkage disequilibrium (LD) between multiple SNPs to test for an overall association of a gene with survival of colorectal cancer patients [165]. Briefly, this approach computed uncorrelated linear combinations of the original SNPs, grouped as PCs, which explain the greatest amount of variance across the gene. Then, PCs that cumulatively explain at least 80% of the variance were retained and included in a Cox proportional hazards regression analysis with colorectal cancer survival as the outcome. Using a likelihood ratio test, we calculated a *P*-value for the global gene-outcome association by comparing models with and without selected PCs with the number

of degrees of freedom equal to the number of PCs. Overall survival and disease-free survival were the main outcomes, each stratified by anatomical site (colon and rectum).

The data were further explored using a single-SNP analysis, followed by the haplotype analysis. For every individual SNP, the associations with overall and disease-free survival in colorectal cancer patients were estimated by Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) while assuming an additive model by Cox regression analysis, adjusted for sex, race, age at diagnosis, disease stage at diagnosis, reported screening procedure, marital status, MSI status, and *BRAF* mutation status when applicable. These covariates were retained in the final model because they either entered the model at $P<0.1$ or altered the parameter estimates by >10%. The proportional hazards assumption was verified by testing the statistical significance of time-by-covariate interactions for each covariate in the Cox model. To control type I error inflation, *P*-values were then adjusted for multiple comparisons using the approach specifically created for correlated tests due to LD by Conneely and Boehnke [166]. For the haplotype analyses, LD plots were generated using the Haploview version 4.2 to evaluate haplotype block structure based on the criteria of Gabriel et al [167]. Haplotype frequencies were estimated using the expectation maximization algorithm accounting for ambiguous linkage phase, and the association between individual haplotype and colorectal cancer survival was assessed by modeling all haplotypes simultaneously with the most frequent haplotype as the reference. Bonferroni correction for multiple testing was performed for thirty-six haplotypes yielding an adjusted *P* value of 0.0014. A global *P* value for each haplotype block was obtained with a Wald test. Haplotype analyses were performed using SimHap GUI version 1.0.2 [168]. Gene-environment (G×E) interactions were tested through stratified analysis and verified with the Wald method by introducing a multiplicative interaction term into the model and assessing its significance. The G×E analyses, a priori, were not adjusted for multiple comparisons. All tests were two-sided. Other data

management and analyses were performed with SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Patient characteristics and clinical predictors

The study sample consisted of 330 men and 201 women (Table 4.2.1). The mean age of the study population was 60.7±9.2 yrs., with 96.9% of the participants being white, 11.5% reporting a bowel screening history, and 66.0% having had tumors at the colon subsite. Information on MSI status was available for a total of 503 patients, with 11.5 % classified as MSI-H and 88.5% as MSS/MSI-L. Salient characteristics were largely comparable between the NFCCS patients included and those excluded from the current study due to lack of genotype/disease-outcome data. At the end of our study (median follow-up time, 6.4 years), 183 (34.5%) of the 531 patients had died. In the univariate analysis, male gender, other ethnicity, advanced stage at diagnosis (III/IV), chemoradiotherapy, and MSS/MSI-L tumors were significantly associated with reduced OS time, whereas bowel screening procedure, tumor location, and *BRAF* mutation status were not associated with OS among the 531 colorectal cancer patients included in this study.

Table 4.2.1 Demographical and clinicopathological characteristics of patients in the Newfoundland Familial Colorectal Cancer Study (NFCCS)

Characteristic	Subjects included in this study (N=531)				Subjects without genotype/ disease-outcome information (N=206)
	No. patients (%)	No. deaths (%)	MST (y) [a]	$P_{log\text{-}rank}$ [a]	No. patients (%)
Age at diagnosis (y) [b]	60.7±9.2	61.3±9.7	-	-	62.1±9.6
Sex					
Female	201 (37.9)	56 (27.9)	6.5		83 (40.3)
Male	330 (62.1)	127 (38.5)	6.3	0.005	123 (59.7)
Race					
White	439 (96.9)	133 (30.3)	6.4		27 (93.1)
Other	14 (3.1)	8 (57.1)	4.7	0.009	2 (6.9)
Reported screening procedure					
Yes	52 (11.5)	10 (19.2)	6.6		7 (24.1)
No	401 (88.5)	131 (32.7)	6.4	0.059	22 (75.9)
Tumor location					
Colon	341 (66.0)	110 (32.3)	6.4		124 (72.1)
Rectum	176 (34.0)	65 (36.9)	6.3	0.444	48 (27.9)
Stage at diagnosis					
I/II	302 (56.9)	76 (25.2)	6.6		56 (27.2)
III/IV	229 (43.1)	107 (46.7)	6.0	<0.001	150 (72.8)
Surgery					
Yes	516 (97.2)	177 (34.3)	6.4		204 (99.0)
No	15 (2.8)	6 (40.0)	6.8	0.790	2 (1.0)
Chemoradiotherapy					
Yes	106 (20.5)	33 (41.51)	6.0		33 (19.2)
No	411 (79.5)	131 (31.87)	6.4	0.036	139 (80.8)
MSI status					
MSS/MSI-L	445 (88.5)	168 (37.8)	6.3		190 (92.2)
MSI-H	58 (11.5)	6 (10.3)	6.7	<0.001	16 (7.7)
BRAF mutation status					
Wild type	432 (89.8)	153 (35.4)	6.4		165 (84.2)
BRAF mutant	49 (10.2)	15 (30.6)	6.3	0.370	31 (15.8)
Dietary vitamin D intake (μg/d) [b]	6.3±3.5	7.0±4.2	-	-	5.5±3.0
Dietary calcium intake (mg/d) [b]	965.6±460.7	1024.8±504.2	-	-	888.3±427.8

Abbreviations: MST, median overall survival time; BMI, body mass index; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS/MSI-L, microsatellite stable/microsatellite instability-low.
[a] The values reported were calculated over the number of subjects with valid information; the numbers of subjects with missing values for each variable are as follows: race (78), reported screening procedure (78), tumor location (14), chemoradiotherapy (14), dietary vitamin D intake (78), dietary calcium intake (78), MSI status (28), *BRAF* mutation status (50).
[b] Continuous variables presented as mean±SD (standard deviation).

Association of *VDR* and *CASR* with survival of colorectal cancer patients

PC analysis was conducted to assess whether there was an overall gene-level association between *VDR* or *CASR* and colorectal cancer survival (Table 4.2.2). At the gene level, we observed no meaningful relationships for *VDR* or *CASR* and colorectal cancer survival. However, after stratification by colorectal subsite, the *VDR* gene exhibited a marginally

significant association with the DFS among patients with rectal cancer (Global *P*=0.037), while the *CASR* gene was related to OS in colon cancer patients at a significance level of 0.05 (Global *P*=0.014).

In analyses of individual SNPs within each gene, a total of four SNPs in *VDR* and two SNPs in *CASR* were related to OS under an additive model (Table 4.2.3 and Table 4.2.4). However, multiple testing adjustment revealed one association of marginally statistical significance for *VDR* (BsmI polymorphism, rs1544410) and the OS of all colorectal cancer ($P_{unadjusted}$=0.002, $P_{adjusted}$=0.058). Specifically, the G-allele was related to worse OS as compared with the A-allele (HR per G allele, 1.15; 95% CI, 1.17-1.94) (Figure 4.2.1). For DFS, no SNPs approached statistical significance after adjusting for multiple comparisons. Given that SNP prevalence varies across populations, analyses were repeated among those with European ancestry ("white") alone, which produced similar findings.

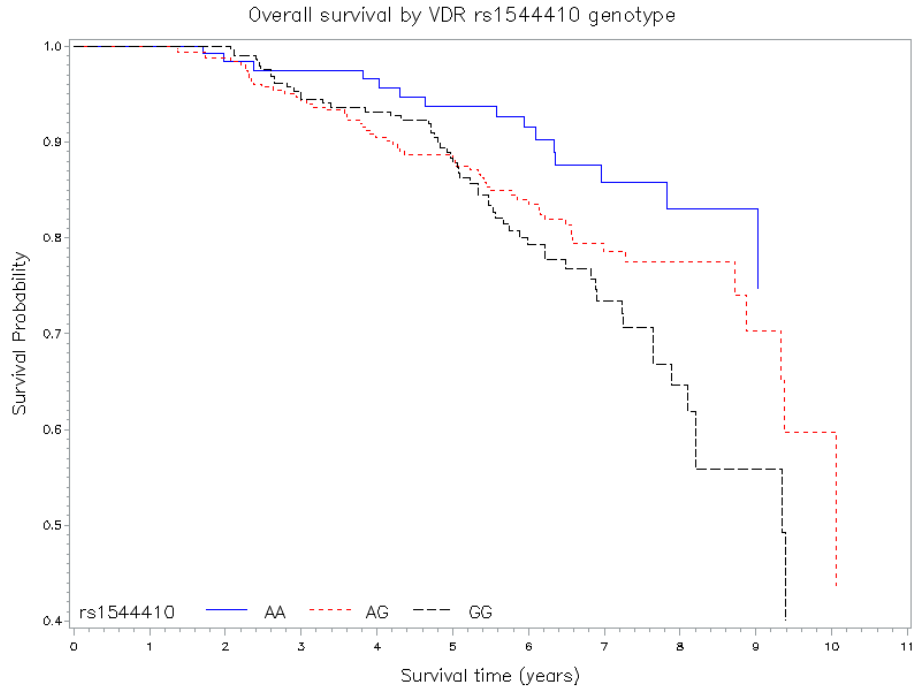


Figure 4.2.1 Overall survival curves by VDR rs1544410 genotype.
Adjusted for age at diagnosis, sex, race, stage at diagnosis, reported screening procedure, marital status, and MSI status.

Table 4.2.2 Association between *VDR* and *CASR* genes and colorectal cancer overall and disease-free survival (n=531)

	Overall survival HR (95%CI) [a]			Disease-free survival HR (95%CI) [a]		
	All CRC	**Colon cancer**	**Rectum cancer**	**All CRC**	**Colon cancer**	**Rectum cancer**
VDR						
PC1	0.80 (0.67-0.95)	0.76 (0.61-0.95)	0.90 (0.64-1.25)	0.83 (0.71-0.98)	0.82 (0.66-1.00)	0.86 (0.64-1.15)
PC2	0.99 (0.83-1.17)	0.85 (0.68-1.07)	1.41 (1.01-1.98)	1.00 (0.84-1.19)	0.84 (0.68-1.04)	1.78 (1.27-2.50)
PC3	0.87 (0.74-1.03)	0.90 (0.73-1.11)	0.81 (0.58-1.11)	0.91 (0.77-1.07)	0.95 (0.77-1.17)	0.74 (0.55-1.00)
PC4	1.03 (0.87-1.21)	1.07 (0.87-1.12)	0.91 (0.66-1.26)	1.05 (0.90-1.23)	1.12 (0.91-1.38)	0.83 (0.62-1.11)
PC5	0.90 (0.75-1.08)	0.89 (0.69-1.14)	0.97 (0.73-1.30)	0.91 (0.77-1.08)	0.92 (0.73-1.15)	1.02 (0.79-1.32)
PC6	0.96 (0.81-1.13)	1.01 (0.82-1.25)	0.84 (0.63-1.12)	1.07 (0.91-1.26)	1.06 (0.85-1.30)	1.10 (0.83-1.45)
PC7	1.05 (0.88-1.24)	1.10 (0.87-1.38)	1.11 (0.83-1.47)	1.09 (0.93-1.28)	1.10 (0.89-1.37)	1.09 (0.85-1.39)
PC8	0.98 (0.83-1.17)	0.90 (0.73-1.11)	1.26 (0.90-1.77)	1.02 (0.86-1.20)	0.87 (0.71-1.07)	1.60 (1.17-2.17)
Global *P* [b]	0.201	0.193	0.206	0.320	0.241	0.037
CASR						
PC1	1.09 (0.91-1.29)	1.04 (0.84-1.30)	1.25 (0.90-1.74)	1.10 (0.93-1.29)	1.02 (0.83-1.26)	1.29 (0.95-1.75)
PC2	0.94 (0.80-1.10)	0.84 (0.68-1.04)	1.20 (0.90-1.61)	0.96 (0.83-1.12)	0.92 (0.76-1.12)	1.15 (0.89-1.49)
PC3	1.12 (0.95-1.31)	1.23 (1.01-1.50)	1.07 (0.81-1.41)	1.13 (0.98-1.30)	1.15 (0.95-1.39)	1.15 (0.91-1.44)
PC4	0.95 (0.80-1.13)	1.02 (0.83-1.26)	0.91 (0.66-1.25)	1.03 (0.89-1.20)	1.07 (0.90-1.28)	1.03 (0.79-1.34)
PC5	0.94 (0.78-1.13)	0.85 (0.68-1.07)	1.19 (0.81-1.76)	0.94 (0.80-1.11)	0.87 (0.71-1.06)	1.15 (0.82-1.60)
PC6	1.10 (0.91-1.32)	1.26 (0.98-1.61)	0.82 (0.60-1.12)	1.12 (0.95-1.33)	1.27 (1.01-1.59)	0.89 (0.69-1.15)
PC7	0.80 (0.65-0.98)	0.72 (0.55-0.94)	0.95 (0.68-1.33)	0.88 (0.73-1.05)	0.81 (0.64-1.03)	0.96 (0.71-1.29)
Global *P* [b]	0.209	0.014	0.486	0.258	0.075	0.492

Abbreviations: CRC, colorectal cancer; HR, hazard ratio; PC, principal component.

[a] Cox proportional hazard model adjusted for age at diagnosis, sex, race, stage at diagnosis, reported screening procedure, marital status, MSI status, and *BRAF* mutation status where applicable; subjects with missing information on tumor location (n = 14) were excluded from the stratified analysis.

[b] Global *P* for association is from a likelihood ratio test with degrees of freedom equal to the number of principal components

Table 4.2.3 Association between *VDR* SNPs and colorectal cancer overall and disease-free survival assuming an additive mode of inheritance

SNP ID	Position [a]	Major/Minor allele (MAF)	All CRC			Colon cancer			Rectum cancer		
			HR (95%CI) [b]	*P*	P_{ACT} [c]	HR (95%CI)	*P*	P_{ACT} [c]	HR (95%CI) [b]	*P*	P_{ACT} [c]
Overall survival											
rs11574143	48234917	G/A (0.089)	0.81 (0.52-1.25)	0.342	0.999	1.14 (0.65-2.02)	0.646	1.000	0.34 (0.16-0.72)	0.005	0.147
rs731236	48238757	T/C (0.417)	1.48 (1.14-1.91)	0.003	0.083	1.41 (1.03-1.91)	0.031	0.568	1.62 (0.99-2.65)	0.051	0.756
rs1544410	48239835	G/A (0.421)	1.50 (1.17-1.94)	0.002	0.058	1.44 (1.06-1.96)	0.020	0.426	1.62 (0.99-2.65)	0.051	0.754
rs2239182	48255411	G/A (0.475)	0.85 (0.67-1.08)	0.182	0.985	0.81 (0.64-1.09)	0.157	0.974	1.01 (0.66-1.55)	0.977	1.000
rs2107301	48255570	C/T (0.230)	1.31 (0.99-1.73)	0.060	0.785	1.35 (0.96-1.90)	0.087	0.884	1.24 (0.73-2.12)	0.431	1.000
rs2239179	48257766	A/G (0.458)	0.77 (0.60-0.98)	0.033	0.605	0.75 (0.55-1.02)	0.062	0.791	0.85 (0.55-1.33)	0.483	1.000
rs12721370	48262073	G/T (0.092)	0.80 (0.52-1.24)	0.322	0.999	0.70 (0.41-1.19)	0.186	0.985	0.94 (0.43-2.08)	0.877	1.000
rs886441	48262964	T/C (0.199)	1.10 (0.82-1.47)	0.542	1.000	0.99 (0.70-1.40)	0.962	1.000	1.52 (0.87-2.65)	0.139	0.968
rs2189480	48263828	C/A (0.373)	0.84 (0.65-1.08)	0.176	0.985	0.88 (0.64-1.21)	0.427	1.000	0.73 (0.46-1.15)	0.178	0.978
rs2239186	48269410	T/C (0.203)	0.66 (0.50-0.88)	0.005	0.146	0.67 (0.47-0.97)	0.031	0.562	0.70 (0.43-1.15)	0.160	0.972
rs6580642	48270596	C/T (0.160)	0.84 (0.60-1.17)	0.291	0.998	0.87 (0.58-1.32)	0.524	1.000	0.63 (0.34-1.15)	0.134	0.967
rs11168275	48272275	A/G (0.242)	1.13 (0.85-1.50)	0.419	0.999	1.07 (0.77-1.50)	0.683	1.000	1.15 (0.65-2.04)	0.634	1.000
rs10735810	48272895	G/A (0.393)	1.00 (0.78-1.28)	0.975	1.000	0.86 (0.63-1.17)	0.337	0.999	1.31 (0.82-2.09)	0.265	0.993
rs2254210	48273714	G/A (0.399)	1.14 (0.90-1.44)	0.291	0.998	1.04 (0.77-1.39)	0.819	1.000	1.36 (0.87-2.13)	0.172	0.977
rs2238136	48277713	G/A (0.243)	0.88 (0.66-1.18)	0.388	0.999	0.96 (0.65-1.41)	0.832	1.000	0.75 (0.48-1.16)	0.196	0.983
rs2238135	48278190	G/C (0.221)	0.86 (0.65-1.14)	0.294	0.998	0.91 (0.62-1.33)	0.620	1.000	0.77 (0.50-1.20)	0.251	0.993
rs2853564	48278487	T/C (0.421)	1.09 (0.86-1.40)	0.478	1.000	1.01 (0.74-1.37)	0.963	1.000	1.31 (0.86-2.00)	0.215	0.986
rs4760648	48280665	C/T (0.408)	1.04 (0.82-1.32)	0.755	1.000	1.01 (0.74-1.38)	0.969	1.000	1.12 (0.75-1.68)	0.575	1.000
rs11168287	48285414	A/G (0.498)	1.09 (0.85-1.39)	0.511	1.000	0.89 (0.65-1.23)	0.478	1.000	1.09 (0.85-1.39)	0.511	1.000
rs4328262	48285648	T/G (0.410)	1.00 (0.78-1.28)	0.991	1.000	0.82 (0.59-1.13)	0.228	0.990	1.35 (0.90-2.01)	0.147	0.965
rs11168293	48293716	G/T (0.324)	1.04 (0.80-1.34)	0.781	1.000	0.86 (0.62-1.19)	0.361	0.998	1.50 (0.95-2.36)	0.083	0.883
rs4760655	48294131	A/G (0.351)	1.08 (0.84-1.40)	0.534	1.000	0.91 (0.65-1.26)	0.567	1.000	1.44 (0.96-2.17)	0.076	0.863
rs7136534	48294626	C/T (0.282)	0.94 (0.72-1.24)	0.668	1.000	1.00 (0.70-1.42)	0.989	1.000	0.95 (0.61-1.49)	0.837	1.000
rs4516035	48299826	T/C (0.431)	1.06 (0.83-1.36)	0.620	1.000	0.88 (0.65-1.21)	0.440	1.000	1.65 (1.06-2.57)	0.026	0.527
Disease-free survival											
rs11574143	48234917	G/A (0.089)	0.98 (0.66-1.45)	0.913	1.000	1.05 (0.64-1.73)	0.836	1.000	0.69 (0.34-1.39)	0.296	0.998
rs731236	48238757	T/C (0.417)	1.34 (1.07-1.69)	0.012	0.313	1.24 (0.93-1.65)	0.143	0.959	1.56 (1.02-2.38)	0.041	0.668
rs1544410	48239835	G/A (0.421)	1.37 (1.09-1.73)	0.007	0.204	1.28 (0.96-1.70)	0.091	0.909	1.56 (1.02-2.38)	0.041	0.666
rs2239182	48255411	G/A (0.475)	0.83 (0.67-1.04)	0.100	0.923	0.79 (0.60-1.04)	0.097	0.915	0.95 (0.65-1.40)	0.809	1.000
rs2107301	48255570	C/T (0.230)	1.10 (0.84-1.43)	0.496	1.000	1.22 (0.89-1.68)	0.225	0.991	0.94 (0.58-1.54)	0.805	1.000
rs2239179	48257766	A/G (0.458)	0.78 (0.62-0.98)	0.031	0.581	0.74 (0.56-0.99)	0.043	0.699	1.01 (0.67-1.53)	0.957	1.000
rs12721370	48262073	G/T (0.092)	0.87 (0.59-1.28)	0.484	1.000	0.65 (0.41-1.04)	0.072	0.861	1.40 (0.68-2.89)	0.364	1.000

