eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

Dietary and genetic factors associated with risk for development of colorectal cancer: Case-control study in a Basque population

Image credits: Own work, by G.steph.rocket, CC by SA 4.0 license

Iker Alegria Lertxundi

PhD Thesis

2020

eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

Dietary and genetic factors associated with risk for development of colorectal cancer: Case-control study in a Basque population

Iker Alegria Lertxundi

PhD Thesis

Directed by:

Dra. Marta Arroyo Izaga

Department of Pharmacy and Food Sciences
PhD in Nutrigenomics and Personalised Nutrition
University of the Basque Country UPV/EHU
Vitoria-Gasteiz, 2020

Regulations:

National, Spain:
Royal decree 99/2011, of 28 January, regulating official teaching for the Ph.D. degree
https://www.boe.es/boe/dias/2011/02/10/pdfs/BOE-A-2011-2541.pdf

Autonomous, Basque Country:
Article 7 of the Official Bulletin of the Basque Country 122-2929
https://www.euskadi.eus/y22-bopv/eu/bopv2/datos/2013/06/1302929e.shtml

University of the Basque Country UPV/EHU:
Regulations Governing the Management of Doctoral Studies
https://www.ehu.eus/eu/web/mde/ekarpen-bilduma-bidezkotesiahttps://www.ehu.eus/documents/1325031/4136559/Tesis+por+compendio+de+contribuciones+eu.pdf/d498be3b-527a-47eb-a112-d470c8b647ca

To my parents

“Let food be thy medicine and medicine be thy food.”

Hippocrates

Acknowledgement

ACKNOWLEDGEMENT FOR THE FINANCIAL SUPPORT

Iker Alegria gratefully acknowledges the support provided by the Basque Government for the fellowship grant (PRE_2014_1_161, PRE_2015_2_0084, EP_2016_1_0098, EP_2016_1_0098 and PRE_2017_2_0006). This research was supported by two projects (from the Department of Health and Consumer Affairs, Basque Government 2011111153; and Saiotek, Basque Government S-PE12UN058), by the CIBERehd and by the U.S. Department of Agriculture—Agricultural Research Service (ARS), under agreement. 58-1950-4-003. Neither the Basque Government nor the U.S. Department of Agriculture—Agricultural Research Service (ARS) had a role in the design, analysis or writing of this article. CIBERehd is funded by the Instituto de Salud Carlos III.

ACKNOWLEDGMENT TO THE PARTICIPANTS AND RESEARCH SERVICES

The authors want to acknowledge particularly the patients enrolled in this study for their participation and the Basque Biobank for Research-OEHUN for its collaboration. The genotyping service was carried out at CEGEN-PRB2-ISCIII; it is supported by grant PT13/0001, ISCIII-SGEFI / FEDER.

ACKNOWLEDGMENT TO THE EDITORIALS

The authors would like to thank the editorials for granting permission to reuse their previously published articles in this doctoral thesis. The links to the final published versions are the following:

- Alegria-Lertxundi *et al.* Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Postsurgery: A Pilot Study. Nutr Cancer. 2016;68(4):577-88. doi: 10.1080/01635581.2016.1158299. Epub 2016 May 4. https://pubmed.ncbi.nlm.nih.gov/27144653/
- Alegria-Lertxundi *et al.* Single nucleotide polymorphisms associated with susceptibility for development of colorectal cancer: Case-control study in a Basque population. PLoS One. 2019 Dec 10;14(12):e0225779. doi: 10.1371/journal.pone.0225779. eCollection 2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0225779

The last two manuscripts have been sent to the following journals:

- Alegria-Lertxundi *et al.* Gene-diet interactions in colorectal cancer: survey design, instruments, participants and descriptive data of a case-control study in the Basque Country has been sent to *Nutrients*.

- Alegria-Lertxundi *et al.* Food groups, diet quality and colorectal cancer risk in the Basque Country is under review by *World Journal of Gastroenterology*.

ACKNOWLEDGMENTS TO THE RESEARCH GROUPS

This thesis has been carried out in the Department of Pharmacy and Food Sciences at the University of the Basque Country (UPV/EHU) in collaboration with:

- BIOMICs Research Group, UPV/EHU.
- BioDonostia Health Research Institute, Donostia University Hospital, Osakidetza/Basque Health Service.
- OSI Bilbao-Basurto, Basurto University Hospital, Osakidetza/Basque Health Service.
- OSI Barrualde-Galdakao, Galdakao-Usansolo University Hospital, Osakidetza/Basque Health Service.
- Center for Colorectal Cancer Screening Programme of Osakidetza/Basque Health Service.
- Basque Biobank for research (O+Ehun), Basque Foundation for Health Innovation and Research (BIOEF).
- Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University.

First of all I would like express my gratitude to my director of thesis, Dr. Marta Arroyo Izaga, for providing me the opportunity to get started in research, for contributing to this work, and for all her support and guidance. In other words, this would not be possible without her. In addition, I am extremely thankful to Prof. Marian Mtz. de Pancorbo for her valuable assistance and cooperation in matters related to genetics. She has taught me that difficult issues can be easily handled and understood. I also gratefully acknowledge the kind help and encouragement received from Prof. José M. Ordovás of the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University. Thanks to him I lived an unforgettable scientific and cultural experience in Boston.

In addition, I am really grateful to all the researchers on this project: Luis Bujanda, Francisco Javier Fernández, Francisco Polo, Mª Carmen Etxezarraga, Iñaki Zabalza, Eduardo de Miguel, Mikel Larzabal, Nerea Segués, Isabel Portillo, Vicente Portugal, Koldo Garcia, Mauro D´Amato, Naiara Garcia and specially to Carmelo Aguirre and Leire Palencia. Thank you, Carmelo, for your time and patience, this thesis would not have been completed without your support. Thank you, Leire for your unconditional support during these years.

OTHER ACKNOWLEDGMENTS

It is a must to mention to Dr. Ana Rocandio for collaborating on this thesis and all the fascinating coffees and meals we had in the cafeteria of the Faculty of Pharmacy. I will not forget the interesting conversations we have had. I would also like to express my gratitude to Estibaliz Mateos, Nekane Basabe, Saioa Telletxea and Sonia Geni Ribeiro De Luca, for all the support and advices offered during these years. I would like to express my gratitude to Diego Rada for giving me the opportunity of cooperating with him in "Nutritional Epidemiology". Thanks to Jorge Ortega, for all those meals and interesting talks we had in Bar Zeppelin together with Estibaliz Mateos.

I am also extremely grateful to Araitz Arantzamendi, "Bikale", for the support and help in some English texts corrections, and to Ibai Arantzamendi for showing interest on Nutrition science. I want to thank June Graña for giving some English advice (I encourage you, June, to undertake a thesis!) and Eneritz Acha for being a great roommate, in which we have spent great moments with Araitz and June. I want to show Naroa Kajarabille and Natalia Romo my gratitude for all the hours we spent talking next to the coffee machine supporting each other. Thanks to all marvellous people I met in these years in Zeppelin: Nagore, Derli, Sandrita, Sandra, Eli, Laura, Silvia and Uli. You have been a huge support sharing interesting anecdotes. Nagore, thank you for your "Pintxo-pote".

On the other hand, there are special people that I have had the opportunity to work and meet in different projects in a dream team, during all these incredible years: Nerea Tellería, Nerea Bermúdez, Ignacio Escribano, Verónica Ovejas, Belén Castro and Alba Martínez. I also want to dedicate this thesis to Dailos Cabrera. Last but not least, I would like to mention one of the most important and special person I have met during this thesis: Daniela Alves. Thank you for all the special moments we spent together. Even though we are far away from each other, we are still together listening to our favourite song "Drive by" composed by "Train". Thank you all!

Glossary

A: adenine
AI: adequate intake
AICR: American Institute for Cancer Research
AMDR: acceptable macronutrient distribution ranges
APC: adenomatous polyposis coli
ASRi: age-estandarized incidence rate
ASRm: age-estandarized mortality rate
BMI: body mass index
C: cytosine
CI: confidence interval
CRC: colorectal cancer
CRCSP: colorectal cancer screening programme
CUP: Continuous Update Project
DHA: docosahexaenoic acid
DI: deprivation index
DUSP10: dual specificity protein phosphatase 10
EAR: estimated average requirements
EER: estimated energy requirement
EPA: eicosapentaenoic acid
ERK: extracellular regulated mitogen-activated protein kinase
FOBT: faecal occult blood test
FS: flexible sigmoidoscopy
G: guanine
gFOBT: guaiac-based faecal occult blood test
GH1: growth hormone 1
GQ: general questionnaire
GWAS: genome-wide association study
HEISD: Healthy Eating Index for Spanish Diet
HRT: hormonal replacement therapy
HWE: Hardy-Weinberg equilibrum
IBD: inflammatory bowel disease
IGF-I: insulin like growth factor 1
iFOBT: immunochemical faecal occult blood test
JNK: c-Jun amino terminal kinase

KOM: Kolon-ondesteko minbizia
L: level
MAPK: mitogen-activated protein kinase
MD: Mediterranean diet
MDS: MedDietScore
MEKK1: MAPK/ERK kinase kinase 1
mRNA: messenger RNA
MTHFR: methylenetetrahydrofolate reductase
MUFA: monounsaturated fatty acids
NA: not available data
NOSP: nutritional objectives for the Spanish population
NSAID: non-steroidal anti-inflammatory drug
OR: odds ratio
PA: physical activity
PE: physical exercise
PI: physical inactivity
PRM: predictive risk modelling
PKG: cGMP-dependent protein kinase
PUFA: polyunsaturated fatty acids
Q: quintile
QoL: quality of life
RCT: randomized controlled trial
RR: relative risk
rs: reference single nucleotide polymorphism
SEK1: dual specificity mitogen-activated protein kinase kinase sek-1
SFFQ: short food frequency questionnaire
SFA: saturated fatty acids
SNP: single nucleotide polymorphism
T: thymine
TEI: total energy intake
WCR: World Cancer Research Fund
WHO: World Health Organization

Abstract

To date, case-control studies have revealed inconsistent evidence on the influence of dietary and genetic factors on colorectal cancer (CRC) risk. In order to better elucidate the role of some of these factors in the aetiology of CRC, the main objective of this study was to analyse dietary and genetic factors in a sample of cases and controls from the population-based CRC screening programme of the Osakidetza/Basque Health Service. In addition, taking into account that an unhealthy diet is associated with the risk of tumour recurrence, metastasis and death, the other aim of this thesis was to assess the adequacy of nutrients consumed and diet quality in a group of CRC patients postsurgery. The results showed that the diet of the studied CRC patients postsurgery is inadequate in many respects, including nutrients and food intakes. In fact, this inadequacy is associated with certain health determinants. On the other hand, there are direct associations between CRC risk and high-fat cheese, and inverse associations with fibre-containing foods and fatty fish, as well as adherence to a Mediterranean Diet pattern, in the case-control sample analysed. With respect to genetic factors, it was confirmed a CRC susceptibility locus and the existence of associations between modifiable factors and the rs6687758 SNP; moreover, the Genetic Risk Score was associated with CRC. However, further studies are needed to better understand the influence of the dietary habits on CRC prevention and to establish the role of the genetic factors, as well as the contribution of the gene-diet interactions to the risk of CRC in this population.