rs886441	48262964	T/C (0.199)	1.02 (0.78-1.33)	0.913	1.000	0.93 (0.67-1.29)	0.647	1.000	1.36 (0.84-2.22)	0.212	0.990
rs2189480	48263828	C/A (0.373)	0.87 (0.69-1.10)	0.238	0.996	0.96 (0.72-1.27)	0.757	1.000	0.75 (0.49-1.13)	0.168	0.980
rs2239186	48269410	T/C (0.203)	0.73 (0.56-0.95)	0.021	0.462	0.83 (0.59-1.16)	0.275	0.997	0.55 (0.34-0.90)	0.017	0.394
rs6580642	48270596	C/T (0.160)	1.03 (0.73-1.45)	0.860	1.000	1.07 (0.69-1.67)	0.772	1.000	0.85 (0.49-1.47)	0.552	1.000
rs11168275	48272275	A/G (0.242)	1.04 (0.81-1.34)	0.773	1.000	1.04 (0.77-1.42)	0.787	1.000	0.95 (0.56-1.59)	0.834	1.000
rs10735810	48272895	G/A (0.393)	1.04 (0.83-1.31)	0.721	1.000	0.87 (0.66-1.16)	0.353	0.999	1.55 (1.03-2.35)	0.038	0.651
rs2254210	48273714	G/A (0.399)	1.07 (0.86-1.33)	0.525	1.000	1.00 (0.77-1.30)	0.994	1.000	1.22 (0.83-1.80)	0.308	0.998
rs2238136	48277713	G/A (0.243)	0.91 (0.70-1.18)	0.479	1.000	0.99 (0.70-1.40)	0.939	1.000	0.86 (0.57-1.30)	0.474	1.000
rs2238135	48278190	G/C (0.221)	0.90 (0.69-1.16)	0.405	0.999	0.97 (0.69-1.38)	0.876	1.000	0.85 (0.56-1.30)	0.453	1.000
rs2853564	48278487	T/C (0.421)	1.10 (0.88-1.37)	0.426	0.999	1.03 (0.78-1.36)	0.837	1.000	1.18 (0.80-1.73)	0.405	1.000
rs4760648	48280665	C/T (0.408)	1.07 (0.86-1.33)	0.568	1.000	1.05 (0.79-1.40)	0.720	1.000	1.10 (0.77-1.57)	0.615	1.000
rs11168287	48285414	A/G (0.498)	1.14 (0.91-1.43)	0.265	0.997	0.91 (0.68-1.21)	0.508	1.000	1.78 (1.18-2.67)	0.006	0.167
rs4328262	48285648	T/G (0.410)	0.98 (0.78-1.24)	0.881	1.000	0.79 (0.58-1.06)	0.111	0.934	1.41 (0.95-2.08)	0.084	0.874
rs11168293	48293716	G/T (0.324)	1.07 (0.85-1.36)	0.553	1.000	0.85 (0.63-1.15)	0.297	0.997	1.51 (0.99-2.31)	0.057	0.760
rs4760655	48294131	A/G (0.351)	1.07 (0.85-1.36)	0.556	1.000	0.92 (0.68-1.23)	0.560	1.000	1.36 (0.92-2.03)	0.126	0.952
rs7136534	48294626	C/T (0.282)	1.01 (0.79-1.29)	0.939	1.000	1.03 (0.74-1.42)	0.881	1.000	0.98 (0.66-1.45)	0.924	1.000
rs4516035	48299826	T/C (0.431)	1.10 (0.87-1.38)	0.433	1.000	0.87 (0.65-1.17)	0.364	0.999	1.58 (1.05-2.38)	0.027	0.544

Abbreviations: CRC, colorectal cancer; HR, hazard ratio; MAF minor allele frequency.

[a] SNP locations were mapped according to the NCBI build 36 coordinates.

[b] Hazard ratio calculated in reference to the allele underlined. Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, race, reported screening procedure, marital status, MSI status, and BRAF mutation status where appropriate.

[c] P-values were adjusted for multiple comparisons using a modification of P_{ACT} for correlated tests developed by Conneely and Boehnke(Conneely & Boehnke, 2007).

Table 4.2.4 Association between *CASR* SNPs and colorectal cancer overall and disease-free survival assuming an additive mode of inheritance

SNP ID	Position [a]	Major/Minor allele (MAF)	All CRC			Colon cancer			Rectum cancer		
			HR (95%CI) [b]	*P*	P_{ACT} [c]	HR (95%CI) [b]	*P*	P_{ACT} [c]	HR (95%CI) [b]	*P*	P_{ACT} [c]
Overall survival											
rs34028592	121902021	A/G (0.185)	0.89 (0.66-1.20)	0.439	0.999	0.97 (0.67-1.41)	0.859	1.000	0.71 (0.40-1.23)	0.219	0.986
rs6762782	121905657	G/A (0.387)	1.03 (0.80-1.32)	0.814	1.000	0.83 (0.61-1.12)	0.220	0.990	1.51 (0.98-2.34)	0.062	0.809
rs1814740	121918491	A/G (0.489)	1.18 (0.93-1.50)	0.168	0.983	1.27 (0.93-1.72)	0.130	0.958	1.05 (0.71-1.56)	0.797	1.000
rs35274320	121937943	G/A (0.160)	1.29 (0.92-1.82)	0.146	0.972	1.17 (0.77-1.78)	0.460	1.000	1.67 (0.85-3.25)	0.135	0.966
rs1354162	121954077	C/A (0.091)	0.57 (0.33-0.98)	0.041	0.668	0.38 (0.20-0.71)	0.003	0.081	1.29 (0.59-2.83)	0.530	1.000
rs7637874	121966952	C/T (0.239)	0.87 (0.65-1.16)	0.342	0.999	0.78 (0.54-1.13)	0.189	0.984	1.07 (0.67-1.72)	0.766	1.000
rs34345120	121968267	C/T (0.055)	1.19 (0.74-1.91)	0.466	1.000	1.37 (0.79-2.39)	0.262	0.995	0.84 (0.33-2.11)	0.703	1.000
rs1463890	121969937	T/C (0.140)	0.92 (0.66-1.27)	0.596	1.000	0.79 (0.53-1.16)	0.220	0.989	1.19 (0.63-2.22)	0.593	1.000
rs7647446	121970020	G/A (0.185)	1.11 (0.82-1.49)	0.510	1.000	1.05 (0.72-1.52)	0.807	1.000	1.30 (0.75-2.23)	0.349	0.999
rs937625	121971512	T/G (0.085)	1.01 (0.66-1.56)	0.948	1.000	1.20 (0.70-2.05)	0.508	1.000	0.88 (0.42-1.87)	0.741	1.000
rs10222633	121976926	G/A (0.492)	1.22 (0.96-1.56)	0.110	0.933	1.41 (1.04-1.91)	0.028	0.540	0.80 (0.50-1.28)	0.354	0.998
rs10934578	121977282	G/T (0.334)	0.98 (0.77-1.26)	0.879	1.000	0.80 (0.50-1.28)	0.354	0.999	1.11 (0.72-1.73)	0.636	1.000
rs3804592	121979229	G/A (0.133)	0.84 (0.56-1.26)	0.401	0.999	0.80 (0.50-1.29)	0.358	0.999	1.07 (0.49-2.36)	0.861	1.000
rs17250717	121980186	G/T (0.095)	1.18 (0.74-1.90)	0.488	1.000	1.64 (0.86-3.12)	0.133	0.958	0.61 (0.29-1.30)	0.200	0.983
rs1801725	122003757	G/T (0.140)	1.23 (0.86-1.75)	0.256	0.997	1.12 (0.71-1.75)	0.635	1.000	1.57 (0.86-2.89)	0.145	0.970
rs1042636	122003769	A/G (0.086)	1.46 (1.02-2.10)	0.039	0.660	1.71 (1.10-2.68)	0.018	0.416	1.14 (0.60-2.17)	0.682	1.000
rs1802757	122005131	C/T (0.145)	1.26 (0.90-1.76)	0.188	0.985	1.17 (0.75-1.83)	0.485	1.000	1.50 (0.87-2.60)	0.146	0.968
Disease-free survival											
rs34028592	121902021	A/G (0.185)	0.80 (0.59-1.09)	0.155	0.977	0.96 (0.67-1.38)	0.821	1.000	0.69 (0.42-1.14)	0.145	0.968
rs6762782	121905657	G/A (0.387)	1.03 (0.82- 1.30)	0.792	1.000	0.87 (0.65-1.17)	0.359	0.999	1.32 (0.90-1.94)	0.152	0.973
rs1814740	121918491	A/G (0.489)	1.11 (0.90-1.39)	0.334	0.999	1.19 (0.90-1.58)	0.221	0.992	1.00 (0.70-1.41)	0.979	1.000
rs35274320	121937943	G/A (0.160)	0.78 (0.46-1.33)	0.367	0.999	0.77 (0.37-1.59)	0.483	1.000	0.89 (0.39-2.05)	0.790	1.000
rs1354162	121954077	C/A (0.091)	0.75 (0.48-1.17)	0.204	0.993	0.53 (0.28-0.97)	0.041	0.691	1.30 (0.65-2.62)	0.462	1.000
rs7637874	121966952	C/T (0.239)	0.85 (0.66-1.11)	0.229	0.995	0.77 (0.55-1.08)	0.127	0.946	1.07 (0.71-1.60)	0.763	1.000
rs34345120	121968267	C/T (0.055)	1.32 (0.88-1.98)	0.177	0.987	1.49 (0.92-2.41)	0.108	0.933	1.04 (0.46-2.33)	0.929	1.000
rs1463890	121969937	T/C (0.140)	0.80 (0.60-1.06)	0.118	0.947	0.77 (0.54-1.10)	0.146	0.958	0.83 (0.50-1.38)	0.467	1.000
rs7647446	121970020	G/A (0.185)	1.16 (0.87-1.55)	0.306	0.999	1.05 (0.73-1.51)	0.785	1.000	1.36 (0.83-2.22)	0.226	0.992
rs937625	121971512	T/G (0.085)	1.18 (0.80-1.73)	0.402	0.999	1.13 (0.68-1.88)	0.645	1.000	1.37 (0.76-2.44)	0.293	0.998
rs10222633	121976926	G/A (0.492)	0.72 (0.47-1.10)	0.138	0.968	0.88 (0.51-1.51)	0.640	1.000	0.46 (0.21-0.99)	0.046	0.699
rs10934578	121977282	G/T (0.334)	1.70 (1.06-2.73)	0.029	0.554	1.62 (0.88-2.99)	0.121	0.946	1.66 (0.79-3.47	0.182	0.981
rs3804592	121979229	G/A (0.133)	0.86 (0.60-1.23)	0.398	1.000	0.79 (0.52-1.21)	0.276	0.996	1.09 (0.54-2.20)	0.819	1.000
rs17250717	121980186	G/T (0.095)	1.24 (0.81-1.89)	0.327	0.999	1.66 (0.92-2.98)	0.092	0.910	0.75 (0.39-1.42)	0.369	0.999

rs1801725	122003757	G/T (0.140)	1.08 (0.79-1.47)	0.628	1.000	1.03 (0.69-1.53)	0.897	1.000	1.25 (0.75-2.08)	0.396	1.000
rs1042636	122003769	A/G (0.086)	1.32 (0.95-1.83)	0.095	0.918	1.39 (0.91-2.12)	0.123	0.947	1.15 (0.67-1.96)	0.618	1.000
rs1802757	122005131	C/T (0.145)	1.19 (0.87-1.62)	0.282	0.998	1.14 (0.75-1.72)	0.535	1.000	1.41 (0.86-2.32)	0.171	0.979

Abbreviations: CRC, colorectal cancer; HR, hazard ratio, MAF, minor allele frequency.

[a] SNP locations were mapped according to the NCBI build 36 coordinates.

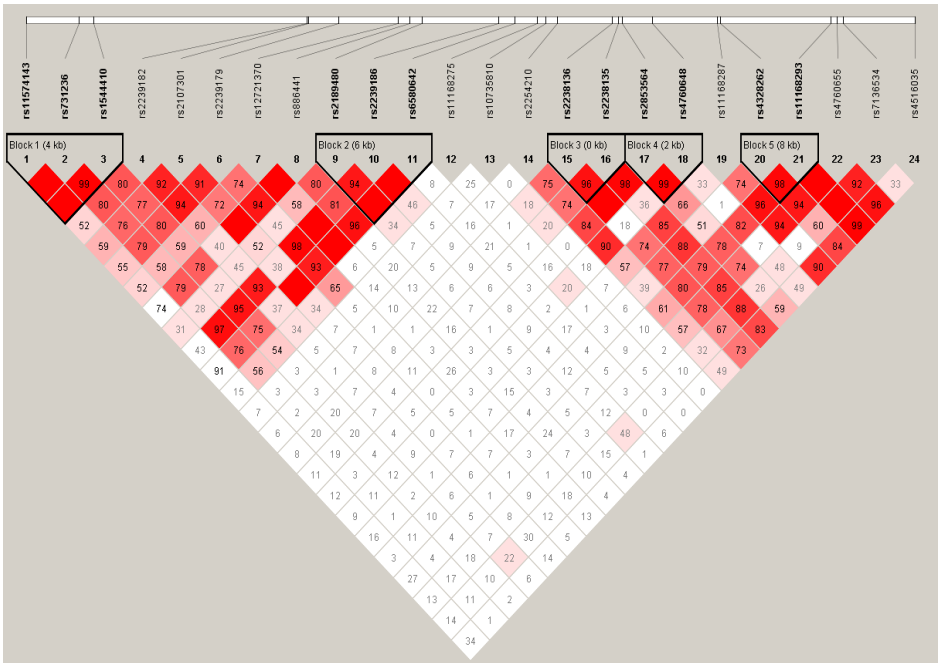
[b] Hazard ratio calculated in reference to the allele underlined. Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, race, reported screening procedure, marital status, MSI status, and BRAF mutation status where appropriate.

[c] *P*-values were adjusted for multiple comparisons using a modification of P_{ACT} for correlated tests developed by Conneely and Boehnke (Conneely & Boehnke, 2007).

Haplotypes and survival of colorectal cancer patients

To evaluate potential epistatic or combined effects of SNPs, haplotype analysis was conducted to derive haplotype groups within linkage equilibrium blocks of each gene. We identified five major blocks on *VDR* and four blocks on *CASR* respectively (Figure 4.2.2, Table 4.2.5). For the VDR gene, the haplotype A-T-G in LD block 1 (rs11574143-TaqI-BsmI), which contained the borderline significant BsmI risk G allele from previous single SNP analysis, was associated with a reduced OS of rectal cancer in comparison to the most common haplotype (HR, 2.53; 95%CI, 1.20-5.34; block global P=0.027), although this association was no longer significant when Bonferroni's correction was applied. For *CASR*, a less frequent (4.2%) haplotype designated as G-G-G-G-G-A-C in block 4 of *CASR* (rs10222633-rs10934578-rs3804592-rs17250717-rs1801725-rs1042636-rs1802757) was associated with a marked increase in odds of all-cause mortality among patients with colon cancers (HR, 3.15; 95%CI, 1.66-5.96; block global *P*=0.001). This association remained significant after Bonferroni correction (*P*<0.0014). Results were similar for DFS.

A.



B.

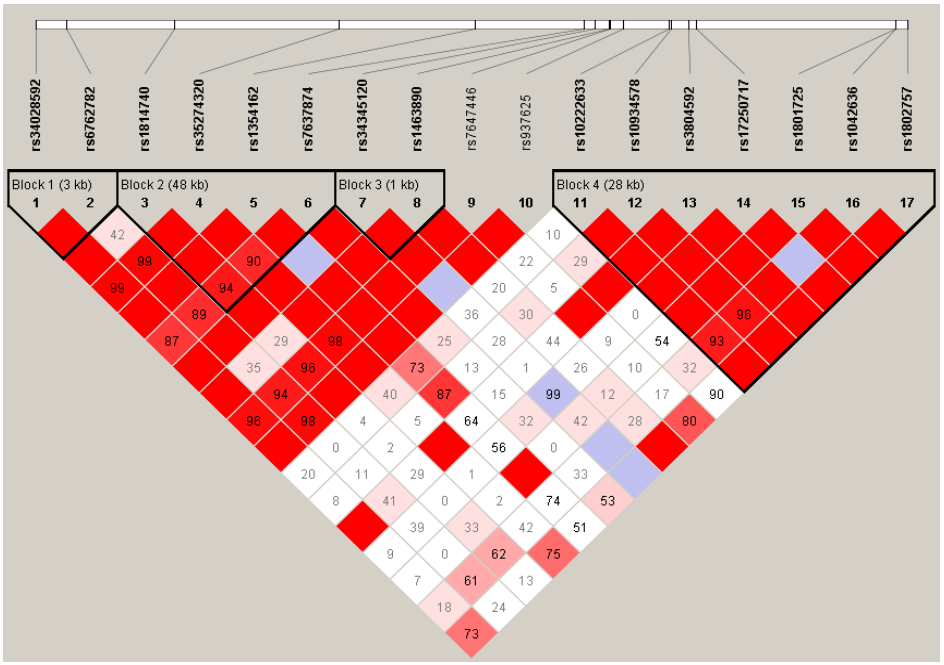


Figure 4.2.2 The linkage disequilibrium (LD) plot of A. *VDR* and B. *CASR* genes. LD strength between the SNPs was indicated by the standard Haploview color scheme based on both D' and LOD values (D'<1 and LOD<2 in white; D'=1 and LOD<2 in blue; D'< 1 and LOD≥2 in shades of pink/red; D'=1 and LOD≥2 in bright red). Numbers in squares are D' (×100), but those with D'=1 are not shown. The black triangle marks the single haplotype block within each gene.