Resumen

Hasta la fecha, los estudios de casos y controles han mostrado contradicciones en las evidencias sobre la influencia de factores dietéticos y genéticos en el aumento del riesgo de cáncer colorrectal (CCR). Con el fin de conocer mejor el papel de algunos de estos factores en la etiología del CCR, planteamos el presente estudio con el objetivo principal de analizar factores dietéticos y genéticos en una muestra de casos y controles procedente del programa de cribado de CCR de Osakidetza/Servicio Vasco de Salud. Además, teniendo en cuenta que una dieta poco saludable se asocia con mayor riesgo de recurrencia del tumor, metástasis y mayor mortalidad, otro objetivo de esta tesis fue evaluar la adecuación de la ingesta de nutrientes y la calidad de la dieta en un grupo de pacientes diagnosticados de CCR, después del tratamiento quirúrgico. Los resultados mostraron que la dieta de los pacientes con CRC estudiados tras la cirugía, era inadecuada en varios aspectos, incluida la ingesta de nutrientes y alimentos, y que esta inadecuación estaba asociada a ciertos determinantes de salud. Por otro lado, en la muestra de casos y controles, se observó asociación directa entre el riesgo de CCR y el consumo de quesos con un alto contenido en grasa, y asociaciones inversas con la ingesta de alimentos ricos en fibra y de pescado azul, así como con la adherencia a un patrón de Dieta Mediterránea. Con respecto a los factores genéticos, se confirmó un locus de susceptibilidad para el CCR y la asociación entre ciertos factores modificables y el SNP rs6687758. También se asoció una mayor puntuación en el riesgo genético con el CCR. En cualquier caso, son necesarios más estudios para comprender mejor la influencia de los hábitos dietéticos sobre el CCR y para establecer el papel de los factores genéticos, así como la contribución de las interacciones gen-dieta al riesgo de CCR en esta población.

Laburpena

Orain arte, kasuen eta kontrolen azterketek agerian utzi dute faktore dietetikoek eta genetikoek kolon-ondesteko minbizirako (KOM) arriskua areagotzean duten eraginari buruzko ebidentziak kontraesankorrak direla. Faktore horietako batzuek KOM etiologian duten zeregina hobeto argitu nahiean, azterlan honen helburu nagusia faktore dietetikoak eta genetikoak aztertzea izan zen, Osakidetza-Euskal osasun zerbitzuaren KOM detekzio goiztiarreko programaren kasuen eta kontrolen lagin batean. Gainera, kontuan hartuta dieta ez oso osasungarria tumorearen errepikatze-arriskuarekin, metastasiarekin eta hilkortasunarekin lotuta dagoela, tesi honen beste helburua izan zen ebaluatzea ea egokia zen mantenugaien kontsumoa eta dietaren kalitatea KOM zuten pazienteen talde batean, kirurgia-tratamendua jaso ondoren. Emaitzek erakutsi zuten kirurgiaren ondoren aztertutako KOM zuten pazienteen dieta desegokia zela alderdi batzuetan, mantenugaien eta elikagaien kontsumoan barne, eta gutxiegitasun hori osasun-determinatzaile jakin batzuekin lotuta zegoela. Bestalde, kasuen eta kontrolen laginean, lotura zuzenak zeuden KOM arriskuaren eta gantz eduki handiko gazta kontsumoaren artean, eta alderantzizko asoziazioak zuntza eta arrain koipetsua duten elikagai-kontsumoarekin, baita Dieta Mediterraneoko patroi batekiko atxikidura ere. Faktore genetikoei dagokienez, baieztatu zen KOM izateko arriskua zegoela, eta faktore aldagarrien eta SNP rs687758 delakoaren arteko loturak zeudela. Gainera, arrisku genetikoan puntuazio handiagoa lortu izana gaixotasunarekin lotu zen. Dena den, azterlan gehiago behar dira elikadura-ohituren eragina KOM arriskuan, faktore genetikoen zeregina, eta geneen eta dietaren arteko elkarrekintzak hobeto ulertzeko.

Motivation of the present study

CRC is both one of the most common and one of the most preventable cancers. Its incidence is steadily rising in western countries and it is already the second most deadly cancer worldwide. The increased incidence of CRC has been attributed to environmental changes, such as a high consumption of processed foods, meat, meat derivatives and alcohol, more frequent sedentary behaviours, greater obesity and longevity. Many risk and protective factors, very common and potentially modifiable lifestyle behaviours, and specifically diet and nutrition have been studied to date. However, many of the results are inconsistent and vary considerably depending on the population and the methodology used. In order to better elucidate the role of some of these factors in the aetiology of CRC, the present work aimed to analyse dietary and genetic factors in a sample, not studied to date in this regard, from the population-based CRCSP of the Osakidetza/Basque Health Service. In addition, taking into account the potential of tertiary prevention measures to improve the prognosis and quality of life of CRC patients, the other aim of this work was to assess the adequacy of the diet of CRC patients postsurgery to the nutritional recommendations. Given the complexity of the subject that we will tackle, the results will be confirmed in subsequent studies with bigger sample sizes. In this sense, the population of the Autonomous Region of Basque Country opens up new opportunities, due to the relatively high incidence of this kind of cancer and the fact of having a public health system that allows us access to clinical histories, pathology reports and tissue samples, all of which are necessary for this study.

Table of Contents

1.INTRODUCTION

1. COLORECTAL CANCER

1.1. DEFINITION AND EPIDEMIOLOGY

Colorectal cancer (CRC) is a malignant neoplasm arising from epithelium anywhere in the large bowel (caecum, ascending, transverse, descending or sigmoid colon, rectum), excluding the appendix and the anus [1]. More than 90% of colorectal carcinomas are adenocarcinomas originating from the glandular epithelial of the large intestine [2]. CRC is already the second most deadly cancer worldwide, with about 881,000 deaths estimated for 2018 [3], and its incidence is steadily rising in western countries [4]. According to GLOBOCAN 2018 data, CRCs are the third most commonly diagnosed form of cancer globally, consisting of 11% of all cancer diagnoses [4]. This type of cancer has a higher incidence in men than women and an increasing age-standardized incidence rate, (ASRi) per 100,000 of CRC in both sexes is 19.7, in males it is 23.6, and in females 16.3 [5].

Incidence and mortality varies geographically, more-developed regions have a higher incidence and mortality than less-developed ones [5]. Europe is among the seven world regions ranked according to ASRi, with an ASRi of 30.0 per 100,000 [6]. In particular, in Spain, the ASRi for CRC is 33.4 per 100,000 (21.1 per 100,000 in males and 12.4 per 100,000 in females) [7]. In the Basque Country, one of the autonomous regions of the North of Spain, this pathology is the most frequent type of cancer (taking into account the incidence rates of both sexes combined) [8]. The main factors that contribute to incidence variation between countries and even within a nation are disparities in access to screenings and in lifestyle behaviours [4,9,10].

Regarding the mortality in CRC in countries with a high human development index, the age-standardized mortality rate (ASRm) is 12.8 per 100,000 among males and 8.5 per 100,000 among females [4]. The CRC is the deadliest cancer among males in Saudi Arabia, Oman and United Arab Emirates, and the most deadly among females in Algeria, Belarus, Japan, Spain, and Portugal [4]. During recent years, mortality rates by CRC have been decreasing due to early screening programs [10,11] and better treatment options [12]. However, by the year 2030, the global burden of CRC is expected to increase by 60% to over 2.2 million new cases. This growth would be probably related to environmental changes, such as a high consumption of processed foods, meat, meat derivatives and alcohol, more frequent sedentary behaviours, greater obesity and longevity [13].

1.2. AETIOLOGY

The aetiology of CRC is complex and still not fully understood. Historically, CRCs have been hypothesized to arise via the gradual stepwise accumulation of mutations [14,15]. The progression from normal tissue to dysplastic epithelium to carcinoma would be accompanied by the accumulation of mutations that perturb specific genetic pathways at each phase of the tumorigenic process. However, several studies demonstrated that 80%–90% of CRC are initiated following the loss of activity of the adenomatous polyposis coli (APC) gene [16,17].

New evolutionary models, for their part, explain better the vast amount of heterogeneity observed within and across colorectal tumours [18]. Good examples of this are the "Big Bang" theory of tumorigenesis [19] and the cancer "punctuated equilibrium" model. This latter model describes the development of tumours as a process characterized by long periods of stasis, punctuated by rapid periods of transformation and molecular changes [20]. In these rapid periods, mutations, epigenetic and transcriptional alterations take place, producing changes in the phenotype and contributing to the development of adenoma.

Notwithstanding these advances, many aspects related to the aetiology of this type of cancer such as the adenoma-to-carcinoma sequence, the timing of mechanisms involved and the dynamics between multiple clones remain unknown. Authors such as Sievers *et al.* [18] have speculated that there are previously unidentified factors, such genetic, epigenetic, or signalling related factors, that make certain cells more susceptible to neoplastic transformation. Understanding better the causes and mechanisms of this disease is critical for long-term prevention, detection and treatment.

1.3. RISK FACTORS

Unlike other cancers, no single risk factor accounts for most cases of CRC. Although inherited susceptibility results in the most striking increases in risk, the majority of CRCs are sporadic rather than familial. Moreover, both genetic and environmental factors play an important role in its aetiology [21]. Males have about a 1.5-fold higher chance than females of developing CRC, taking into account all ages and nations [4]. However, women are more susceptible to suffer from right-sided colon cancer, which is characterised by a greater aggressive growth compared to the left-sided one [22]. In addition, people over 65 years old are about three times more likely to suffer from CRC than those between 50-64 years of age, and about 30 times more likely to suffer from CRC than those who are 25-49 years old [3].

Besides age and male sex, other risk factors identified in epidemiological studies are the following: family history of CRC [23], inflammatory bowel disease (IBD), smoking, excessive alcohol consumption, high consumption of red and processed meat, physical inactivity (PI), obesity, and diabetes. The risk increase is strongest for people with CRC history in first-degree and people with IBD, for both factors the estimated relative risks (RRs) are greater than 2. More than 30% of CRC patients have a family history of this type of cancer. Those with a relative with CRC history in first-degree suffer a 2-4 times higher risk. The most common hereditary syndrome, the Lynch syndrome, accounts for 2%-4% all cases. The second one is the familial adenomatous polyposis, which is less than 1% of all cases [3].