Table 4.2.5 Haplotypes on *VDR* and *CASR* genes and associations with overall and disease-free survival among colorectal cancer patients (n=531)

Haplotypes	Frequency [k]	Overall survival HR (95%CI) [j]			Disease-free survival HR (95%CI) [j]		
		All CRC	Colon cancer	Rectum cancer	All CRC	Colon cancer	Rectum cancer
VDR, block 1 [a]							
GTG	0.5010	1.00	1.00	1.00	1.00	1.00	1.00
GCA	0.4060	0.67 (0.51-0.87)	0.65 (0.47-0.90)	0.66 (0.40-1.08)	0.72 (0.57-0.92)	0.77 (0.57-1.04)	0.66 (0.43-1.02)
ATG	0.0846	0.96 (0.61-1.50)	0.64 (0.46-1.16)	2.53 (1.20-5.34)	0.87 (0.58-1.31)	0.81 (0.48-1.36)	1.31 (0.65-2.64)
Global *P* [l]		0.236	0.790	0.027	0.293	0.773	0.229
VDR, block 2 [b]							
CTC	0.4473	1.00	1.00	1.00	1.00	1.00	1.00
ACC	0.1992	1.39 (1.01-1.92)	1.37 (0.91-2.07)	1.30 (0.73-2.32)	1.36 (1.00-1.84)	1.18 (0.81-1.72)	1.71 (0.98-2.99)
ATC	0.1749	0.94 (0.65-1.35)	0.89 (0.56-1.40)	1.12 (0.58-2.14)	0.99 (0.72-1.38)	0.95 (0.62-1.45)	1.03 (0.59-1.81)
CTT	0.1729	0.90 (0.62-1.29)	0.92 (0.58-1.44)	0.71 (0.36-1.41)	1.02 (0.74-1.42)	1.05 (0.69-1.60)	0.86 (0.48-1.54)
Global *P* [l]		0.325	0.559	0.470	0.923	0.967	0.880
VDR, block 3 [c]							
GG	0.7469	1.00	1.00	1.00	1.00	1.00	1.00
AC	0.2178	1.13 (0.84-1.51)	1.04 (0.70-1.55)	1.30 (0.83-2.040	1.09 (0.83-1.43)	0.99 (0.69-1.43)	1.16 (0.76-1.78)
AG	0.0238	1.38 (0.62-3.04)	1.21 (0.46-3.16)	3.07 (0.62-14.59)	1.31 (0.63-2.71)	1.39 (0.58-3.30)	1.30 (0.28-5.81)
GC	0.0115	0.80 (0.25-2.52)	0.59 (0.14-2.41)	2.18 (0.21-18.83)	0.76 (0.24-2.36)	0.67 (0.16-2.69)	1.35 (0.17-9.76)
Global *P* [l]		0.638	0.897	0.228	0.550	0.678	0.456
VDR, block 4 [d]							
CC	0.4176	1.00	1.00	1.00	1.00	1.00	1.00
TT	0.4091	1.06 (0.82-1.38)	0.99 (0.72-1.38)	1.26 (0.80-2.00)	1.10 (0.86-1.39)	1.05 (0.77-1.42)	1.17 (0.78-1.77)
TC	0.1698	1.09 (0.77-1.53)	0.95 (0.60-1.51)	1.36 (0.80-2.33)	1.09 (0.80-1.50)	0.98 (0.65-1.48)	1.19 (0.72-1.97)
Global *P* [l]		0.762	0.926	0.447	0.171	0.438	0.289
VDR, block 5 [e]							
GG	0.4044	1.00	1.00	1.00	1.00	1.00	1.00
TT	0.3264	0.97 (0.73-1.30)	1.25 (0.86-1.83)	0.63 (0.38-1.04)	0.96 (0.74-1.26)	1.29 (0.92-1.82)	0.59 (0.37-0.97)
TG	0.2667	1.03 (0.77-1.38)	1.19 (0.81-1.75)	0.87 (0.55-1.38)	1.08 (0.82-1.41)	1.26 (0.88-1.80)	0.82 (0.53-1.27)
Global *P* [l]		0.707	0.383	0.119	0.283	0.313	0.038
CASR, block 1 [f]							
AG	0.6175	1.00	1.00	1.00	1.00	1.00	1.00
AA	0.1983	1.04 (0.77-1.42)	1.37 (0.95-1.99)	0.67 (0.38-1.19)	1.08 (0.81-1.42)	1.27 (0.89-1.81)	0.85 (0.53-1.37)
GA	0.1842	0.90 (0.65-1.24)	1.06 (0.72-1.57)	0.65 (0.37-1.14)	0.87 (0.64-1.17)	1.03 (0.70-1.50)	0.66 (0.39-1.11)
Global *P* [l]		0.841	0.125	0.123	0.792	0.627	0.357
CASR, block 2 [g]							

GGCC	0.3272	1.00	1.00	1.00	1.00	1.00	1.00
AGCT	0.2284	0.74 (0.54-1.02)	0.70 (0.46-1.06)	0.91(0.55-1.50)	0.76 (0.57-1.02)	0.72 (0.49-1.05)	0.91 (0.59-1.41)
AGCC	0.1860	0.89 (0.63-1.25)	1.13 (0.73-1.74)	0.62(0.34-1.13)	0.94 (0.69-1.28)	1.11 (0.75-1.66)	0.70(0.41-1.18)
GACC	0.1617	0.65 (0.45-0.96)	0.74 (0.47-1.19)	0.50 (0.24-1.05)	0.67 (0.47-0.96)	0.80 (0.51-1.23)	0.49(0.25-0.95)
AGAC	0.0911	0.49 (0.28-0.85)	0.29 (0.13-0.68)	0.92 (0.39-2.12)	0.65 (0.41-1.04)	0.50 (0.26-0.94)	0.95(0.45-2.02)
Global *P* [l]		0.070	0.047	0.477	0.300	0.113	0.490
CASR, block 3 [h]							
CT	0.8618	1.00	1.00	1.00	1.00	1.00	1.00
CC	0.0846	1.02 (0.66-1.57)	1.19 (0.70-2.05)	0.86 (0.40-1.84)	1.19 (0.81-1.76)	1.13 (0.68-1.89)	1.37 (0.77-2.46)
TC	0.0536	1.19 (0.74-1.92)	1.37 (0.78-2.39)	0.82 (0.32-2.09)	1.33 (0.89-2.01)	1.49 (0.91-2.43)	1.08 (0.48-2.45)
Global *P* [l]		0.910	0.741	0.819	0.677	0.617	0.908
CASR, block 4 [i]							
AGGGGAC	0.3440	1.00	1.00	1.00	1.00	1.00	1.00
AGGGGAT	0.1598	1.54 (1.03-2.31)	1.55 (0.93-2.59)	1.55 (0.78-3.07)	1.46 (1.01-2.10)	1.54 (0.95-2.50)	1.48 (0.82-2.66)
GTGGTAC	0.1363	1.10 (0.73-1.65)	1.36 (0.81-2.28)	0.68 (0.32-1.42)	1.19 (0.83-1.70)	1.39 (0.88-2.20)	0.86 (0.47-1.58)
GGAGGAC	0.1231	1.42 (0.91-2.22)	1.62 (0.96-2.76)	0.75 (0.30-1.85)	1.40 (0.94-2.09)	1.59 (0.99-2.56)	0.88 (0.39-1.99)
GTGTGAC	0.0968	1.11 (0.66-1.88)	0.84 (0.42-1.68)	1.66 (0.70-3.93)	1.03 (0.65-1.64)	0.82 (0.44-1.54)	1.38 (0.64-2.96)
GTGGGGC	0.0808	1.83 (1.20-2.79)	2.34 (1.38-3.97)	1.18 (0.54-2.58)	1.56 (1.07-2.28)	1.73 (1.06-2.82)	1.21 (0.64-2.28)
GGGGGAC	0.0423	2.30 (1.33-3.98)	**3.15 (1.66-5.96)**	0.75 (0.21-2.69)	2.15 (1.31-3.52)	**3.25 (1.80-5.85)**	0.73 (0.24-2.24)
GTGGGAC	0.0151	1.69 (0.60-4.68)	3.05 (0.85-10.63)	0.59 (0.07-4.39)	1.16 (0.39-3.31)	1.89 (0.51-6.64)	0.45 (0.06-3.21)
Global *P* [l]		0.015	0.001	0.423	0.014	0.001	0.944

Abbreviations: CRC, colorectal cancer; HR, hazard ratio.

[a] *VDR*, block 1 includes rs11574143, rs731236, and rs1544410.

[b] *VDR*, block 2 includes rs2189480, rs2239186, and rs6580642.

[c] *VDR*, block 3 includes rs2238136 and rs2238135.

[d] *VDR*, block 4 includes rs2853564 and rs4760648.

[e] *VDR*, block 5 includes rs4328262 and rs11168293.

[f] *CASR*, block 1 includes rs34028592 and rs6762782.

[g] *CASR*, block 2 includes rs1814740, rs35274320, rs1354162, and rs7637874.

[h] *CASR*, block 3 includes rs34345120 and rs1463890.

[i] *CASR*, block 4 includes rs10222633, rs10934578, rs3804592, rs17250717, rs1801725, rs1042636, and rs1802757.

[j] Cox proportional hazard model adjusted for age at diagnosis, sex, race, stage at diagnosis, reported screening procedure, marital status, and MSI status where applicable; subjects with missing information on tumor location (n = 14) were excluded from the stratified analysis.

[k] Rare haplotypes with frequencies less than 1% were excluded from analyses.

[l] Global P for association is from a Wald test with degrees of freedom equal to the number of haplotypes

Those with significant *P*-values after Bonferroni correction for thirty-six haplotypes are shown in bold (i.e. the adjusted *P*-value at the 0.05 significance level is 0.0014).

Gene-diet interactions

We evaluated relationships between *VDR* or *CASR* variations and overall survival among colorectal cancer patients after stratification by dietary vitamin D and calcium intakes (Table 4.2.6). We saw HRs>1 for *CASR* SNP rs1042636 (R990G) (HR, 2.21; 95%CI, 1.37-3.56; P_{int}=0.040) and the haplotype G-T-G-G-G-G-C in *CASR* LD block 4 (HR, 2.21; 95%CI, 1.36-3.58; P_{int}=0.017) in the stratum with calcium intake below the median, although the associations lost significance after adjustment for multiple tests. No evidence for modification by dietary vitamin D intake was observed.

Table 4.2.6 Association between selected genetic variations in *VDR* and *CASR* and colorectal cancer overall survival stratified by dietary vitamin D and calcium intakes

Variant	Alleles [a]/ Haplotypes	Dietary vitamin D HR (95% CI) [b]		P_{int}	Dietary calcium HR (95% CI) [b]		P_{int} [d]
		<Median [c]	≥Median [c]		<Median [c]	≥Median [c]	
No. of deaths/At risk		63/214	71/217		65/218	69/213	
VDR							
rs731236 (*TaqI*)	T/C	1.40 (0.95-2.05)	1.38 (0.96-1.98)	0.976	1.03 (0.99-1.06)	1.30 (0.92-1.82)	0.386
rs1544410 (*BsmI*)	G/A	1.46 (0.99-2.13)	1.38 (0.96-1.98)	0.880	1.63 (1.10-2.40)	1.31 (0.93-1.84)	0.336
rs10735810 (*FokI*)	G/A	1.43 (0.95-2.13)	0.82 (0.59-1.14)	0.052	1.35 (0.91-2.01)	0.78 (0.56-1.10)	0.047
Linkage block 1 [e]	GTG	1.49 (1.03-2.16)	1.21 (0.85-1.73)	0.138	1.57 (1.09-2.26)	1.18 (0.84-1.68)	0.123
	GCA	0.72 (0.49-1.05)	0.73 (0.50-1.05)	0.406	0.64 (0.43-0.94)	0.77 (0.55-1.09)	0.290
	ATG	0.86 (0.42-1.77)	1.43 (0.80-2.55)	0.300	0.97 (0.49-1.92)	1.45 (0.80-2.61)	0.280
Linkage block 2 [f]	CTC	0.76 (0.52-1.11)	1.04 (0.74-1.48)	0.207	0.85 (0.59-1.23)	1.00 (0.70-1.41)	0.749
	ACC	1.59 (1.00-2.51)	1.30 (0.86-1.95)	0.342	1.69 (1.06-2.70)	1.10 (0.71-1.73)	0.313
	ATC	1.04 (0.62-1.72)	0.87 (0.56-1.36)	0.925	0.83 (0.50-1.39)	0.94 (0.60-1.46)	0.636
	CTT	0.85 (0.53-1.39)	0.74 (0.45-1.20)	0.438	0.84 (0.53-1.35)	0.89 (0.55-1.45)	0.864
CASR							
rs1801725 (A986S)	G/T	1.36 (0.81-2.30)	1.30 (0.79-2.14)	0.995	1.13 (0.70-1.85)	1.41 (0.84-2.39)	0.619
rs1042636 (R990G)	A/G	1.93 (1.16-3.22)	1.20 (0.71-2.03)	0.216	2.21 (1.37-3.56)	1.01 (0.57-1.81)	0.040
Linkage block 2 [g]	GGCC	1.65 (1.15-2.38)	1.27 (0.88-1.82)	0.104	1.37 (0.95-1.98)	1.46 (1.02 -2.10)	0.705
	AGCT	0.76 (0.48-1.22)	0.90 (0.62-1.31)	0.099	0.78 (0.50-1.21)	0.88 (0.59-1.33)	0.371
	AGCC	1.06 (0.68-1.64)	1.15 (0.76-1.77)	0.809	1.06 (0.70-1.61)	1.24 (0.80-1.94)	0.957
	GACC	0.70 (0.40-1.22)	0.84 (0.54-1.33)	0.905	0.87 (0.51-1.49)	0.71 (0.44-1.13)	0.370
	AGAC	0.55 (0.25-1.22)	0.58 (0.27-1.25)	0.670	0.67 (0.30-1.48)	0.51 (0.24-1.09)	0.801
Linkage block 4 [h]	AGGGGAC	0.63 (0.41-0.96)	0.82 (0.56-1.21)	0.456	0.73 (0.49-1.09)	0.73 (0.49-1.10)	0.753
	AGGGGAT	1.44 (0.89-2.34)	1.11 (0.67-1.85)	0.465	1.02 (0.61-1.71)	1.44 (0.89-2.33)	0.892
	GTGGTAC	0.74 (0.43-1.26)	0.77 (0.46-1.27)	0.628	0.88 (0.54-1.45)	0.70 (0.41-1.20)	0.895
	GGAGGAC	1.72 (0.96-3.10)	0.83 (0.46-1.48)	0.131	1.31 (0.70-2.47)	0.97 (0.55-1.71)	0.793
	GTGTGAC	0.62 (0.28-1.34)	1.11 (0.59-2.07)	0.100	0.51 (0.23-1.11)	1.20 (0.64-2.25)	0.208
	GTGGGGC	1.96 (1.16-3.29)	1.20 (0.71-2.05)	0.058	2.21 (1.36-3.58)	1.04 (0.58-1.87)	0.017
	GGGGGAC	1.18 (0.45-3.01)	2.58 (1.36-4.84)	0.163	1.22 (0.51-2.90)	2.79 (1.46-5.31)	0.181

[a] Two variants at the locus presented as: major allele/minor allele. Hazard ratio calculated in reference to the allele underlined.

[b] Cox proportional hazard model adjusted for age at diagnosis, sex, race, stage at diagnosis, reported screening procedure, marital status, and MSI status where appropriate.

[c] Median dietary intakes are 5.6 μg/d for vitamin D and 862.1 mg/d for calcium.

[d] *P* for interaction is computed with Wald method testing significance of multiplicative interaction term between genetic variants and respective stratified variable; not adjusted for multiple comparisons.

[e] *VDR*, linkage block 1 includes rs11574143, rs731236, and rs1544410.

[f] *VDR*, linkage block 2 includes rs2189480, rs2239186, and rs6580642.
[g] *CASR*, linkage block 2 includes rs1814740, rs35274320, rs1354162, and rs7637874.
[h] *CASR*, linkage block 4 includes rs10222633, rs10934578, rs3804592, rs17250717, rs1801725, rs1042636, and rs1802757.

Discussion

In this study, *VDR* and *CASR* genes were associated with DFS and OS of colorectal cancer, respectively, at the gene level. Particularly, *VDR* BsmI polymorphism exhibited marginally significant association with the OS of colorectal cancer patients after adjustment for multiple comparisons. Haplotype analyses showed that the *CASR* block 4 haplotype G-G-G-G-G-A-C, defined by rs10222633-rs10934578-rs3804592-rs17250717-rs1801725-rs1042636-rs1802757, was associated with a reduced OS among colon cancer patients.

Experimental work indicates that vitamin D has pleiotropic biological activities with complex anticancer properties which include inhibition of cell proliferation, invasion, induction of apoptosis, cell cycle arrest, as well as simulation of differentiation [18]. The VDR has been proposed as a potential mediator that could modulate the effects of vitamin D. For example, VDR interacts with β-catenin and inhibits β-catenin signaling that is deregulated in most colorectal cancers [18, 34]. Through the nuclear VDR, $1,25(OH)_2D_3$ induces E-cadherin, increases β-catenin nuclear export, and inhibits β-catenin gene regulatory activity to hinder proliferation and loss of differentiation in the early stage of carcinogenesis [18]. *VDR* is highly polymorphic with over 100 known allelic variants. Some polymorphisms may have functional importance and thus have been evaluated in previous association studies on colorectal cancer. Specifically, the FokI translational start codon polymorphism alters the VDR structurally, with the F-variant VDR being three amino acids shorter and functionally more effective than the protein produced from the f allele [87, 88]. BsmI (intron 8), ApaI (intron 8) and TaqI (exon 9) polymorphisms have been reported to influence *VDR* expression and thus serum levels of $1,25(OH)_2D_3$, but they are speculated to affect VDR function through linkage disequilibrium with other mutations in the 3-UTR region that alter mRNA transcriptional activity and stability [89, 90]. Although not a universal finding, the B allele of the *VDR* BsmI polymorphism has been found

to be linked with a reduced risk of colorectal cancer [22, 28]. Carriage of the variant FokI allele revealed either null [91, 92] or conflicting associations [88, 93, 94]. With regard to survival after cancer diagnosis, several SNPs and haplotypes in *VDR* gene that are related to lower *VDR* function or expression (Cdx2, FokI and G-T-C for Cdx2-FokI-BsmI) have been linked with poorer survival for a variety of cancers such as non-small cell lung [169, 170] and epithelial ovarian [171] cancers. Few studies have been published on *VDR* polymorphisms and colorectal cancer survival [15, 32-34, 127]. Nevertheless, these studies did not observe any significant associations of *VDR* variants with colorectal cancer survival. In our study, the variant B (A) allele of the *VDR* BsmI appeared to be associated with an improved overall survival among colorectal cancer patients; yet no association was detected for *VDR* FokI polymorphism. The inconsistencies in findings with previous studies may be explained by differences in population, other environmental factors (e.g., diet and lifestyle), and incomplete coverage of the gene in some studies [15, 32, 33, 127]. While we do not presume to know how the *VDR* BsmI SNP influences survival for colorectal cancer, the pathway may be involved in the biological response to treatments. As such, the BsmI polymorphism warrants further investigation in terms of its role in treatment response and patient prognosis in colorectal cancer.