Patients with IBD have a two-fold risk of developing CRC. The primary causes behind IBD are ulcerative colitis and Crohn´s disease [24,25]. Both IBDs are auto-immunes and characterised by inflammation that results in the abnormal release of growth cytokines, metabolic free radicals, excess blood flow and other factors that have an influence on carcinogenesis. There are other, more common and potentially modifiable, risk factors, such as lifestyle and certain diseases that contribute greatly to CRC process at the population level, even if their RR is low (between 1.2 and 2.0) [26]. Thus toxic habits such as tobacco and alcohol have been associated with CRC risk. The relative CRC risk of regular smoking is 1.18 [27] and this risk has been associated with mutagens of tobacco [28]. A meta-analysis [29] and more recent studies [30] have also found that associations between alcohol consumption and CRC risk is dose-dependent. Although the mechanisms underlying alcohol-induced CRC are still not well defined, plausible events include: genotoxic effect of acetaldehyde, increased estrogen concentration, cellular stress, altered folate metabolism, and inflammation [31,32].

With regard to diet, red meat and meat derivatives are known to increase the risk of CRC [33]. Prospective studies found a RR of 1.22 among those who consumed the most red meat and meat derivatives [34]. In fact, the International Agency for Research on Cancer (IARC) designated processed meat as “carcinogenic” and red meat as “probably carcinogenic”, due to its impact on CRC risk [35]. This effect has been related to the high fat and inflammatory substances content [36,37] and the high-temperature cooking of meat [37,38].

On the other hand, both obesity and PI constitute the most significant behavioural contributors to CRC development, especially in countries with a high human development index, Studies found that sedentary people can have up to a 50% risk of CRC. It has been estimated that obesity is associated with a 50% greater risk of colon

cancer in men and 20% in women, and 20% greater risk of rectal cancer in men and 10% in women [3]. The changes that occur in the obese state and that connect obesity with increased CRC risk are the following: altered levels of insulin, insulin-like growth factor-1, leptin, adiponectin, steroid hormones, and cytokines [39]. In the same way, Diabetes mellitus is linked to a predisposition to suffer from CRC [40], due to shared risk factors between diabetes, obesity and sedentary behaviours. Finally, emerging evidence suggests that infection with *Helicobacter pylori*, *Fusobacterium* spp, and other potential infectious agents might be associated with an increase of CRC [41-43].

1.4. PREVENTIVE FACTORS

Established preventive factors against CRC include physical activity (PA) and use of certain medications with risk reduction in the order of 20%-30% [22,44-46]. Many studies have emphasized the effect of PA in reducing the risk of susceptibility to CRC [47,48]. Authors such Golshiri *et al.* [49] found that CRC risk in people that practice PA in leisure-time is 27% less than people who do not. Possible events related to the mechanisms underlying PA-mediated CRC include: changes in the material in gastrointestinal transmit time, immune function as well as changes in prostaglandin levels, insulin, insulin-like growth factors, bile acid secretion, serum cholesterol as well as pancreatic and gastrointestinal hormone profiles [50,51].

Certain medications that are commonly used for other diseases, such as non-steroidal anti-inflammatory drugs (NSAIDs) and hormonal replacement therapy (HRT) in postmenopausal women have also been shown to protect against CRC. For the first of these drugs, the benefit has not been quantified, and due to the side effects that these drugs can cause they are not used in primary prevention of CRC [3]. And with regard to the latter, the protective effects remain controversial: although observational studies found a long-term decreased risk of CRC, randomized trials did not replicate these results [3]. Although not as consistent as PA and certain medications, several data suggest a protective effect of a diet especially rich in fruits and vegetables, fibre, resistant starch, dairy products, as well as calcium supplements [33,52,53]. This protective effect of diet and nutrition against the development of CRC is independent of obesity and is estimated to explain 30%-50% of the CRC incidences.

Fibre found commonly in vegetables, fruits, and whole grains are protective because they increase transit time and consequently reduce the carcinogens exposition. In addition, bacterial fermentation of fibre produces short-chain fatty acids with anticarcinogenic properties [54,55]. The Continuous Update Project (CUP) on CRC of

2011, led by the World Cancer Research Fund and American Institute of Cancer Research (WCRF/AICR), concluded that there was now “convincing” evidence that increased fibre intake was protective against the risk of colorectal cancer [56]. Regarding dairy product consumption, two meta-analyses [57,58] reported a significant inverse association when comparing the highest and lowest levels of intake. Observed inverse associations between intake of dairy products and CRC development have been largely attributed to their content of calcium, as well as lactic acid-producing bacteria and other constituents or bio-active compounds [59,60].

The last CUP on CRC, led by WCRF/AICR, determined that there is strong evidence that the consumption of dairy products may help to protect against CRC [33]. However, the CRC risk associated with the consumption of different types of dairy products (e.g., yogurt or hard cheese), as well as the consumption of dairy product subtypes according to their fat composition (e.g., skimmed/semi-skimmed or full milk), remains unclear [61-63]. The WCRF/AICR, in the last CUP on CRC, concluded that calcium supplement diets probably decrease CRC risk [33]. This mineral may reduce CRC risk via stimulating differentiation, reducing proliferation, and inducing apoptosis [64]. In addition, a few large randomized controlled trials (RCTs), have reported that this supplementation can reduce recurrence of adenomas, precursors of most sporadic CRCs [65-66]. Nevertheless, some issues on the association between calcium and CRC risk are still not well understood, as is the case of differences according to the anatomic subsite of the tumour, the dose-response or when exposure to this mineral may play the most significant role.

Other nutrients supplements apart from calcium, such as folate seem to inhibit carcinogenesis but promote the growth of existent tumours. Therefore, its use is not recommended, except in case of pregnancy or MTHFR mutation, which have a predisposition to high homocysteine levels [3]. In any case, foods and nutrients are not consumed in isolation but as part of a dietary pattern; therefore, the actual effect of diet on disease risk may be observed only when all components are considered jointly [67]. For this purpose, several diet quality indexes have been developed using point systems to measure whole diet quality based on the alignment of food choices with dietary recommendations. Some of these indices have been used to begin assessing the relationships between overall diet quality and CRC risk, and the results show that high scores in these indices are associated with a lower CRC risk [68-71]. In particular, the association between Mediterranean Diet (MD) and CRC has been examined by many case-control [72-74] and cohort studies [75].

This dietary pattern is characterised by high intakes of vegetables, fruits, whole grains, nuts, and olive oil; moderate intakes of fish, poultry, and low-fat dairy foods; and low intakes of red meat, processed meat, and sugar-sweetened drinks. Although most of studies showed an inverse association between the use of MD and the risk for CRC, some inconsistencies have been observed, firstly relating to the different scoring indexes utilized to determine MD adherence. In spite of these inconsistencies, results of observational studies and three meta-analyses showed a decreased risk of CRC associated with adherence to a MD [76]. The health benefits of the MD have been extensively documented in the literature about protection against various diseases, including cancers. This is also reflected in its inclusion as a recommended dietary pattern, among others in the 2015 Dietary Guidelines for Americans [77].

Lastly, it should be noted that protective and risk factors are not present in isolation, but coexist and interact with each other and with other factors, for example, both dietary and genetic factors affect CRC risk, through an interactive manner [78]. The recognition of these interactions as a driver in CRC may open up new areas of research in disease epidemiology, risk assessment, and treatments.

1.5. GENETIC SUSCEPTIBILITY

Although environmental factors are undoubtedly major risk factors for this type of cancer, early onset and familial clustering suggest that CRC has a substantial heritable component [79]. In a study carried out in a large twin sample [80], researchers noted that 35% of CRC risk might be attributable to heritable factors. While rare genetic variants with high penetrance do confer a predisposition for inherited forms of CRC, as is also the case in familial adenomatous polyposis and Lynch syndrome, which account for 5% of CRC cases [81], the remaining genetic heritability appears to be a consequence of joint inheritance of multiple common low-penetrance genetic variants [82,83]. It seems that susceptibility single-nucleotide polymorphisms (SNPs) confer weak but cumulative and increasing effects on CRC development [84].

To investigate common low-penetrance genetic variants for CRC, genome-wide screening using high-throughput DNA sequencing has been tried. A previous genome-wide association study (GWAS) had identified several CRC-susceptibility SNPs that confer a modest increased risk of CRC in populations of European ancestry [85-91]. Despite many candidate gene [92] and genome-wide association studies (GWAS) [93] evaluating common genetic risk factors for CRC, only a few of these have been replicated in subsequent studies [94].

1.6. PREVENTION

Colorectal cancer (CRC) is both one of the most common and one of the most preventable cancers globally, with an important potential for primary, secondary and tertiary prevention. Epidemiological evidence suggests that a significant proportion of CRCs could be avoided by changing lifestyle-related factors [96]. Several of the aforementioned risk factors, especially maintenance of a reasonable level of PA and a healthy weight, should be included in comprehensive primary prevention strategies. Regarding diet and nutrition, since foods and nutrients are not consumed in isolation and there is controversy about the role of specific foods groups and nutrients, consideration of dietary pattern as a whole appears useful for establishing recommendations.

The role of many nutritional supplements, including, folate, vitamin B_6 and omega-3, remains unknown. Apparently, only calcium and vitamin D supplementation appear to add a modest benefit, particularly in those with a low daily intake [97]. On the other hand, the role of medications such as aspirin and NSAIDs drugs, and postmenopausal HRT might be associated with substantial reductions in CRC risk. However, due to lack of data and possible side-effects, these medications are not recommended in primary prevention of CRC [98]. Since most cases of CRC develop slowly over more than 10 years and early detection allows efficient treatment, perspectives for secondary prevention by screening are much better for this cancer than for most other cancers. Faecal occult blood testing (FOBT) (that can either be guaiac-based (gFOBT) or immunochemical (iFOBT)), flexible sigmoidoscopy (FS) and colonoscopy are recognized as being cost-efficient [100] and the majority of guidelines suggest 1-2 year intervals for FOBT screening [99].

National and international screening guidelines also recommended starting at 50 years of age for average-risk individuals, with use of gFOBT or iFOBT. A positive gFOBT or iFOBT has to be followed up by colonoscopy. Population-based CRC screening programmes (CRCSPs) have been implemented from 2013 nationally or regionally in 20 of the EU Member States, in the 50+ year age group. Modelling studies [101] suggest that the cost-effectiveness ratio of theses CRCSPs is dependent on several factors such as background risk, screening method and organization of the programme, resources in health care and on the targeted age range, among others. iFOBT has been the most common screening test because of its higher sensitivity and the logistic advantages it has over gFOBT [102,103].

The Spanish Society of Medical Oncology, for its part, recommends screening for average-risk individuals aged >= 50, without any other added risk factors, with use of biennial iFOBT. As an alternative to iFOBT, annual or biennial high-sensitivity gFOBT, FS repeated every 5 years or colonoscopy repeated every 10 years can be used. Population-based CRCSP is covered in all the Autonomous Communities, and it was estimated that the entire population would be included in organized population-based programmes by the year 2024 [104]. In particular, in 2008, in the Autonomous Community of the Basque Country the implementation of a regional population-based screening programme for CRC was approved. The programme was aimed at men and women between 50 and 69 years old, using one sample of iFOBT biennially and a colonoscopy under sedation as a diagnostic confirmation in positive cases. The programme started in 2009, reaching almost the whole target population (approximately 586,700 people) at the beginning of 2014. The main results found in the first period showed a high participation rate, as well as high adenoma and CRC detection rates [105,106].