Another gene analyzed in the present study is the *CASR*. The *CASR* is crucial for the maintenance of extracellular calcium homeostasis by affecting parathyroid hormone secretion and calcium reabsorption [21, 22]. It may also influence vitamin D metabolism. CASR has been implicated in breast and prostate cancers [99]. Indeed, several commonly studied *CASR* polymorphisms, including rs1801725 [96], rs1042636 [98], rs10934578, rs12485716, rs2270916 and rs4678174 [24], have been related to the risk of colorectal cancer in some studies, but not others [22, 30, 97]. In the work by Kim et al [90], these SNPs were significant only under low calcium intake. Despite the many studies

investigating *CASR* and colorectal cancer risk, we found only two published papers [15, 31] that evaluated the association of *CASR* and colorectal cancer survival and observed no relationship, though only one SNP (rs1801725) was included in both studies. In the current study, none of these high-interest SNPs in the literature (rs10934578, rs1801725 and rs1042636) were significantly related to colorectal cancer survival. But, there was a suggestion that an intronic SNP in *CASR*, rs1354162, was associated with more favorable survival in colon cancer patients. The possible mechanisms explaining this association remain undetermined, particularly since rs1354162 is within an intron. Recent studies suggest that intronic SNPs have the potential to influence alternative splicing of RNA [172, 173]. Interestingly, we found that the wild-type haplotype, G-G-G-G-G-A-C, in block 4 of *CASR* was associated with worse OS of colon cancer patients compared with the most common haplotype. Several SNPs defining this haplotype are nonsynonymous coding SNPs (i.e., rs1801725 and rs1042636) or intron variants shown to be robustly associated with serum levels of calcium in recent GWAS studies (i.e., rs10222633 and rs10934578) [174]. We speculate that the region where these SNPs located may harbor a site of causative variants that in conjunction with each other impact on disease outcome; and these seven SNPs should be considered as candidate tag SNPs within the *CASR* gene for future association studies. Our results confirm with the theoretical expectation that haplotype-based approaches may have greater power than single-locus tests [175].

While it is unclear why *CASR* variation is related to survival only among colon cancer patients, and not rectal cancer patients, the difference in structure and cellular composition of the surface epithelium between colon (ciliated columnar epithelium) and lower rectum (squamous epithelium) may partially account. Alternatively, bile acids, formed in the liver and absorbed from the intestine has been shown to enhance intestinal proliferation and tumor yield [176]. Calcium could bind to secondary bile acids to neutralize mucosal toxicity and

reduce cell proliferation. The longer transit time in colon than rectum might simply allow more time for calcium to exert its action. Therefore, the *CASR* variants may be more influential in the progression of colon cancer where calcium may have a stronger protective effect.

A novel aspect of our study is the inclusion of gene-diet interaction. A previous study [90] on *CASR* polymorphisms (rs10934578, rs12485716, rs2270916, and rs4678174) and colorectal cancer risk has linked all four of these SNPs to an elevated risk of colorectal cancer only in the lower calcium category, which is consistent with our findings of a stronger effect of *CASR* variants in the low calcium group. Presumably the influence of subtle differences between genotypes was overwhelmed by the protective effect of high-dose calcium [88]. Although the probability that these interactions are false-positive findings is high, this study still contributes to the overall evidence that calcium may act as a potential effect modifier in relation to the relationship between *CASR* genotypes and colorectal cancer survival. If the gene-nutrient interaction will be replicated in further studies, then cancer patients, especially those with detrimental genotypes, may benefit from the use of calcium supplements to improve their survival. Such supplementations should be based on well-designed and carefully conducted RCTs.

The strengths of the current study include its relatively large size, long follow-up period (up to 10 years), and detailed information on potential confounders and effect modifiers. Limitations to this study include a lack of the cause of death data for all deceased patients; however, we obtained the cause of death defined by ICD codes for 104 of 183 deceased patients; thereof the majority (90.4%) was due to colorectal cancer. In addition, the lack of serum levels of 25-hydroxyvitamin D (25(OH)D) and calcium impeded us to test possible 25(OH)D/calcium-diet/gene interactions and to evaluate the extent to which the gene-colorectal cancer outcome association is mediated through serum levels of 25(OH)D/calcium. Moreover, post-diagnosis diet was not measured in this study. Therefore we were unable to

assess how post-diagnostic dietary factors interact with *VDR* and *CASR* genotypes to influence colorectal cancer prognosis. Besides, we cannot rule out effects of other genes with polymorphisms in the vitamin D and calcium metabolism pathway that may also influence the overall colorectal cancer initiation and progression. It is also notable that most SNPs examined in this work are tagging SNPs, which are selected merely as indicators for specific regions of interest; thus, there is a low probability that they are the causal SNPs [34]. Therefore, first the replication of this work in other populations and then in detail examination of the other polymorphisms in the *VDR* and *CASR* genes is necessary to identify truly causal variants.

Conclusions

Our results suggest that polymorphic variations of *VDR* and *CASR* are associated with survival in patients with colorectal cancer. These findings indicate that certain variants of the *VDR* and *CASR* genes may be utilized as novel biomarkers for predicting prognosis in colorectal cancer patients.

4.3 Paper 3. Prediagnostic Consumption of Calcium and Dairy Products and Colorectal Cancer Survival: Results from the Newfoundland Familial Colorectal Cancer Cohort Study

Authors

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Abstract

Background

Vitamin D, calcium, and dairy products are inversely associated with colorectal cancer incidence, but little is known of their influence on colorectal cancer survival. We investigated prediagnostic intakes of vitamin D, calcium, and dairy products for their relevance to colorectal cancer prognosis.

Methods

We analyzed 504 colorectal cancer patients enrolled in the Newfoundland Familial Colorectal Cancer Study who were diagnosed for the first time with colorectal cancer from 1999 to 2003. Mortality and cancer recurrence followed-up was through April 2010. Data on diet and lifestyle factors were gathered via epidemiological questionnaires. Multivariate Cox models estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for the relationship of prediagnostic intakes of vitamin D, calcium, and dairy products with all-cause mortality (overall survival, OS) and disease-free survival (DFS) among colorectal cancer patients.

Results

Prediagnostic calcium intake from foods, but not total calcium intake, was negatively associated with all-cause mortality (HR for Q2 vs. Q1, 0.44; 95% CI, 0.26-0.75, P trend=0.980). An inverse relationship was also seen in a dose-response fashion for prediagnostic cheese intake (HR for Q4 vs. Q1, 0.57, 95% CI, 0.34-0.95, P trend=0.029). No evidence for modification by factors known to be associated with colorectal cancer survival was observed.

Conclusions

High prediagnostic intakes of cheese and calcium from foods may be associated with increased survival among colorectal cancer patients.

Background

Colorectal cancer, one of the most common cancer types in Western countries, accounts for an estimated total death of 9,300 per year in Canada (12% of all cancer deaths) [38]. The etiology of colorectal cancer involves various modifiable lifestyle factors, especially diet; but to date, only intakes of alcoholic drinks and red and processed meat are regarded as convincing dietary risk factors for colorectal cancer [37].

Dairy products have been hypothesized to be inversely associated with colorectal cancer risk due to their high content of calcium, which may induce apoptosis [6], inhibit heme-associated colon carcinogenesis [7], and reduce cell proliferation directly [8] and indirectly through binding proinflammatory secondary bile acids and fatty acids to render them inert [9, 10]. As adequate vitamin D level is crucial for proper calcium absorption, the anticancer effects of diary products and calcium are strongly dependent on vitamin D status. A published meta-analysis of data from 19 prospective cohort studies on colorectal cancer (the WCRF/AICR Continuous Update Project (CUP)) [52] reported a 17% decreased risk per 400g/day of total dairy products and a 9% decreased risk per 200 g/day of milk intake. The CUP meta-analyses for dietary vitamin D and calcium also showed inverse associations with colorectal cancer risk, but indicated that evidence for vitamin D and calcium was limited and/or suggestive [37].

Although the inverse associations between intakes of vitamin D, calcium and dairy products have been consistently seen, their impact on survival after colorectal cancer diagnosis is largely unknown. The only four available studies on prediagnostic consumption of dairy products in relation to colorectal cancer survival have yielded inconclusive results. In the Japan Collaborative Cohort Study [11], high yogurt intake was associated with reduced risk of rectal cancer mortality in male, whereas others [10, 12, 13] yield null associations. The Cancer Prevention Study-II Nutrition Cohort Study [12] reported that only postdiagnosis

intake of dairy products were relevant to survival in patients with colorectal cancer. Findings for dietary vitamin D (or 25-hydroxyvitamin D), calcium and colorectal cancer survival are similarly mixed [12, 14, 15]. In addition, these published reports were mainly conducted in the United States, Japan, and western Europe, but none of these were from the Canadian population. We therefore investigated whether prediagnostic consumption of vitamin D, calcium, and dairy products (total, milk, yogurt, and cheese) is associated with colorectal cancer overall (all-cause death) and disease-free survival in a population-based cohort of colorectal cancer patients enrolled in the Newfoundland Familial Colorectal Cancer Study (NFCCS).

Materials and methods

Study population

The Newfoundland Familial Colorectal Cancer Study has been previously described [4, 146, 163]. Briefly, patients aged 20-75 years with a first-time diagnosis of pathologically confirmed colorectal cancer between 1997 and 2003 were enrolled through the Newfoundland Colorectal Cancer Registry (NFCCR) (N=737). Consenting patients received mail-in epidemiological questionnaires that assessed their diet and lifestyle habits from one year prior to diagnosis and were asked for access to their archived tumor tissue and medical records. The current analysis excluded patients with unknown vital status at end of follow-up, those who provided insufficient dietary information, and those who reported total caloric intakes in the upper or lower 2.5% (870 and 4330 kcal/d for men, 1010 and 5050 kcal/d for women, respectively). After exclusion, 504 eligible colorectal cancer patients remained in the final analytical cohort. The study protocol was approved by

the Human Investigation Committee of Memorial University of Newfoundland. All participants provided informed consent.

Diet assessment and baseline information collection

Information on demographics, socio-economic status, individual behaviors, medical history cancer, and family history was gathered by detailed Family History Questionnaires (FHQ) and Personal History Questionnaires (PHQ). Participants in the current study also completed a self-administered Food Frequency Questionnaire (FFQ) that included 169 food items, beverages, and vitamin and mineral supplements at the time of recruitment. The semi-quantitative FFQ applied in this study had been previously validated in the Newfoundland and Labrador population [143]. The dietary nutrient intakes for individuals were calculated by multiplying the frequency of consumption of each food item from the FFQ by the nutrient content of a typical portion size [132]. Nutrient values were adjusted for total energy using the residuals method [177]. The microsatellite instability (MSI) status for the tumor DNA have been determined in our previous studies [146].

Study outcomes

Study participants were followed for deaths, recurrence, and metastasis from the date of cancer diagnosis until April 2010. Data on vital status were collected trough a combination of active follow-up (follow-up FHQ) and record linkage to death certificates, pathology reports, autopsy records, physicians' notes, surgical reports, and the records from the Dr. H. Bliss Murphy Cancer Care Foundation. The main outcomes were overall survival (OS) and disease-free survival (DFS). The end-point for the OS analysis was death from all-causes and for the DFS analysis was all-cause death, colorectal cancer recurrence, or metastasis,

whichever came first. Participants did not suffer an event of interest within the study duration were censored on the date of last follow-up.

Statistical analysis

Survival distributions across groups of baseline characteristics were compared with a log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) representing the associations between a defined endpoint event and quartiles of prediagnostic intakes of vitamin D, calcium, and dairy products were calculated with multivariable Cox regression models using the first quartile as the reference. Potential confounders were identified as those that altered the effect estimates by ≥10% or a P<0.1 from the univariable analysis; those included sex, age at diagnosis, stage at diagnosis, marital status, total energy intake, reported chemoradiotherapy and MSI status. The assumption of proportional hazard rates over time was verified for each covariate by checking the parallelism of the Kaplan-Meier curves and testing the significance of an interaction term between the explanatory variable and the natural logarithm of follow-up time. Statistical linear trend was examined based on category median values. Potential interactions were assessed by testing the significance of multiplicative interaction terms using a Wald test for interaction as well as stratified analyses. Bonferroni correction for multiple comparisons was performed for eight nutrients/foods yielding an adjusted P value of 0.0083. P values were two-sided. All data analyses and management were performed in SAS software version 9.4 (SAS Institute Inc, Cary, North Carolina, USA).

Results

Patient characteristics

Of the 504 colorectal cancer patients that were included in this study, a total of 159

(31.5%) subjects died during follow-up (median follow-up time, 6.4 years, Table 4.3.1). The mean age of the study population was 60.9±9.0 yrs., with 65.1% having tumors at the colon subsite. In univariate analyses, old age at diagnosis, male gender, advanced stage at diagnosis (III/IV), and MSS/MSI-L tumors were significantly associated with worse all-cause mortality in this cohort.

Table 4.3.1 Demographical and clinicopathological characteristics of study population (N=504)

Characteristics	No. of patients	No. of deaths (%)	Univariate HR (95% CI) [a]
Age at diagnosis (y) [b]	60.9±9.0	62.0±8.9	1.02 (1.00-1.03)
Sex			
Male	306	106 (34.6)	1.00
Female	198	53 (26.8)	0.70 (0.50-0.98)
BMI (kg/m^2)			
<25.0	140	43 (30.7)	1.00
25.0-29.9	203	70 (34.5)	1.06 (0.72-1.55)
≥30	146	41 (28.1)	0.91 (0.60-1.40)
Marital status			
Single	109	40 (36.7)	1.00
Married or living as married	395	119 (30.1)	0.85 (0.60-1.22)
Tumor location			
Colon	328	97 (29.6)	1.00
Rectum	176	62 (35.2)	1.19 (0.86-1.63)
Stage at diagnosis			
I/II	293	66 (22.5)	1.00
III/IV	211	93 (44.1)	2.36 (1.72-3.24)
Chemoradiotherapy			
No	100	38 (38.0)	1.00
Yes	404	121 (30.0)	1.36 (0.94-1.95)
MSI status			
MSS/MSI-L	423	146 (34.5)	1.00
MSI-H	55	4 (7.3)	0.17 (0.06-0.46)
Smoking status			
Never smokers	138 (27.4)	36 (26.1)	1.00
Ever smokers	366 (72.6)	123 (33.6)	1.27 (0.87-1.84)
Total energy intake (kcal/d) [b]	2455.3±849.4	2491.53±796.7	1.11 (0.96-1.27)

Abbreviations: BMI, body mass index; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS/MSI-L, microsatellite stable/microsatellite instability-low; HR, hazard ratio; CI, confidence interval.

[a] Cox proportional hazard regression.

[b] Continuous variables presented as mean±SD (standard deviation).

Calcium, vitamin D and colorectal cancer survival

We found longer OS time with higher dietary vitamin D intake; compared with the lowest quartile, the HRs (95% CI) were 0.52 (0.31-0.87) for the second quartile and 0.57 (0.34-0.95) for the third quartile (Table 4.3.2). This inverse association was limited to patients diagnosed with rectal cancer only. Likewise, for dietary calcium intake, the risk of all-cause death decreased by 56% for subjects in the second quartile compared with those in the lowest

quartile of consumption. No associations were observed among total vitamin D, total calcium intake and OS in colorectal cancer patients. Results were similar but less significant for DFS.

Table 4.3.2 Association of prediagnosis vitamin D and calcium intakes with colorectal cancer overall and disease-free survival (N=478)

		Overall Survival				Disease-Free Survival			
	Median Intake	**No. of Events [a] /No. at Risk**	**Overall CRC HR (95% CI) [b]**	**Colon cancer HR (95% CI) [b]**	**Rectal cancer HR (95% CI) [b]**	**No. of Events [a] /No. at Risk**	**Overall CRC HR (95% CI) [b]**	**Colon cancer HR (95% CI) [b]**	**Rectal cancer HR (95% CI) [b]**
Dietary vitamin D (μg/d)									
Q1	3.4	37/119	1.00	1.00	1.00	42/119	1.00	1.00	1.00
Q2	5.1	30/120	0.52 (0.31-0.87)	0.68 (0.36-1.30)	0.36 (0.14-0.89)	37/120	0.62 (0.39-0.99)	0.65 (0.36-1.17)	0.68 (0.31-1.47)
Q3	6.6	32/119	0.57 (0.34-0.95)	0.73 (0.37-1.44)	0.44 (0.19-0.99)	41/119	0.77 (0.49-1.20)	0.80 (0.44-1.46)	0.70 (0.35-1.40)
Q4	9.4	52/120	0.93 (0.59-1.45)	1.35 (0.74-2.46)	0.56 (0.27-1.18)	58/120	1.09 (0.72-1.64)	1.36 (0.79-2.32)	0.80 (0.41-1.57)
P for trend [c]			0.709	0.142	0.290		0.273	0.083	0.634
Total vitamin D (diet+supplements; μg/d)									
Q1	3.7	35/119	1.00	1.00	1.00	40/119	1.00	1.00	1.00
Q2	5.6	30/119	0.55 (0.33-0.94)	0.73 (0.37-1.44)	0.47 (0.19-1.14)	39/119	0.76 (0.48-1.21)	0.91 (0.50-1.68)	0.64 (0.30-1.36)
Q3	7.6	42/120	0.90 (0.56-1.45)	1.08 (0.56-2.08)	0.83 (0.39-1.74)	46.120	0.95 (0.61-1.48)	1.14 (0.63-2.09)	0.80 (0.40-1.57)
Q4	16.5	44/120	1.00 (0.62-1.60)	1.47 (0.79-2.71)	0.63 (0.27-1.44)	53/120	1.25 (0.81-1.91)	1.43 (0.81-2.52)	1.03 (0.52-2.08)
P for trend [c]			0.334	0.045	0.463		0.072	0.098	0.514
Dietary calcium (mg/d)									
Q1	630.3	43/119	1.00	1.00	1.00		1.00	1.00	1.00
Q2	826.6	27/120	**0.44 (0.26-0.75)**	0.55 (0.28-1.07)	0.29 (0.10-0.82)	48/119	0.59 (0.37-0.95)	0.67 (0.36-1.24)	0.51 (0.22-1.16)
Q3	1002.0	34/119	0.72 (0.45-1.15)	0.87 (0.46-1.65)	0.55 (0.24-1.23)	32/120	0.85 (0.55-1.31)	1.00 (0.56-1.80)	0.66 (0.33-1.32)
Q4	1330.8	47/120	0.82 (0.53-1.26)	1.05 (0.58-1.92)	0.58 (0.29-1.18)	42/119	1.10 (0.74-1.63)	1.40 (0.81-2.42)	0.82 (0.45-1.49)
P for trend [c]			0.980	0.307	0.199	56/120	0.237	0.051	0.660
Total calcium (diet+supplements; mg/d)									
Q1	639.6	40/119	1.00	1.00	1.00	46/119	1.00	1.00	1.00
Q2	853.7	33/119	0.64 (0.38-1.07)	0.90 (0.45-1.80)	0.42 (0.16-1.13)	37/119	0.79 (0.50-1.26)	1.04 (0.55-1.95)	0.57 (0.25-1.30)
Q3	1056.7	31/120	0.70 (0.43-1.15)	0.95 (0.48-1.91)	0.49 (0.22-1.09)	40/120	0.84 (0.54-1.31)	1.02 (0.55-1.91)	0.67 (0.34-1.32)
Q4	1462.8	47/120	1.03 (0.66-1.60)	1.49 (0.79-2.83)	0.66 (0.32-1.37)	55/120	1.20 (0.79-1.80)	1.58 (0.88-2.84)	0.91 (0.49-1.69)
P for trend [c]			0.056	0.076	0.500		0.048	0.061	0.367

Abbreviations: CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval.

[a] Events are defined as all-cause deaths for overall survival and death/recurrence/metastasis (which occurred earliest) for disease-free survival; subjects with missing information on prediagnosis vitamin D or calcium intakes (n = 26) were excluded from the analysis.

[b] Cox proportional hazard model adjusted for age at diagnosis, sex, stage at diagnosis, marital status, MSI status, chemoradiotherapy, and total energy intake where applicable.

[c] Test for linear trend was based on the median values for each quartile of intake.

Diary products and colorectal cancer survival

Compared with the lowest quartile, the third quartile of total dairy product intake was associated with a decreased risk of all-cause mortality in rectal cancer patients (Table 4.3.3). We also saw better survival in colorectal cancer patients with pre-diagnostic milk in the third quartile compared to those in the lowest quartile of consumption. A significant dose-survival advantage for high vs. low cheese intake was observed (HR for Q4 vs. Q1, 0.57; 95% CI, 0.34-0.95; P-trend=0.029). Of note, we analyzed 8 diet-colorectal cancer survival relationships; Bonferroni adjustment for multiple testing yielded only one significant association between higher calcium intake and longer OS time for this agnostic analysis ($P<0.0083$)

Interaction

Although the protective effect of cheese intake was only evident in patients with physical activity level>22.4 MET h/week (HR for Q4 vs. Q1, 0.42; 95% CI, 0.20-0.86; P-trend=0.012) (Table 4.3.4), none of these examined factors showed statistically significant interaction with the intake of calcium and dairy products (milk and cheese) in relation to colorectal cancer overall survival.

Table 4.3.3 Association of prediagnosis dairy intakes with colorectal cancer overall and disease-free survival (N=504)

		Overall Survival				Disease-Free Survival			
	Median Intake	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]
Total-diary product (g/d)									
Q1	55.1	35/126	1.00	1.00	1.00	40/126	1.00	1.00	1.00
Q2	220.2	46/125	1.03 (0.64-1.67)	1.58 (0.83-3.02)	0.50 (0.22-1.14)	55/125	1.41 (0.91-2.19)	2.02 (1.12-3.65)	0.70 (0.34-1.42)
Q3	369.1	30/127	0.69 (0.40-1.18)	0.99 (0.48-2.04)	0.40 (0.16-0.97)	37/127	0.85 (0.52-1.38)	1.13 (0.59-2.14)	0.51 (0.24-1.11)
Q4	697.5	48/126	1.10 (0.67-1.81)	1.57 (0.80-3.10)	0.68 (0.29-1.55)	56/126	1.45 (0.92-2.29)	1.88 (1.02-3.48)	1.01 (0.50-2.06)
P for trend [d]			0.712	0.293	0.582		0.207	0.136	0.722
Milk (g/d)									
Q1	2.6	39/123	1.00	1.00	1.00	47/123	1.00	1.00	1.00
Q2	164.8	41/121	0.84 (0.51-1.36)	0.73 (0.38-1.39)	1.04 (0.46-2.34)	45/121	0.97 (0.62-1.52)	0.90 (0.50-1.62)	1.02 (0.49-2.12)
Q3	257.0	30/131	0.59 (0.35-0.99)	0.67 (0.36-1.25)	0.57 (0.21-1.48)	38/131	0.68 (0.43-1.07)	0.66 (0.37-1.17)	0.75 (0.34-1.65)
Q4	578.3	49/129	1.03 (0.64-1.65)	1.09 (0.61-1.96)	1.13 (0.49-2.64)	58/129	1.22 (0.80-1.87)	1.19 (0.70-2.03)	1.34 (0.64-2.80)
P for trend [d]			0.629	0.471	0.731		0.232	0.353	0.357
Yogurt (g/d)									
Q1	0	69/217	1.00	1.00	1.00	83/217	1.00	1.00	1.00
Q2	8.6	9/33	1.21 (0.57-2.56)	1.04 (0.40-2.69)	2.24 (0.57-8.77)	10/33	1.07 (0.53-2.15)	0.89 (0.35-2.26)	2.59 (0.86-7.86)
Q3	46.0	39/136	1.04 (0.68-1.60)	1.12 (0.63-2.00)	0.92 (0.45-1.88)	47/136	1.01 (0.69-1.47)	1.41 (0.86-2.32)	0.58 (0.32-1.06)
Q4	175.0	42/118	1.08 (0.67-1.73)	1.20 (0.65-2.18)	0.99 (0.44-2.23)	48/118	1.12 (0.75-1.67)	1.57 (0.94-2.62)	0.64 (0.32-1.27)
P for trend [d]			0.826	0.582	0.815		0.544	0.096	0.299
Cheese (g/d)									
Q1	0	46/128	1.00	1.00	1.00	54/128	1.00	1.00	1.00
Q2	5.3	35/135	0.88 (0.54-1.41)	0.77 (0.42-1.42)	1.14 (0.50-2.58)	42/135	0.77 (0.50-1.18)	0.69 (0.40-1.20)	1.09 (0.54-2.17)
Q3	12.8	40/115	0.89 (0.56-1.43)	0.78 (0.43-1.41)	1.12 (0.48-2.57)	45/115	0.81 (0.53-1.24)	0.71 (0.41-1.23)	1.10 (0.55-2.22)
Q4	30.0	38/126	0.57 (0.34-0.95)	0.61 (0.33-1.15)	0.43 (0.17-1.11)	47/126	0.65 (0.41-1.02)	0.77 (0.44-1.35)	0.43 (0.19-0.94)
P for trend [d]			0.029	0.167	0.048		0.098	0.592	0.019

Abbreviations: CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval.

[a] Events are defined as all-cause deaths for overall survival and death/recurrence/metastasis (which occurred earliest) for disease-free survival.

[b] Cox proportional hazard model adjusted for age at diagnosis, sex, stage at diagnosis, marital status, MSI status, chemoradiotherapy, and total energy intake where applicable.

[d] Test for linear trend was based on the median values for each quartile of intake.

Table 4.3.4 Overall colorectal cancer survival in relation to quartiles of milk, cheese and calcium intakes by selected demographic and lifestyle characteristics of participants

	No. of Events [a] /No. at Risk	Quartiles HR （95% CI） [b]				P trend [c]	P interaction [d]
		Q1	Q2	Q3	Q4		
Milk							
Sex							
Male	106/306	1.00	0.69 (0.37-1.28)	0.53 (0.28-1.01)	1.28 (0.73-2.25)	0.131	
Female	53/198	1.00	1.07 (0.47-2.43)	0.67 (0.26-1.71)	0.77 (0.32-1.83)	0.469	0.225
Physical activity							
≤22.4 MET h/week	67/235	1.00	0.79 (0.39-1.59)	0.61 (0.28-1.33)	0.64 (0.29-1.40)	0.268	
>22.4 MET h/week	92/268	1.00	0.88 (0.43-1.77)	0.61 (0.30-1.22)	1.13 (0.60-2.13)	0.478	0.313
Alcohol drinking							
≤7 drinks/week	99/338	1.00	0.76 (0.41-1.40)	0.51 (0.26-0.99)	0.92 (0.52-1.63)	0.809	
>7 drinks/week	51/138	1.00	0.90 (0.37-2.18)	0.69 (0.28-1.67)	1.61 (0.65-3.97)	0.286	0.550
Cigarette smoking							
Ever smokers	123/366	1.00	0.95 (0.52-1.76)	0.72 (0.39-1.30)	1.24 (0.71-2.17)	0.279	
Never smokers	36/138	1.00	0.73 (0.31-1.75)	0.30 (0.08-1.12)	0.66 (0.21-2.13)	0.244	0.508
Cheese							
Sex							
Male	106/306	1.00	0.88 (0.49-1.59)	1.19 (0.67-2.11)	0.63 (0.33-1.20)	0.165	
Female	53/198	1.00	0.97 (0.41-2.30)	0.53 (0.22-1.29)	0.59 (0.25-1.39)	0.184	0.418
Physical activity							
≤22.4 MET h/week	67/235	1.00	0.89 (0.43-1.85)	1.29 (0.62-2.66)	0.68 (0.31-1.51)	0.385	
>22.4 MET h/week	92/268	1.00	0.88 (0.46-1.69)	0.68 (0.36-1.32)	0.42 (0.20-0.86)	0.012	0.748
Alcohol drinking							
≤7 drinks/week	99/338	1.00	0.84 (0.47-1.52)	1.00 (0.57-1.74)	0.62 (0.33-1.17)	0.164	
>7 drinks/week	51/138	1.00	0.87 (0.36-2.14)	0.72 (0.29-1.82)	0.43 (0.15-1.21)	0.093	0.519
Cigarette smoking							
Ever smokers	123/366	1.00	1.03 (0.35-3.03)	0.87 (0.24-3.12)	1.00 (0.28-3.59)	0.995	
Never smokers	36/138	1.00	0.81 (0.46-1.42)	0.86 (0.51-1.45)	0.47 (0.26-0.87)	0.016	0.129
Calcium							
Sex							
Male	106/306	1.00	0.54 (0.29-1.00)	0.90 (0.50-1.61)	1.06 (0.62-1.79)	0.342	
Female	53/198	1.00	0.30 (0.11-0.82)	0.48 (0.21-1.09)	0.52 (0.23-1.16)	0.344	0.199
Physical activity							
≤22.4 MET h/week	67/235	1.00	0.40 (0.19-0.88)	0.60 (0.27-1.32)	0.49 (0.23-1.05)	0.187	
>22.4 MET h/week	92/268	1.00	0.40 (0.19-0.87)	0.67 (0.34-1.30)	0.82 (0.94-1.67)	0.529	0.221
Alcohol drinking							

≤7 drinks/week	99/338	1.00	0.38 (0.20-0.75)	0.71 (0.39-1.30)	0.73 (0.43-1.26)	0.952	
>7 drinks/week	51/138	1.00	0.90 (0.37-2.19)	0.65 (0.28-1.50)	1.24 (0.55-2.78)	0.769	0.593
Cigarette smoking							
Ever smokers	123/366	1.00	0.56 (0.18-1.72)	0.53 (0.19-1.51)	0.77 (0.24-2.49)	0.395	
Never smokers	36/138	1.00	0.42 (0.23-0.81)	0.77 (0.43-1.36)	0.89 (0.53-1.50)	0.497	0.733

Abbreviations: HR, hazard ratio; CI, confidence interval.

[a] Events are defined as all-cause deaths for overall survival and death/recurrence/metastasis (which occurred earliest) for disease-free survival.

[b] Cox proportional hazard model adjusted for age at diagnosis, sex, stage at diagnosis, marital status, MSI status, chemoradiotherapy, and total energy intake where applicable.

[c] Test for linear trend was based on the median values for each quartile of intake.

[d] *P* for interaction is computed with Wald method testing significance of multiplicative interaction term between dietary calcium/dairy intakes and respective stratified variable.

Discussion

Results of the present study demonstrate a nonlinear association between higher prediagnostic consumption of calcium from foods and lower risk of all-cause mortality in patients with colorectal cancer. Stratified analysis by selected demographic and lifestyle characteristics of patients found no statistically significant effect modification with calcium, milk, or cheese in relation to colorectal cancer survival.

Increasing consumption of calcium and dairy products is associated with a reduced colorectal cancer risk on the basis of several meta-analyses [52, 178]. However, only minimal studies have reported results on the prediagnostic intake of calcium and dairy products in relation to survival after colorectal cancer diagnosis. In a large Japanese cohort study of 45,181 men and 62,643 women [11], yogurt intake was negatively associated with rectal cancer mortality in men. The results of other three studies [10, 12, 13] showed however no association between prediagnostic intake of diary and calcium and colorectal cancer survival. Nevertheless, a significant inverse association was observed for postdiagnosis consumption of calcium and milk in relation to all-cause death amongst colorectal cancer patients in the Cancer Prevention Study-II Nutrition Cohort Study [12].

Dairy products are the main foods rich in calcium, which is thought to lower colorectal cancer risk through its antiproliferative effects in the colorectal epithelium by binding toxic bile and fatty acids, thereby neutralizing mucosal toxicity [9]. In addition, calcium has been shown to prevent K-ras mutations [179] and to influence

multiple intracellular signaling pathways thus inducing differentiation in normal cells and apoptosis in malignant cells [6]. A number of clinical trials have reported a reduction in epithelial cell proliferation of the colorectum with high dietary intake of calcium and dairy products [180, 181]. These mechanisms are consistent with our observations of an inverse association between intake of calcium prediagnosis and the risk of all-cause death amongst colorectal cancer patients in the current study. Intriguingly, we observed a dose-response survival advantage of high prediagnostic cheese intake. Findings of previous research on cheese consumption and colorectal cancer mortality have mostly been null [11, 182]. A possible explanation for the null associations in prior research may be that the cheese intake in the populations studied was too low a dose for a reduction in the risk of death [183]. Our results suggest that a greater than 20 g/d of cheese consumption is required for a protective effect and yet the cutoff value for the highest group of cheese intake was much lower than this in previous studies [11, 182]. Compared with other dairy products, cheese is high in fatty acids and whey protein. Conjugated linoleic acid (CLA) and phytanic acid are two common fatty acids in cheese which have demonstrated putative beneficial effects on health and may inhibit the growth of colon cancer cells [184, 185]. Also, the hydrolysis of protein during cheese production could produce multiple bioactive peptides that have shown biological activities including antioxidant, antitumor, immunomodulatory, and anti-inflammatory effects [185]. The distinct nutrient composition of cheese could explain in part the diet-cancer survival association that is only observed for cheese but not other dairy products.

A novel feature of our study is the inclusion of effect modification by known prognostic factors in the calcium/dairy products and colorectal cancer survival relationship. None of these factors examined showed significant interaction with dietary calcium or dairy product intakes, which is in line with the only available previous study that reported no interactions of dairy products with patients' baseline characteristics in relation to colorectal cancer mortality [10].

The strengths of our study include its relatively large sample size, a long-time follow up from 1997 to 2010, and detailed questionnaire information. Limitations include a lack of information on postdiagnosis diet, which impeded us to evaluate the effect of diet and dietary modifications postdiagnosis on survival outcome. In addition, information on causes of death (ICD codes) was not available for the entire deceased patients; however, of those with such data (91 out of 159 patients), the majority (91.2%) was due to colorectal cancer.

Conclusions

Our results suggest that prediagnostic intake of cheese and calcium from foods may be associated with survival after a diagnosis of colorectal cancer. More well-designed observational studies are needed to provide insights into the dietary modulation of colorectal cancer, especially the role of postdiagnostic diet in relation to colorectal cancer survival.

Chapter 5 – Summary

5.1 Summary of findings and limitations

This thesis comprises three subprojects. The first mainly investigated the associations of the *GC* SNP rs2282679 with colorectal cancer risk and survival and whether the associations vary by dietary vitamin D intake and tumor molecular phenotype. We found that the *GC* SNP rs2282679 was not associated with overall colorectal cancer risk, but may be associated with survival after cancer diagnosis. The association of this SNP on survival among colorectal cancer patients may differ according to dietary vitamin D intake and according to tumor *BRAF* mutation status. These results may provide relevant information for identification of patients with increased susceptibility to colorectal cancer incidence and mortality and for patient assignment to interventions that are tailored to the individual.

One limitation of this study is that only one genetic variant of the *GC* gene was evaluated, thereby providing incomplete coverage of this gene; and we cannot exclude that genetic polymorphisms in other genes in the vitamin D metabolism pathway may also influence overall colorectal cancer initiation and progression. It is also possible that rs2282679 is not the true causal variant in itself but acts as a proxy through linkage disequilibrium. Moreover, despite our best funding efforts, serum levels of 25(OH)D or calcium were not determined in this study; but instead, dietary intakes of vitamin D and calcium were evaluated. It is often assumed that serum levels of 25(OH)D and calcium are closely related to the amount of nutrient present in the diet. Also, dietary intakes

may have the advantage of being less susceptible to short-term fluctuations in concentration than in plasma or serum and thereby being more reflective of the average status and the usual long-term exposure of the population. However, we admit that the lack of serum markers impeded us to test the relations of *GC* rs2282679 polymorphisms with plasma vitamin D concentration and to evaluate the extent to which the high risk of colorectal cancer mortality associated with the C allele is mediated through low 25(OH)D levels. Furthermore, dietary vitamin D intake may not accurately reflect each participant's vitamin D status since dietary history as measured by the FFQ is imprecise, and neither dermatic synthesis of vitamin D from sun exposure nor long-term dietary vitamin D intake was taken into account. Additionally, individuals were asked to report dietary exposures from one year prior to diagnosis for cases and one year prior to recruitment for controls; therefore, cases recalled dietary intakes from years earlier than controls. The longer recall period increases the rate of recall error resulting in higher likelihood of exposure misclassification in the case group. Further studies should be addressed to investigate the role of rs2282679/MSI classification in predicting the response to therapeutic lifestyle interventions.

The second component of this thesis investigated genetic variants in *VDR* and *CASR* for their relevance to colorectal cancer survival. Results demonstrated that polymorphic variations in *VDR* and *CASR* may be associated with survival after a diagnosis of colorectal cancer. Potential interactions were seen among prediagnostic dietary calcium intake with the *CASR* R990G and the *CASR* G-T-G-G-G-G-C haplotype for rs10222633-rs10934578-rs3804592-rs17250717-A986S-R990G-rs1802757, with

decreased OS time associated with these variants limited to patients consuming dietary calcium below the median.