Increasing evidence shows also that the prognosis and quality of life (QoL) of CRC patients can be substantially improved by tertiary prevention measures [107]. In particular, the use of aspirin, increasing PA level and cessation of smoking could improve the survival and the quality of life of CRC patients. In observational studies there is increasing evidence that use of low-dose aspirin could enhance survival after CRC diagnosis [108-110], through mechanisms related to cyclooxygenase (COX) inhibition and non-COX mechanisms [111]. Some randomized controlled trials have begun to study the potential role of this medication in tertiary prevention [112]. A smoking habit and excessive alcohol consumption, in addition to being risk factors for CRC, are associated with lower survival rates in CRC patients [113]. Although the mechanism of these associations remain unclear, it is known that these unhealthy lifestyle habits increase surgical complications and decrease response to radiotherapy and chemotherapy, among other effects [114-116].

Moreover, there is evidence that PA appears to have a favourable influence on cancer outcomes, including common cancer symptoms, quality of life and survival [117,118]. In this sense, some of the most studied mechanisms include changes in whole-body and visceral fatness, metabolic dysregulation, adipokines, and sex hormones; chronic, low-grade inflammation; oxidative stress causing DNA damage and gene mutations; and impaired immune surveillance/function [119-121]. Finally, there is no convincing evidence about the association with improved survival in CRC patients that follow

guidelines [122]. However, having a healthy body weight, being physically active and following a healthy diet after diagnosis were all associated with a longer survival of colon cancer patients in stage III in a chemotherapy trial [123]. Taking into account that after the "teachable moment" of a cancer diagnosis patients are more likely to change their habits, then it seems likely that the promotion of healthy lifestyle in CRC patients could improve prognoses considerably.

References:

1. Oxford Medical Education. Available from: http://www.oxfordmedicaleducation.com/wp-content/uploads/2016/03/Colorectal-cancer.protected.pdf (Accesed: on 04 June 2020).
2. Hamilton SR, Bosman FT, Boffetta P, *et al.* Carcinoma of the colon and rectum. In: WHO Classification of Tumours of the Digestive System. Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. Lyon: IARC Press, 2010:134-46.
3. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol*. 2019;14(2):89-103. doi: 10.5114/pg.2018.81072. Epub 2019 Jan 6. PMID: 31616522; PMCID: PMC6791134.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018 Nov;68(6):394-424. doi: 10.3322/caac.21492. Epub 2018 Sep 12. PMID: 30207593.
5. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2018). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today (Accessed on 02 November 2018)
6. Globocan 2018. Available from: https://gco.iarc.fr/today/online-analysis-table?v=2018&mode=cancer&mode_population=continents&population=900&populations=908&key=asr&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&group_cancer=1&include_nmsc=1&include_nmsc_other=1#collapse-group-1-4-0 (accesed on 02 November 2018)
7. Globocan 2018. Available from https://gco.iarc.fr/today/data/factsheets/populations/724-spain-fact-sheets.pdf) (accessed on 02 November 2018).

8. Lopez de Munain A, Audicana C. Cáncer en la Comunidad Autónoma de Euskadi 2001-2017. Departamento de Salud de Gobierno Vasco/Eusko Jaurlaritza. 2019. Available from: https://www.euskadi.eus/contenidos/informacion/registros_cancer/es_def/adjuntos/Cancer-CAE-2001-2017.pdf (accessed on 02 November 2018)
9. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence – SEER 18 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2015 Sub (1973–2013 varying) – Linked To County Attributes – Total U.S., 1969–2014 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2016, based on the November 2015 submission.
10. Roquette R, Painho M, Nunes B. Geographical patterns of the incidence and mortality of colorectal cancer in mainland Portugal municipalities (2007-2011). *BMC Cancer*. 2019 May 29;19(1):512. doi: 10.1186/s12885-019-5719-9. PMID: 31142284; PMCID: PMC6542026.
11. Bosetti C, Levi F, Rosato V, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Recent trends in colorectal cancer mortality in Europe. *Int J Cancer*. 2011 Jul 1;129(1):180-91. doi: 10.1002/ijc.25653. Epub 2010 Nov 16. PMID: 20824701.
12. Thanikachalam K, Khan G. Colorectal Cancer and Nutrition. *Nutrients*. 2019 Jan 14;11(1):164. doi: 10.3390/nu11010164. PMID: 30646512; PMCID: PMC6357054.
13. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017 Apr;66(4):683-691. doi: 10.1136/gutjnl-2015-310912. Epub 2016 Jan 27. PMID: 26818619.
14. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med*. 1988 Sep 1;319(9):525-32. doi: 10.1056/NEJM198809013190901. PMID: 2841597.
15. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. *Cancer*. 1975 Dec;36(6):2251-70. doi: 10.1002/cncr.2820360944. PMID: 1203876.
16. Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Mori T, Nakamura Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet*. 1992 Jul;1(4):229-33. doi: 10.1093/hmg/1.4.229. PMID: 1338904.
17. Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. *Hum Mutat*. 1993;2(6):425-34. doi: 10.1002/humu.1380020602. PMID: 8111410.