Limitations to this study include a lack of the cause of death data for all deceased patients. In addition, the lack of serum levels of 25-hydroxyvitamin D (25(OH)D) and calcium impeded us to test possible 25(OH)D/calcium-diet/gene interactions and to evaluate the extent to which the gene-colorectal cancer outcome association is mediated through serum levels of 25(OH)D/calcium. Besides, we cannot rule out effects of other genes with polymorphisms in the vitamin D and calcium metabolism pathway that may also influence the overall colorectal cancer initiation and progression. It is also notable that, due to the limitations related to secondary use of research data, only genetic markers available from the NFCCS study were explored and most SNPs examined in this work are tagging SNPs, which are selected merely as indicators for specific regions of interest; thus, there is a low probability that they are the causal SNPs. Therefore, first the replication of this work in other populations and then in detail examination of the other polymorphisms in the *VDR* and *CASR* genes is necessary to identify truly causal variants.

The third subproject examined the relationship of prediagnostic intakes of vitamin D, calcium, and dairy products with colorectal cancer all-cause (OS) and disease-free survival (DFS), and further explored if the association was modified by sex, physical activity, alcohol drinking, and cigarette smoking. Results showed that high prediagnostic intake of calcium from foods was associated with increased survival among colorectal cancer patients. No evidence for modification by factors known to be

associated with colorectal cancer survival was observed. These findings underscore the importance of maintaining a healthy diet containing sufficient calcium before diagnosis.

However, this study is limited in a lack of information on postdiagnosis diet, which impeded us to evaluate the effect of diet and dietary modifications postdiagnosis on survival outcome. In addition, information on causes of death (ICD codes) was not available for the entire deceased patients; however, of those with such data, the majority (91.2%) was due to colorectal cancer. Besides, the estimation of food and or nutrient intakes from an FFQ is imprecise and there will always be a potential for measurement error. Although the FFQ applied in the Newfoundland population has been validated, it requires further accurate evaluation. These findings need to be replicated in future studies.

5.2 Future research

In this thesis, the roles of vitamin D, calcium, and dairy intakes and genes involved in their metabolic pathways in colorectal cancer survival were described and examined in the context of a population-based cohort study in Newfoundland. Due to the limitations related to secondary use of research data, only prediagnostic consumption of nutrients/foods were explored in this dissertation. Since colorectal cancer patients may make changes in diet post-diagnosis, it is important that future nutrition research on cancer survival include postdiagnostic diet. Furthermore, in this dissertation the primary focus was on allelic variations in the *GC*, *VDR* and *CASR* genes and a limited

number of tagging SNPs were explored. Future studies should first replicate this work in other populations and then examine in detail other DNA polymorphisms in the genes of interest to identify truly causal variants. This research will benefit the evaluation of other genes with polymorphic variations in relation to the initiation and progression of colorectal cancer within NFCCS.

Current studies examining associations between gene and disease seldom consider the modification effects of environmental exposures. Our data suggest potential gene-diet interactions in relation to colorectal cancer survival. These associations, however, were not statistically significant after Bonferroni's correction for multiple testing, which might be explained by insufficient power. Thus, large-scale prospective studies with greater power are needed to validate these interaction findings. If the gene-nutrient interaction will be replicated in further studies, then cancer patients, especially those with detrimental genotypes, may benefit from the use of calcium supplements to improve their survival. Such supplementations should be based on well-designed and carefully conducted randomized controlled trials.

Last but not least, further research is required to reveal the exact mechanisms underlying the relationship of prediagnostic consumption of vitamin D, calcium, and dairy intakes, and related gene variants with colorectal cancer survival reported in this thesis.

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Appendices

Appendix 1. Personal History Questionnaire

Please write in your answers where space is provided, or place tick marks in circles O

What date are you filling out this questionnaire? _____/_____/_____

Day Month Year

Identifying information

1. Are you male or female?
O male
O female

2. What is your date of birth?
___ ___ years
O don't know

2. What is your age?
day ___ ___
month ___ ___
year ___ ___
O don't know day
O don't know month
O don't know year

3. Are you a twin or triplet?
┌ O yes, a twin
├ O yes, other multiple (triplet, quadruplet, etc.): _____
│
Please specify
│ O no
│ O don't know
│
└ If yes, please read the following statement and answer the question.

Non-identical twins are no more alike than ordinary brothers and sisters. Genetically identical twins, on the other hand, look so much alike *that is, they have a strong resemblance to each other in height, colouring, features of the face, etc.) that people often mistake one for the other, especially during their childhood.

Do you have a genetically identical twin or triplet?

O yes
O no
O don't know

5. What is your marital status?

O currently married or living as married
O separated
O divorced
O widowed
O single or never married
O don't know

Bowel Screening and Health

6. Have you ever had a test for blood in your stool, called a smear test or a hemoccult? This test is frequently done as part of a routine physical examination, or it can be done at home.

O yes
O no → Please go to # 7
O don't know → Please go to # 7

6a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

6b. What were the reasons for your first test? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O don't know

7. Have you ever had a sigmoidoscopy? sigmoidoscopy involves looking inside the lower bowel and rectum with a lighted instrument. This examination is usually done in a doctor's office without anesthesia.

O yes
O no → Please go to # 8
O don't know → Please go to # 8

7a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

7b. What were the reasons for your first sigmoidoscopy? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O don't know

6c. How many times have you had a hemoccult test?

___ number of hemoccult tests
O don't know

6d. If you have had a hemoccult test more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

8. Have you ever had a colonoscopy? colonoscopy is an examination of the entire large bowel using a long flexible instrument. This examination is usually done under sedation.

O yes
O no → Please go to # 9
O don't know → Please go to # 9

8a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

8b. What were the reasons for your first colonoscopy? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O other: _____
Please specify
O don't know

8c. How many times have you had a colonoscopy?

7c. How many times have you had a sigmoidoscopy?

___ number of sigmoidoscopies
O don't know

7d. If you have had a sigmoidoscopy more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

9. Has a doctor ever told you that you had polyps in your large bowel or colon or rectum? Polyps are growths in the lining of the colon which vary in size from a tiny dot several inches.

O yes
O no → Please go to # 10
O don't know → Please go to # 10

9a. When did your doctor first tell you that you have had polyps?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

9b. Have you been told more than once that you had polyps?

O yes
O no
O don't know

9c. When did you your doctor last tell you that you had polyps?

age at last diagnosis ___ ___
or

___ number of colonoscopies
O don't know

8d. If you have had a colonoscopy more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

year of last diagnosis ___ ___ ___ ___
O don't know

9d. Do you know what kind of polyps they were?

O benign
O adenomatous (pre-cancerous)
O hyperplastic
O other: _____
Please specify
O don't know

9e. Did you have the polyps removed (by a procedure called a polypectomy)? (This can be done during a sigmoidoscopy or colonoscopy.)

O yes
O no → Please go to # 10
O don't know → Please go to # 10

9f. When did you first have polyps removed?

age at first polypectomy ___ ___
or
year of first polypectomy ___ ___ ___ ___
O don't know

9g. Have you had polyps removed more than once?

O yes
O no
O don't know

9h. If you have had polyps removed more than once, when did you last have polyps removed?

age at first polypectomy ___ ___
or
year of first polypectomy ___ ___ ___ ___
O don't know

10. Has a doctor ever told you that you had familial adenmotaous polyposis, known also as FAP? This is a condition, sometimes occurring in families, in which numerous polyps line the inside of the large bowel or colon.

11. Has a doctor ever told you that you had Crohn's disease? This is where you have an inflammation that extends into the deeper layers of the intestinal wall. It may also affect other parts of the digestive tract, including the mouth, esophagus, stomach, and small intestine.

O yes
O no → Please go to # 12
O don't know → Please go to # 12

11a. When did your doctor first tell you that you had Crohn's disease?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

12. Has a doctor ever told you that you had ulcerative colitis? This is an inflammation and ulceration of the lining of the bowel (colon) & rectum. It is not a stomach ulcer.

O yes
O no → Please go to # 13
O don't know → Please go to # 13

12a. When did your doctor first tell you that you had ulcerative colitis?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

13. Has a doctor ever told you that you had

O yes
O no → Please go to # 11
O don't know → Please go to # 11

10a. When did your doctor first tell you that you had FAP?
age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

irritable bowel syndrome? This is a disorder of the bowels leading to cramping, gassiness, bloating and alternating diarrhea and constipation. It is sometimes called IBS, or spastic colon.

O yes
O no → Please go to # 14
O don't know → Please go to # 14

13a. When did your doctor first tell you that you had irritable bowel syndrome?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

14. Has a doctor ever told you that you had diverticular disease? This may also be called diverticulosis or diverticulitis. It's a condition in which the bowel may become infected, and can lead to pain and chronic problems with bowel habits. and small intestine.

O yes
O no → Please go to # 15
O don't know → Please go to # 15

14a. When did your doctor first tell you that you had diverticular disease?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

15. Have you ever had any of your large bowel or colon removed?

O yes
O no → Please go to # 16
O don't know → Please go to # 16

Was it completed removed, or was only part of it removed?
O completed removed
O partly removed
O don't know

15a. When did you first have any of your bowel or colon removed?

15b. Have you had more than one surgery to remove your bowel or colon?

O yes
O no → Please go to # 16
O don't know → Please go to # 16

15c. When did you last have any of your bowel or colon removed?

age at last operation ___ ___
or
year of last operation ___ ___ ___ ___
O don't know

16. Have you had your gallbladder removed?

O yes
O no → Please go to # 17
O don't know → Please go to # 17

16a. When did you have your gallbladder removed?

age at operation ___ ___
or
year of operation ___ ___ ___ ___
O don't know

17. Has a doctor ever told you that you had diabetes, also known as diabetes mellitus? Please do not include diabetes which you had only during pregnancy.

O yes
O no → Please go to # 14
O don't know → Please go to # 14

17a. When did your doctor first tell you that you had diabetes?

age at first operation ___ ___
Or
year of first operation ___ ___ ___ ___
O don't know

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

17b. Did you ever take medication to control your diabetes?

O yes
O no → Please go to # 18
O don't know → Please go to # 18

17c. What type of medication did you use, pill or insulin injections?

O pills
O insulin injections
O both
O don't know → Please go to # 18

17d. How often did you usually take it? Please choose the most appropriate category.

	Pills	Insulin
times per day or	___ ___	___ ___
times per week or	___ ___	___ ___
times per month or	___ ___	___ ___
times per year	___ ___	___ ___
don't know	O	O

17e. About one year before your recent cancer diagnosis, were you taking it?

	Pills	Insulin
O yes	O	O
O no	O	O
O don't know	O	O

17f. How long, in total, have you taken this medication?

	Pills	Insulin

18. Has a doctor ever told you that you had high cholesterol? If your doctor told you it borderline, please tick no.

O yes
O no → Please go to # 19
O don't know → Please go to # 19

18a. When did your doctor tell you that you had high cholesterol?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

18b. How you ever take medication to control your high cholesterol?

O yes
O no → Please go to # 19
O don't know → Please go to # 19

18c. How often did you usually take it? Please choose the most appropriate category.

___ ___ times per day or
___ ___ times per week or
___ ___ times per month or
___ ___ times per year or
O don't know

18d. About one year before your recent cancer diagnosis, were you taking it?

O yes
O no
O don't know

number of moths or	___ ___	___ ___
number of years	___ ___	___ ___
don't know	O	O

18e. How long, in total, have you taken this medication?

___ ___ number of months or
___ ___ number of years
O don't know

19. Has a doctor ever told you that you had high levels of fat (other than cholesterol) in your blood, also called high triglycerides? If your doctor told you it was borderline, Please tick no.

O yes
O no → Please go to # 20
O don't know → Please go to # 20

19a. What did your doctor first tell you that you had high triglycerides?

age at diagnosis
or
year of diagnosis
don't know

19b. Did you ever take medication to control the high levels of fat in your blood?

O yes
O no → Please go to # 20
O don't know → Please go to # 20

19c. How often did you usually take it? Please choose the most appropriate category.

___ ___ times per day or
___ ___ times per week or
___ ___ times per month or
___ ___ times per year or
O don't know

19d. About one year before your recent cancer diagnosis, were you taking it?

O yes
O no
O don't know

19e. How long, in total, have you taken this

20. Has a doctor ever told you that you had any type of cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

20a. What type of cancer was it?
_____ cancer

20b. When did your doctor tell you that you had this type of cancer?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

20c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

21. Has a doctor ever told you that you had any other cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

21a. What type of cancer was it?
_____ cancer

21b. When did your doctor tell you that you had this type of cancer?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

21c. Were you treated with radiation therapy

medication?

___ ___ number of months or
___ ___ number of years
O don't know

(radiotherapy) for this cancer?

O yes
O no
O don't know

19. Has a doctor ever told you that you had any cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

22a. What type of cancer was it?

_____cancer

22b. When did your doctor first tell you that you had this type of cancer?

age at diagnosis
or
year of diagnosis
don't know

22c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

23. Has a doctor ever told you that you had any other cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

22a. What type of cancer was it?

_____cancer

23b. When did your doctor first tell you that you had this type of cancer?

age at diagnosis
or
year of diagnosis
don't know

23c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

Medications

Have you ever taken any of the following medications regular (at least twice a week for more than a month)?

24. Aspirin (such as Anacin, Bufferin, Bayer, Excedrin, Ecotrin)

O yes
O no → Please go to # 25
O don't know → Please go to # 25

24a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? __ Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

24b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

24c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly
(at least twice a week for more than a month)? (continued)

25. Acetaminophen (such as Tylenod, Anacin-3, Panadol)

O yes
O no → Please go to # 26
O don't know → Please go to # 26

25a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

25b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

25c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _

26. Ibuprofen medications (such as Advil, Motrin, Medipren, Indocid, Naprosyn, NSAIDS (NSAIDS are non-steroidal anti-inflammatory drugs)

O yes
O no → Please go to # 27
O don't know → Please go to # 27

26a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

26b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

26c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _

count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

27. Bulk-forming laxatives (such as Metamucil, Citrucel, FibreCon. Serutan, psyllium)

O yes
O no → Please go to # 28
O don't know → Please go to # 28

28. Other laxatives (such as Ex-Lax, Correctol, Dulcolax, Senokot, Colace, castor, cod liver oil, mineral oil, milk of magnesia, lactulose, Epsom salts)

O yes
O no → Please go to # 29
O don't know → Please go to # 29

27a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

28a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

27b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

28b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

27c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

28c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

29. Multivitamin supplements (such as One-A-Day, Theragram, Centrum, Unicap) (not individual vitamins)

O yes
O no → Please go to # 28
O don't know → Please go to # 28

30. Folic acid or folate pills or tablets

O yes
O no → Please go to # 31
O don't know → Please go to # 31

29a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

30a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

29b. About one year before your recent cancer

30b. About one year before your recent cancer

diagnosis, were you taking it regularly?

O yes
O no
O don't know

29c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

diagnosis, were you taking it regularly?

O yes
O no
O don't know

30c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

31. Calcium pills or tablets

O yes
O no → Please go to # 32
O don't know → Please go to # 32

29a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or

32. Calcium-based antacids (such as Tums, Rolaids, Extra-strength Rolaids, Alka-Mints, Chooz Antacid gum)

O yes
O no → If female, Please go to # 33
If male. Please go to # 44
O don't know → If female, Please go to # 33
If male. Please go to # 44

32a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or

___ ___ times per week
O don't know

29b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

29c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

___ ___ times per week
O don't know

32b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

32c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Men: please go to #44 on page 17
Women: please continue with #33 on page 13

Menstruation, Pregnancy, and Menopause

33. How old were you when you had your first menstrual period?

___ ___ years of age
O don't know
O never had a menstrual period

34. Have you ever been pregnant?

O yes
O no → Please go to # 35
O don't know → Please go to # 35

└>How many times have you been pregnant? Please include miscarriages, stillbirths, tubal pregnancies and

34c. How many of your pregnancies resulted in live births?

O never
___ ___ number of pregnancies with live-born children
O don't know

34d. How old were you at the first live birth?

age at first birth ___ ___ or
year of first birth ___ ___ ___ ___
O don't know

34e. How old were you at the last live birth?

abortions.

___ ___ number of pregnancies
O don't know

34a. How many times were you pregnant with more than one baby (twins, triplets or ___ more)? If you are pregnant now, please do not include your current pregnancy. _

O never
___ ___ number of pregnancies
with more than one baby
O don't know

34b. How many of your pregnancies lasted 6 months or longer? (Pregnancy usually lasts 9 months. Six months is about the earliest a baby could survive.) If you are pregnant now, please do not include your current _ pregnancy.

O never
___ ___ number of pregnancies lasting
6 months or longer
O don't know

35b. In total, how long did you take these hormonal contraceptives? If you started and stopped and then started again, please count only the time you were taking these contraceptives.

___ ___ number of years
O don't know

36. Have you had a menstrual period in the last

age at last birth ___ ___ or
year of last birth ___ ___ ___ ___
O don't know

35. Have you ever used birth control pills or other hormonal contraceptives (implants or injections) for at least one year?

┌ O yes
│ O no → Please go to # 36
│ O don't know → Please go to # 36
└>How old were you when you first used
Any of these hormonal contraceptives?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

35a. Were you still using hormonal contraceptives about one year before your recent cancer diagnosis?

O yes
O no
O don't know

Please complete the next few questions which ask about surgeries you may have had.

39. Hysterectomy (only the uterus or womb Removed)

┌ O yes
│ O no
│ O don't know
└>age when removed ___ ___ or

12 months? Please include only menstrual bleeding, not bleeding that results from hormonal replacement therapy (HRT) or progesterones, progesttins or withdrawal bleeding.

O yes → Please go to #42
O no
O don't know → Please go to #42

Have your periods stopped permanently or only temporarily due to pregnancy, breast-feeding, or other conditions?

O permanently
O temporarily → Please go to #42

37. How old were you when your periods stopped permanently?

age they stopped ___ ___ or
year they stopped ___ ___ ___ ___
O don't know

38. Why did your menstrual periods stop permanently? Please tick all that apply.

O natural menopause
O surgery
O radiation or chemotherapy
O other reason
Please specify: _____
O Don't know

39d. Both ovaries removed without hysterectomy

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

years when removed ___ ___ ___ ___
O don't know

39a. Hysterectomy with one ovary or part of an Ovary removed)

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39b. Hysterectomy with both ovaries removed

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39c. One ovary removed, completely or partly, without hysterectomy

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

42a. Were you still having menstrual periods when you first took these hormones?

O yes
O no
O don't know

40. If you had radiation or chemotherapy, when did you first have it?

O had radiation or chemotherapy
>age when this was given ___ ___ or
year when this was given ___ ___ ___ _
O don't know
O never had radiation or chemotherapy

41. if your periods stopped permanently for any reason other than surgery, radiation or chemotherapy, when did you this occur?

O other reason
Please specify: _____
>age when occurred ___ ___ or
year when occurred ___ ___ ___ ___
O don't know
O not applicable

42. Doctors prescribe hormonal replacement therapy for many reasons, including menopausal symptoms, surgical removal of the ovaries, osteoporosis, and heart disease prevention. (Menopausal symptoms include hot flashes, sweating, and depression.)

Have you ever taken hormonal replacement therapy prescribed by a doctor and in the form of a pill or a patch?

Please do not include hormonal therapy that was prescribed for birth control, infertility, hormone therapy delivered by injections, vagina creams or vaginal suppositories, or herbal or soy products.

O yes
O no → Please go to #43
O don't know → Please go to #43

42b. Were you prescribed either an estrogen-only pill or patch (such as Premarin) for hormone replacement therapy?

O yes
O no
O don't know
>How old were you when you first took estrogen-only medication?

age when first taken ___ ___ or
years when first taken _____ ___ _
O don't know

42c. Were you still using estrogen-only medication for hormone replacement therapy about one year before your recent cancer diagnosis?

O yes
O no
O don't know

42d. In total, how long did you take estrogen-only medication for hormone replacement therapy? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

42e. Progesterone or progestin is frequently prescribed by doctors together with estrogen for hormone replacement therapy. One common brand name is Provera. Another one is Prometrium. Have you ever taken progesterone or progestin together with estrogens for hormone replacement therapy?

O yes

43. Have you ever taken tamoxifen, raloxifene, or other anti-estrogen medication (such as Lupron or Depo-Provera)?

O yes
O no → Please go to #44
O possibly – I have participated in a clinical trial for tamoxifen or other anti-estrogen medication

| O no → Please go to #43
| O don't know → Please go to #43
└>How old were you when you first took progesterone or progestin together with estrogens?

age when first taken ___ ___ or
year when first taken ___ ___ ___ ___
O don't know

42f. Were you still using progesterone or progestin medication about one year before your recent cancer diagnosis?

O yes
O no
O don't know

42g. In total, how long did you take progesterone or progestin together with estrogens? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

| O don't know
|
└>What anti-estrogen medication did you take? Please tick all that apply.

O tamoxifen
O raloxifene
O other: _____
Please specify

43a. How old were you when you first took tamoxifen, raloxifene or other anti-estrogen medication?

age when first taken ___ ___ or
year when first taken ___ ___ ___ ___
O don't know

43b. Were you still using tamoxifen, raloxifene or other anti- estrogen medication about one year before your recent cancer diagnosis?

O yes
O no
O don't know

43c. In total, how long did you take tamoxifen, raloxifene or other anti-estrogen medication? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Diet

44. About one year before your recent cancer diagnosis, on average, how often did you eat a piece serving of fruit?
(A serving of fruit is: 1 medium-sized fresh fruit; ½ cup of chopped, cooked or canned fruit;

¼ cup of dried fruit; 6 ounces of fruit juice (50%-100% pure juice).) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't know

45. About one year before your recent cancer diagnosis, on average, how often did you eat a piece serving of vegetables?
(A serving of vegetables is: 1 medium-sized fresh vegetables; ½ cup of chopped, cooked or chopped vegetables; 6 ounces of vegetable juice (50%-100% pure juice).) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't know

46. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of red meat (not chicken or fish)?

(A serving of red meat is: 2-3 ounces of red meat (a piece of meat about the size of a deck of cards). Red meats include: beef, steak, hamburger, prime rib, ribs, beef hot dogs, beef-based processed meat, veal, pork, bacon, pork sausage, ham, lamb, venison.)

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat → Please go to #47
O don't know

46a. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of red meat that was cooked by broiling, grilling, barbecueing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat that was cooked by these methods → Please go to #47
O don't know

46b. On average, when you ate red meat cooked by these methods, which of the following best describes its appearance?

What was its outside appearance?	What was its inside appearance? (how well done it was)?
O lightly browned	O red (rare)
O medium browned	O pink (medium)
O heavily browned or blackened	O brown (well-done)
O don't know	O don't know

47. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of chicken? Please do not include turkey or any other bird.
(A serving of chicken is: 2-3 ounces of chicken meat; 1 drumstick; 1 thigh; half a breast; 2 wings; 3 nuggets.) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat that was cooked by these methods → Please go to #48
O don't know

47a. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of chicken that was cooked by broiling, grilling, barbecueing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat chicken that was cooked by these methods → Please go to #48
O don't know

47b. On average, when you ate chicken cooked by these methods, which of the following best describes its appearance?

What was its outside appearance?

O lightly browned
O medium browned
O heavily browned or blackened
O don't know

We would like you to think back to when you were in your 20s and remember the physical activities you participated in then.

48. In your 20s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Jogging (running slower than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 20s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
		___ ___ years	___ ___ months	__ minutes per week / __hours per week

49. When you were in your 20s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don’t know

If you are younger than 31, please go to the next section (Alcohol Consumption) on page 25. Otherwise, please continue with #50.

Now, please think back to your 30s and 40s.

50. In your 30 and 40s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Jogging (running slower than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 30s and 40s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
		___ ___ years	___ ___ months	__ minutes per week / __hours per week

51. When you were in your 30s and 40s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don't know

If you are younger than 31, please go to the next section (Alcohol Consumption) on page 25. Otherwise, please continue with #50.

Now, please think back to since you turned 50s.

52. In your 50s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes →	___ ___ years	___ ___ months	__ minutes per week /

	O no →			__hours per week
Jogging (running slower than a mile in 10 minutes)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 50s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week /

__hours per week

_____ → ___ ___ years ___ ___ months __ minutes per week / __hours per week

_____ → ___ ___ years ___ ___ months __ minutes per week / __hours per week

_____ → ___ ___ years ___ ___ months __ minutes per week / __hours per week

_____ → ___ ___ years ___ ___ months __ minutes per week / __hours per week

_____ → ___ ___ years ___ ___ months __ minutes per week / __hours per week

___ ___ years ___ ___ months __ minutes per week / __hours per week

53. When you were in your 50s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don't know

We would like you to think back to when you were in your 20s.

54. In your 20s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

For how many During those years,

		years?	how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know
Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

55. When you were in your 20s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

    ___ ___ years consumed
    O never consumed alcohol

56. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

    ___ ___ years consumed
    O never consumed alcohol

If you are younger than age 31, please go to the next section (Smoking) on page 28.
Otherwise, please continue with #57.

Now, please think back to your 30s and 40s.

57. In your 30s and 40s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day

			O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know
Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

58. When you were in your 30s and 40s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

___ ___ years consumed
O never consumed alcohol

56. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ years consumed
O never consumed alcohol

If you are younger than age 51, please go to the next section (Smoking) on page 28.
Otherwise, please continue with #60.

Now, please think back to since you turned 50s.

60. In your 50s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day

			O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know
Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

61. When you were in your 30s and 40s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

___ ___ years consumed
O never consumed alcohol

62. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ years consumed
O never consumed alcohol

Smoking

63. Have you ever smoked at least one cigarette a day for 3 months or longer?

O yes
O no → Please go to #64
O don't know → Please go to #64

64. Have you ever smoked at least one cigar a month for at least 3 months?

O yes
O no → Please go to #65
O don't know → Please go to #65

64a. When did you first start smoking at least one cigar a month?

63a. When did you first start smoking at least one cigarette a day?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

63b. During periods when you smoked regularly, how many cigarettes did you typically smoke in a day?

___ ___ ___ ___ cigarettes per day
O don't know

63c. About one year before your recent cancer diagnosis, were you still smoking at least one cigarette a day?

O yes
O no
O don't know

63d. Do you still smoke at least one cigarette a day?

O yes
O no → Please go to #63f
O don't know → Please go to #63f

63e. When did you stop smoking at least one cigarette a day (we mean stop smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

63f. How many years, in total, did you smoke at least one cigarette a day for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

___ ___ ___ ___ total number of years
O don't know

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

64b. During periods when you smoked regularly, how many cigar did you typically smoke in a month?

___ ___ ___ ___ cigarettes per month
O don't know

64c. About one year before your recent cancer diagnosis, were you still smoking at least one cigar a month?

O yes
O no
O don't know

64d. Do you still smoke at least one cigar a month?

O yes
O no → Please go to #64f
O don't know → Please go to #64f

64e. When did you stop smoking at least one cigar a month (we mean stop smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

64f. How many years, in total, did you smoke at least one cigar a month for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

___ ___ ___ ___ total number of years
O don't know

65. Have you ever smoked at least one pipe a month for at least 3 months?

O yes
O no → Please go to #66
O don't know → Please go to #66

65a. When did you first start smoking at least one pipe a month?

age at first use ___ ___ or
year of first use ___ ___ ___ ___

Height and Weight

66. About how tall are you, without your shoes on?

___ feet ___ ___ inches
or
___ ___ ___ centimeters
O don't know

67. How much did you weigh about one year before your recent cancer diagnosis?

O don't know

65b. During periods when you smoked regularly, how many pipe did you typically smoke in a month?

___ ___ ___ ___ pipe per month
O don't know

65c. About one year before your recent cancer diagnosis, were you still smoking at least one pipe a month?

O yes
O no
O don't know

65d. Do you still smoke at least one pipe a month?

O yes
O no → Please go to #65f
O don't know → Please go to #65f

65e. When did you stop smoking at least one pipe a month (we mean stop smoking smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

65f. How many years, in total, did you smoke at least one pipe a month for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

___ ___ ___ ___ total number of years
O don't know

___ pounds
Or
___ ___ ___ kilograms
O don't know

Additional Information

69. Previous to this study, have you and your relatives ever taken part in any family health studies?

O yes
O no
O don't know

Background Information

70. What is the highest level of education that you completed?

O less than 8 years
O 8 to 11 years
O high school graduate
O vocational or technical school
O some college or university
O bachelor's degree
O graduate degree
O don't know

71. Country of birth sometimes affects disease risk. Please fill in country of birth for yourself, you parents and your grandparents.

In addition, scientists have found that some genetic traits are more common or less

common among Jewish people of different ethnic backgrounds. Please answer the questions about Jewish descent for each person.

	Country of birth	Is this person of Jewish descent?	Ashkenazi (East European)	Sephardic	Other	Don't know
You	_____	O yes O no O don't know	O	O	O	O
Your mother	_____	O yes O no O don't know	O	O	O	O
Your father	_____	O yes O no O don't know	O	O	O	O
Your mother's mother	_____	O yes O no O don't know	O	O	O	O
Your mother's father	_____	O yes O no O don't know	O	O	O	O
Your father's mother	_____	O yes O no O don't know	O	O	O	O
Your father's father	_____	O yes O no O don't know	O	O	O	O

72. How many years have you lived in Canada?

O all my life
___ ___ number of years
O don't know

73. Ethnicity and race sometimes affect disease risk. Scientists have found that some genetic traits are more common or less common among people of different backgrounds. We would like to know if this is true for genes associated with colorectal cancer.

Please fill in the background for yourself, your parents and your grandparents.
Please tick all that apply.

	You	Your mother	Your father	Your Mother's mother	Your Mother's father	Your Father's mother	Your Father's Father

Black, From Africa	O	O	O	O	O	O	O
Black, from Caribbean (Trinidad, Jamaica, Haiti)	O	O	O	O	O	O	O
Black from North America	O	O	O	O	O	O	O
Black, other	O	O	O	O	O	O	O
White	O	O	O	O	O	O	O
First Nations (Indian, Inuit)	O	O	O	O	O	O	O
North African (Egyptian)	O	O	O	O	O	O	O
Middle East (Iranian)	O	O	O	O	O	O	O
Filipino	O	O	O	O	O	O	O
Japanese	O	O	O	O	O	O	O
Korean	O	O	O	O	O	O	O
Chinese	O	O	O	O	O	O	O
Other South East Asian (Vietnamese)	O	O	O	O	O	O	O
South Asian (East Indian, Pakistani)	O	O	O	O	O	O	O
Other: Please specify	_____	_____	_____	_____	_____	_____	_____
Don't know	O	O	O	O	O	O	O

74. Which of the following categories best describes your total annual household income about one year before your recent diagnosis?

O no income
O less than $6,000
O $6,000 - $11,999
O $12,000 - $19,999
O $20,000 - $29,999
O $30,000 - $39,999
O $40,000 - $49,999
O $50,000 - $59,999
O $60,000 - $69,999
O $70,000 - $79,999
O $80,000 +
O don't know

75. In case we need to contact you in the future and you have moved, could we have the name of someone who is not living with you to whom we might write or call for your new address?

Name of relative or friend: _____

His or her address: _____

_____

His or her telephone number: (___ ___ ___) ___ ___ ___ - ___ ___ ___ ___

Thank you very much for taking the time to fill out this questionnaire.
We appreciate your participation.

Please mail this completed questionnaire in the return envelope provided.

Appendix 2. Food Frequency Questionnaire

Canadian Study of Diet and Health

Who this questionnaire is for and what it asks about:

This questionnaire is to be completed by the person taking part in this study:

Part I asks about the foods you ate about **one year before your diagnosis**.

Part II asks about vitamins and other dietary supplements that you may have used.

If possible, please return this questionnaire within two weeks.

The completed questionnaire should be sealed in the pre-paid envelope and mailed back to:

CRC-IHRT,
Room 1758E, Level 1, Health Science Centre,
300 Prince Phillip Drive,
St. John's, NL, Canada, A1B 9Z9.

If you have any questions about this form or the study, call our toll-free number, **1-888-908-4988.**

The information given to us in this questionnaire will be kept confidential.

Thank you for your time and assistance.

1

HOW TO COMPLETE THIS QUESTIONNAIRE

We would like to know how often you ate certain foods **about one year before diagnosis,** and their amounts.

Section A ***(lists foods and portion sizes)***
Amounts are described in various ways, including the number of:

cups, teaspoons (tsp), ounces (oz), inches ("), pieces (e.g., 1 apple)
grams (gm), tablespoons (tbsp), millilitres (ml), centimetres (cm).

We want to know the ***Portion Size*** of your **USUAL SERVING.** We have given an example of an average portion size. If your portion size was different than the average, you can indicate this by putting an **X** or ✓in the circles for ***Smaller*** or ***Larger*** portion sizes. ***Smaller*** than average is about 25% or less than the average portion size, while ***Larger*** than average is about 25% or more than the average size. Leave the circle blank if your typical portion size was average.

Included with this questionnaire is a ***FOOD PHOTOGRAPH PAGE*** that shows small, medium and large portion sizes for vegetables, meat and chicken. Some questions ask you to refer to the photo page to help you choose your usual portion size.

Section B ***(asks about how often you ate certain foods one year before diagnosis)***
For each food item listed, choose **one** column (Per Day, Per Week, Per Month, or Never / Rarely) that best describes ***HOW OFTEN*** you ate or drank that item. For example, if you ate CREAM CHEESE 3 times a month during the year of interest, you would write (3) in the *PER MONTH* column. If you ate SWEET POTATOES only 2 times during the year of interest, you can place a checkmark (✓) in *the NEVER OR RARELY* column.

Section C ***(To be completed only for seasonal foods)***
Some foods (for example fresh fruit and vegetables) are not available throughout the year. **For foods that you do not eat all year round** (i.e. in season only), indicate the number of months of the year that you ate them.

Please complete each question as best you can. We know that it is difficult to recall exactly how often you ate something. If you are not certain, try to give your best estimate.

EXAMPLE

		Section A			*Section B* YEAR BEFORE DIAGNOSIS				*Section C*
	FOOD	**Average Portion Size**	**Your Portion Size, if NOT Average** Smaller	Larger	**HOW OFTEN?** (Complete one column only) per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	**If Ate Food in Season Only** enter Months per Year
1	CREAM CHEESE	2 tbs/ 30 ml/ 1 oz	O	O			***3***		
2	CANTELOUPE	1/8 or 1 slice	O	O		***1***			***4***
3	SWEET POTATOES	1 medium/ ½ cup	O	O				√	

Section A				Section B YEAR BEFORE DIAGNOSIS				Section C
FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
		Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
Beverages								
1 WHOLE MILK (any, if in cereal & drinks)	1 cup/ 250 ml	O	O					
2 2% MILK, 2% Evaporated milk (any, if in cereal & drinks)	1 cup/ 250 ml	O	O					
3 SKIM MILK (any, if in cereal & drinks)	1 cup/ 250 ml	O	O					
4 MILK SHAKE	1 cup/ 250 ml	O	O					
5 YOGURT DRINK	1 cup/ 250 ml	O	O					
6 COFFEE (not decaffeinated)	1 cup/ 250 ml	O	O					
7 COFFEE (decaffeinated)	1 cup/ 250 ml	O	O					
8 TEA (not herbal)	1 cup/ 250 ml	O	O					
9 TEA (herbal)	1 cup/ 250 ml	O	O					
10 SUGAR (in tea and coffee)	1tsp or 1 cube	O	O					
11 COCA COLA, PEPSI, OTHER COLA	1 cup/ 250 ml	O	O					
12 DIET SOFT DRINKS	1 cup/ 250 ml	O	O					
13 OTHER SOFT DRINKS (not diet/dry or cola)	1 cup/ 250 ml	O	O					
14 ORANGE or GRAPEFRUIT JUICE	¾ cup/ 175 ml	O	O					
15 APPLE or GRAPE JUICE	¾ cup/ 175 ml	O	O					
16 OTHER FRUIT JUCES (pineapple, cranberry, etc)	¾ cup/ 175 ml	O	O					
17 FRUIT DRINK/ LEMONADE	¾ cup/ 175 ml	O	O					
18 FRUIT DRINKS, ICED TEA	¾ cup/ 175 ml	O	O					
19 VEGTABLE JUICES	¾ cup/ 175 ml	O	O					
20 BEER or ALE	335 ml/ 1 bottle	O	O					
21 WHITE WINE	150 ml / 5 oz	O	O					
22 RED WINE, SHERRY, PORT (or other fortified wine)	150 ml / 5 oz	O	O					
23 LIQUOR (for example, whiskey, rum etc)	45 ml/ 1.5 oz	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
	Dairy Products								
24	EGG (boiled, poached)	1 egg	O	O					
25	EGG (fried, scrambled, omelette)	1 egg	O	O					
26	CREAM CHEESE, Regular fat	2 tbs/ 30 ml/ 1 oz	O	O					
27	CHEESE, Regular fat (such as cheddar, Swiss, processed)	1 slice/ 30 g/ 1oz	O	O					
28	CHEESE, Light (6-15% fat, such as cream cheese, cheddar)	1 slice/ 30 g/ 1oz	O	O					
29	CHEESE, Ultra Light (5% fat or less, such as cheddar)	1 slice/ 30 g/ 1oz	O	O					
30	COTTAGE or RICOTTA CHEESE	125 ml/ ½ cup	O	O					
31	CREAM (coffee, whipping, sour or regular)	1 tbs/ 15 ml	O	O					
32	CREAM (half and half, light sour cream)	1 tbs/ 15 ml	O	O					
33	COFFEE WHITENER (non-dairy)	1 tbs/ 15 ml	O	O					
34	YOGURT, Regular (plain, 2.4% fat or more)	¾ cup/ 175 ml	O	O					
35	YOGURT, Light (plain, less than 2.4% fat)	¾ cup/ 175 ml	O	O					
36	YOGURT, Regular (fruit flavoured or frozen, 2.4% fat or more)	¾ cup/ 175 ml	O	O					
37	YOGURT, Light (fruit flavoured or frozen, less than 2.4% fat)	¾ cup/ 175 ml	O	O					
	Mixed Dishes								
38	SOUPS (creamed)	1 cup/ 250 ml	O	O					
39	SOUPS (non-creamed)	1 cup/ 250 ml	O	O					
40	PEA SOUP	1 cup/ 250 ml	O	O					
41	PASTA with meat sauce (spaghetti, lasagna)	1 cup/ 250 ml	O	O					
42	PASTA with tomato sauce (spaghetti)	1 cup/ 250 ml	O	O					
43	MIXED DISHES with cheese or cheese sauce (macaroni and cheese)	1 cup/ 250 ml	O	O					
44	PIZZA with meat	1 Medium slice	O	O					
45	PIZZA with vegetable only	1 Medium slice	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
46	MEAT STEW with carrots, other vegetables	1 cup/ 250 ml/ photo A, medium	O	O					
47	CHILI with meat or Con Carne	1 cup/ 250 ml	O	O					
	Vegetables								
48	POTATOES (mashed, boiled, baked etc)	1 medium/ ½ cup/ 125 ml	O	O					
49	FRENCH FRIES or FRIED POTATOES	1 cup/ 250 ml	O	O					
50	CARROTS (raw or cooked)	1 medium/ ½ cup /125 ml	O	O					
51	BROCCOLI	1 cup/ 250 ml	O	O					
52	CABBAGE, COLESLAW	½ cup/ 125 ml	O	O					
53	CAULIFLOWER	½ cup/125 ml	O	O					
54	CORN	1 ear / ½ cup	O	O					
55	PEAS or LIMA BEANS	½ cup/125 ml	O	O					
56	GREEN or YELLOW BEANS	½ cup/125 ml	O	O					
57	BEANS or LENTILS (baked or boiled beans, kidney beans, chickpeas)	½ cup/125 ml cooked	O	O					
58	SPINACH and other green leafy vegetables (greens, collards, kale, mustard greens etc)	½ cup/125 ml cooked or 1 cup raw	O	O					
59	GREEN SALAD (with lettuce)	1 cup/ 250 ml	O	O					
60	CUCUMBER	½ cup/ 125 ml sliced	O	O					
61	TOMATOES (fresh)	1 medium/ ½ cup/ 125 ml	O	O					
62	TOMATOES (canned, pureed or sauce)	½ cup/125 ml	O	O					
63	ONIONS (raw or cooked)	½ cup/125 ml	O	O					
64	BEETS (boiled or pickled)	½ cup/125 ml	O	O					
65	TURNIPS or RUTABAGAS	1 medium/ ½ cup/125 ml	O	O					
66	OTHER ROOT VEGETABLES (sweet potatoes, yams, radish, etc)	½ cup/125 ml	O	O					
67	YELLOW SQUASH (winter type)	½ cup/125 ml	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
90	LIVER	85 g/ 3 oz	O	O					
91	FRIED CHICKEN	photo C, medium	O	O					
92	CHICKEN / TURKEY (roasted or stewed)	photo C, medium	O	O					
93	CHICKEN / TURKEY, SKIN REMOVED	photo C, medium	O	O					
94	SALTED/ DRIED MEAT	photo C, small	O	O					
95	PICKLED MEAT (brined)	photo C, small	O	O					
96	SHELLFISH (shrimp, lobster, crab)	85 g/ 3 oz/ photo C, small	O	O					
97	FRIED FISH	175 g/ 6 oz/ photo B, medium	O	O					
98	FISH (baked or broiled)	175 g/ 6 oz/ photo B, medium	O	O					
99	CANNED FISH (tuna, salmon)	½ can/ 48 ml/ 1.7 oz	O	O					
100	SMOKED FISH or LOX	85 g/ 3 oz/ photo C, small	O	O					
101	SALTED/ DRIED FISH	85 g/ 3 oz/ photo C, small	O	O					
102	PICKLED FISH	85 g/ 3 oz/ photo C, small	O	O					
103	SEA-BIRDS, SEAL	85 g/ 3 oz/ photo C, small	O	O					
104	CARIBOU, MOOSE	85 g/ 3 oz/ photo C, small	O	O					
105	PARTRIDGE, OTHER WILD BIRDS	85 g/ 3 oz/ photo C, small	O	O					
	Cereals and Grains								
106	BRAN or GRANOLA CEREALS (including All Bran)	½ cup/ 125 ml	O	O					
107	WHOLE WHEAT CEREALS (such as shredded wheat)	½ cup/ 125 ml/ 1 biscuit	O	O					
108	CEREALS, NOT SUGAR COATED (such as Special K)	½ cup/ 125 ml	O	O					
109	HOT CEREALS (for example: oatmeal)	½ cup/ 125 ml	O	O					
110	SUGAR COATED CEREALS	½ cup/ 125 ml	O	O					
111	OTHER BREAKFAST CEREALS	½ cup/ 125 ml	O	O					
112	SUGAR ON CEREAL	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
90	LIVER	85 g/ 3 oz	O	O					
91	FRIED CHICKEN	photo C, medium	O	O					
92	CHICKEN / TURKEY (roasted or stewed)	photo C, medium	O	O					
93	CHICKEN / TURKEY, SKIN REMOVED	photo C, medium	O	O					
94	SALTED/ DRIED MEAT	photo C, small	O	O					
95	PICKLED MEAT (brined)	photo C, small	O	O					
96	SHELLFISH (shrimp, lobster, crab)	85 g/ 3 oz/ photo C, small	O	O					
97	FRIED FISH	175 g/ 6 oz/ photo B, medium	O	O					
98	FISH (baked or broiled)	175 g/ 6 oz/ photo B, medium	O	O					
99	CANNED FISH (tuna, salmon)	½ can/ 48 ml/ 1.7 oz	O	O					
100	SMOKED FISH or LOX	85 g/ 3 oz/ photo C, small	O	O					
101	SALTED/ DRIED FISH	85 g/ 3 oz/ photo C, small	O	O					
102	PICKLED FISH	85 g/ 3 oz/ photo C, small	O	O					
103	SEA-BIRDS, SEAL	85 g/ 3 oz/ photo C, small	O	O					
104	CARIBOU, MOOSE	85 g/ 3 oz/ photo C, small	O	O					
105	PARTRIDGE, OTHER WILD BIRDS	85 g/ 3 oz/ photo C, small	O	O					
	Cereals and Grains								
106	BRAN or GRANOLA CEREALS (including All Bran)	½ cup/ 125 ml	O	O					
107	WHOLE WHEAT CEREALS (such as shredded wheat)	½ cup/ 125 ml/ 1 biscuit	O	O					
108	CEREALS, NOT SUGAR COATED (such as Special K)	½ cup/ 125 ml	O	O					
109	HOT CEREALS (for example: oatmeal)	½ cup/ 125 ml	O	O					
110	SUGAR COATED CEREALS	½ cup/ 125 ml	O	O					
111	OTHER BREAKFAST CEREALS	½ cup/ 125 ml	O	O					
112	SUGAR ON CEREAL	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
113	100% WHOLE GRAIN or DARK BREAD	1 slice	O	O					
114	60% WHOLE GRAIN, LIGHT RYE	1 slice	O	O					
115	WHITE BREAD	1 slice	O	O					
116	WHITE BREAD ROLLS (including hot dog buns etc)	1 roll	O	O					
117	WHOLE WHEAT ROLLS	1 roll	O	O					
118	CRACKERS (snack or soda type)	2	O	O					
119	BRAN/OAT MUFFIN	1 medium, ½ extra large	O	O					
120	OTHER MUFFIN (plain cake, with berries)	1 medium, ½ extra large	O	O					
121	PANCAKES, WAFFLES	1	O	O					
122	MACARONI, SPAGHETTI, NOODLES (plain)	1 cup cooked/ 250 ml	O	O					
123	RICE	½ cup cooked/ 125 ml	O	O					
124	CRISP SNACKS (potato chips, popcorn, pretzels etc)	small bag or 1 cup	O	O					
	Fruits								
125	APPLE, PEAR	1	O	O					
126	CITRUS FRUITS (orange, grapefruit)	1 orange, ½ grapefruit	O	O					
127	BERRIES (strawberries, blueberries, bakeapples)	½ cup/ 125 ml	O	O					
128	GRAPES	½ cup/ 125 ml	O	O					
129	BANANA	1	O	O					
130	PEACH, PLUM, NECTARINE, APRICOT	1	O	O					
131	CANTALOUPE	1/8 or 1 slice	O	O					
132	WATERMELON	1 wedge, 3" base	O	O					
133	HONEYDEW MELON	1/8 or 1 slice	O	O					
134	MANGO	1	O	O					
135	PAPAYA	1	O	O					
136	APPLESAUCE	½ cup/ 125 ml	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
137	DRIED FRUITS (raisins, dates, prunes)	2 tbsp/ 2 dates	O	O					
138	CANNED FRUIT (all kinds)	½ cup/ 125 ml	O	O					
139	ALL OTHER FRUIT (fresh kiwi, pomegranate, etc.)	1	O	O					
	Desserts and Sweets								
140	CAKES	1 slice, 2" x 4" x 1"	O	O					
141	PIES and TARTS	1 slice	O	O					
142	DONUTS and SWEET ROLLS	1	O	O					
143	COOKIES	1	O	O					
144	ICE CREAM	½ cup/ 125 ml	O	O					
145	LIGHT or DIET ICE CREAM	½ cup/ 125 ml	O	O					
146	PUDDING	½ cup/ 125 ml	O	O					
147	DIET or LIGHT PUDDING	½ cup/ 125 ml	O	O					
148	JELLO	½ cup/ 125 ml	O	O					
149	POPSICLES, FREEZIES	1	O	O					
150	CHOCOLATE BAR and CHOCOLATE CANDY	1 bar / 50g or 5 candy size	O	O					
151	CANDY (without chocolate)	1 caramel	O	O					
	Miscellaneous								
152	TOFU, TEMPEH	½ cup, 2" x 2" x 1" piece	O	O					
153	KETCHUP	1 tbs	O	O					
154	MAYONNAISE/ MIRACLE WHIP, Regular fat (on bread, salad, meat, etc)	1 tbs	O	O					
155	MAYONNAISE/ MIRACLE WHIP, Light (on bread, salad, meat, etc)	1 tbs	O	O					
156	SALAD DRESSING, Regular fat (French, Italian etc)	1 tbs	O	O					
157	OIL (in cooking)	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
158	BUTTER (on vegetables or bread; exclude use in baked and mixed dishes)	1 pat/ 1 tsp	O	O					
159	MARGARINE (on vegetables or bread; exclude use in baked or mixed dishes)	1 pat/ 1 tsp	O	O					
160	PEANUT BUTTER	1 tbs	O	O					
161	PEANUTS	30g/ 1 oz	O	O					
162	OTHER NUTS	30g /1 oz	O	O					
163	JAM, JELLY, HONEY, SYRUP	1 tbs	O	O					
164	GRAVY	4 tbs	O	O					
165	CHOCOLATE or STRAWBERRY SYRUP	1 tbs	O	O					
166	CHOCOLATE SPREADS	1 tbs	O	O					
167	SAUCES (white, cream, Mornay)	30 ml/ 1oz/ 2 tbs	O	O					
168	WHEAT BRAN	1 tbs	O	O					
169	WHEAT GERM	1 tbs	O	O					

Continue on next page ⟶

Now we would like to ask you a few questions about how you prepared certain foods ABOUT ONE YEAR BEFORE DIAGNOSIS and whether you followed any special diets. For the following questions, please check the circle or fill in the appropriate answer:

1. About 1 year before diagnosis, how much of the visible fat on your meat did you eat?

O Most of it.
O Some of it.
O As little as possible.
O Do not eat meat

2. About 1 year before diagnosis, how often did you eat the skin on chicken?

O Most of it.
O Some of it.
O As little as possible.
O Do not eat chicken

3. About 1 year before diagnosis, what kind of fat did you usually use for stir/pan frying?

O Vegetable oil
O Vegetable shortening
O Lard/ pork fat
O Butter
O Margarine
O Do not add fat or oil
O Other, please specify _____

4. About 1 year before diagnosis, what kind of fat did you usually use for deep frying?

O Vegetable oil
O Vegetable shortening
O Lard/ pork fat
O Butter
O Margarine
O Do not fry
O Other, please specify _____

5. About 1 year before diagnosis, what kind of fat did you usually use for baking?

O Butter
O Margarine
O Vegetable Oil
O Vegetable shortening
O Lard/ pork fat
O Do not bake

6. About 1 year before diagnosis, what type of oil did you use in other preparations (for example, in salad dressings)?

_____

7. About 1 year before diagnosis, what type of the following items did you usually use? *Please check one box per line.*

Mayonnaise/Miracle Whip
O regular O light O both O none

Cream cheese
O regular O light O both O none

8. About 1 year before diagnosis, were you a (*please check one box only*):

O Non-vegetarian (eats all meat, chicken, fowl)
O Partly non-vegetarian (eats chicken, fish, no meat)
O Vegan (eats no dairy, no eggs, no meat)
O Lacto-vegetarian (eats dairy, no eggs, no meat)
O Lacto-ovo vegetarian (eats dairy & eggs, no meat)

9. About 1 year before diagnosis, were you on a special diet?

O No O Yes

If yes, what type of diet?

O To lose Weight
O To lower cholesterol
O Diabetes
O Heart disease
O Hypertension
O Gastric ulcer
O Bowel disease
O Low fat
O High fibre
O Other type: _____

If yes, how long were you on the special diet?

_____

PART 2 - USE OF VITAMINS AND DIETARY SUPPLEMENTS

Now we would like to know about your use of vitamins and dietary supplements. <u>ABOUT ONE YEAR BEFORE DIAGNOSIS,</u> did you take any of the following? If *Yes*, then specify usual brand and amount and how long you took them.

Vitamin and Amount – if used, ⇨	<u>How many</u> pills did you take per week?	<u>How long</u> had you taken them?
Vitamin C EXAMPLE O None O Below 500 Ø 500-1000 O above 1000 mg	0 5 per week	2 4 months
Multivitamins that include minerals ⇨ O No O Yes If yes, usual brand _____	per week	months
Multivitamins, no minerals O No O Yes If yes, usual brand _____	per week	months
B Complex vitamins O No O Yes If yes, usual brand _____	per week	months
In the following items, DO NOT INCLUDE use of the above MULTIVITAMINS		
Vitamin A O None O Below 10000 O 10000-15000 O above 15000 IU	per week	months
Vitamin C O None O Below 500 O 500-1000 O above 1000 mg	per week	months
Vitamin E O None O Below 400 O 400-800 O above 800 IU	per week	months
Beta-carotene O None O Below 10000 O 10000-15000 O above 15000 IU	per week	months
Folic acid O None O Below 1.0 O 1.0 mg O above 1.0 mg*	per week	months
Calcium O None O Below 250 O 250-500 O above 500 mg	per week	months
Iron O None O Below 100 O 100-200 O above 200 mg	per week	months
Other dietary supplements (e.g., yeast, cod liver oil, etc) O No O Yes, specify type: _____	per week	months
_____	per week	months

* 1 mg = 1000 micrograms

We welcome any other information or comments that you would like to give us:

_____

_____

_____

THANK YOU VERY MUCH for your assistance in this research!

For Office Use Only

Study #: _____

Interviewer: _____

Date completed (D/M/Y): _____

Appendix 3. Publications/In Preparation

Manuscripts

1. Zhu Y, Wang PP, Zhai G, Savas S et al: Vitamin D Receptor and Calcium Sensing Receptor Polymorphisms and Colorectal Cancer Survival in Newfoundland Population. British Journal of Cancer 2017.

2. Sharma I, Wang PP, Zhu Y, Woodrow J, Mulay S, Parfrey PS et al: Inflammatory diet and risk of colorectal cancer: A population based Case-Control Study in Newfoundland, Canada. Nutrition 2017: 101(5).

3. Chen Z, Wang PP, Shi L, Zhu Y, Liu L, Gao Z, Woodrow J, Roebothan B: Comparison in dietary patterns derived for the Canadian Newfoundland and Labrador population through two time-separated studies. Nutrition Journal 2015: 14:75.

4. Chen Z, Wang PP, Woodrow J, Zhu Y, Roebothan B, Mclaughlin JR, Parfrey PS: Dietary patterns and colorectal cancer: results from a Canadian population-based study. Nutrition Journal 2015: 14(1):8.

5. Yan J, Liu L, Zhu Y, Huang G, Wang PP: The association between breastfeeding and childhood obesity: a meta-analysis. BMC Public Health 2014: 14(1):1267.

6. Zhu Y, Yang SR, Wang PP, Savas S, Wish T, Zhao J, Green R, Woods M, Roebothan B et al: Influence of pre-diagnostic cigarette smoking on colorectal cancer survival: overall and by tumour molecular phenotype. Br J Cancer 2014: 110(5):1359-66.

7. Zhu Y, Wang PP, Zhai G, Savas S et al: Association of rs2282679 A>C polymorphism in vitamin D binding protein gene with colorectal cancer risk and survival: effect modification by dietary vitamin D intake. *Submitted to BMC Cancer (manuscript ID: BCAN-D-16-02298).*

8. Zhao J, Zhu Y, Wang PP et al: Examining the direct and indirect effects of socioeconomic status (SES) on colorectal cancer risk using structural equation modeling. *Submitted to Clinical Epidemiology (manuscript ID: 146950).*

9. Zhu Y, Wang PP, Zhai G, Savas S et al: Prediagnostic Consumption of Calcium and Dairy Products and Colorectal Cancer Survival: Results from the Newfoundland Familial Colorectal Cancer Cohort Study. *In preparation*

10. Zhu Y, Zhao J, Wang PP, Zhai G et al: Walking, Non-walking Exercise and

Colorectal Cancer - a large population based case-control study in Canada. *In preparation*

11. Sharma I, Wang PP, Zhu Y, Woodrow J et al: Assessing agreement amongst dietary patterns: Findings from Newfoundland Colorectal Cancer survival Cohort. *In preparation*

12. Li Q, Zhu Y, Zhai G et al: DNA methylation profiling in MZ twins discordant for colon cancer. *In preparation*

Abstracts

1. Zhu Y, Wang PP, Zhai G, Bapat B, Sevtap S. Vitamin D receptor and calcium sensing receptor polymorphisms and colorectal cancer survival in Newfoundland population. EUR J CANCER 2017; 72(S):56.

2. Zhao J, Zhu Y, Wang PP. Examining the direct and indirect effects of socioeconomic status (SES) on colorectal cancer risk using structural equation modeling. EUR J CANCER 2017; 72(S):56.