18. Sievers CK, Grady WM, Halberg RB, Pickhardt PJ. New insights into the earliest stages of colorectal tumorigenesis. *Expert Rev Gastroenterol Hepatol*. 2017 Aug;11(8):723-729. doi: 10.1080/17474124.2017.1330150. Epub 2017 May 26. PMID: 28503955; PMCID: PMC5859121.
19. Sottoriva A, Kang H, Ma Z, Graham TA, Salomon MP, Zhao J, Marjoram P, Siegmund K, Press MF, Shibata D, Curtis C. A Big Bang model of human colorectal tumor growth. *Nat Genet.* 2015 Mar;47(3):209-16. doi: 10.1038/ng.3214. Epub 2015 Feb 9. PMID: 25665006; PMCID: PMC4575589.
20. Cross WCh, Graham TA, Wright NA. New paradigms in clonal evolution: punctuated equilibrium in cancer. *J Pathol.* 2016 Oct;240(2):126-36. doi: 10.1002/path.4757. Epub 2016 Aug 24. PMID: 27282810.
21. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ, Watanabe T. Colorectal cancer. *Nat Rev Dis Primers*. 2015 Nov 5;1:15065. doi: 10.1038/nrdp.2015.65. PMID: 27189416; PMCID: PMC4874655.
22. Kim SE, Paik HY, Yoon H, Lee JE, Kim N, Sung MK. Sex- and gender-specific disparities in colorectal cancer risk. *World J Gastroenterol*. 2015 May 7;21(17):5167-75. doi: 10.3748/wjg.v21.i17.5167. PMID: 25954090; PMCID: PMC4419057.
23. Taylor DP, Burt RW, Williams MS, Haug PJ, Cannon-Albright LA. Population-based family history-specific risks for colorectal cancer: a constellation approach. *Gastroenterology*. 2010 Mar;138(3):877-85. doi: 10.1053/j.gastro.2009.11.044. Epub 2009 Dec 21. PMID: 19932107; PMCID: PMC2831153.
24. Lutgens MW, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm Bowel Dis*. 2013 Mar-Apr;19(4):789-99. doi: 10.1097/MIB.0b013e31828029c0. PMID: 23448792.
25. Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol*. 2012 Jun;10(6):639-45. doi: 10.1016/j.cgh.2012.01.010. Epub 2012 Jan 28. PMID: 22289873.
26. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014 Apr 26;383(9927):1490-1502. doi: 10.1016/S0140-6736(13)61649-9. Epub 2013 Nov 11. PMID: 24225001.
27. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. *JAMA*. 2008 Dec 17;300(23):2765-78. doi: 10.1001/jama.2008.839. PMID: 19088354.
28. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, Slager SL, Smyrk

TC, Thibodeau SN, Cerhan JR, Limburg PJ. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. *J Natl Cancer Inst*. 2010 Jul 21;102(14):1012-22. doi: 10.1093/jnci/djq201. Epub 2010 Jun 29. PMID: 20587792; PMCID: PMC2915616.

29. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, Negri E, Straif K, Romieu I, La Vecchia C, Boffetta P, Jenab M. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Ann Oncol*. 2011 Sep;22(9):1958-72. doi: 10.1093/annonc/mdq653. Epub 2011 Feb 9. PMID: 21307158.
30. Ibáñez-Sanz G, Díez-Villanueva A, Alonso MH, Rodríguez-Moranta F, Pérez-Gómez B, Bustamante M, Martin V, Llorca J, Amiano P, Ardanaz E, Tardón A, Jiménez-Moleón JJ, Peiró R, Alguacil J, Navarro C, Guinó E, Binefa G, Fernández-Navarro P, Espinosa A, Dávila-Batista V, Molina AJ, Palazuelos C, Castaño-Vinyals G, Aragonés N, Kogevinas M, Pollán M, Moreno V. Risk Model for Colorectal Cancer in Spanish Population Using Environmental and Genetic Factors: Results from the MCC-Spain study. *Sci Rep*. 2017 Feb 24;7:43263. doi: 10.1038/srep43263. Erratum in: Sci Rep. 2017 May 17;7:46807. Navarro, Pablo Fernández [corrected to Fernández-Navarro, Pablo]. Erratum in: *Sci Rep*. 2017 May 17;7(1):46807. PMID: 28233817; PMCID: PMC5324108.
31. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer*. 2007 Aug;7(8):599-612. doi: 10.1038/nrc2191. PMID: 17646865.
32. Huang X, Li X, Ma Q, Xu Q, Duan W, Lei J, Zhang L, Wu Z. Chronic alcohol exposure exacerbates inflammation and triggers pancreatic acinar-to-ductal metaplasia through PI3K/Akt/IKK. *Int J Mol Med*. 2015 Mar;35(3):653-63. doi: 10.3892/ijmm.2014.2055. Epub 2014 Dec 29. PMID: 25573338; PMCID: PMC4314411.
33. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. 2017. Available from: https://www.wcrf.org/sites/default/files/Colorectal-cancer-report.pdf (accesed on 15 january 2019).
34. Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, Norat T. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. PLoS One. 2011;6(6):e20456. doi: 10.1371/journal.pone.0020456. Epub 2011 Jun 6. PMID: 21674008; PMCID: PMC3108955.
35. Kim E, Coelho D, Blachier F. Review of the association between meat consumption and risk of colorectal cancer. *Nutr Res*. 2013 Dec;33(12):983-94. doi: 10.1016/j.nutres.2013.07.018. Epub 2013 Oct 7. PMID: 24267037.

36. Gamage SMK, Dissabandara L, Lam AK, Gopalan V. The role of heme iron molecules derived from red and processed meat in the pathogenesis of colorectal carcinoma. *Crit Rev Oncol Hematol*. 2018 Jun;126:121-128. doi: 10.1016/j.critrevonc.2018.03.025. Epub 2018 Apr 7. PMID: 29759553.
37. Turesky RJ. Mechanistic Evidence for Red Meat and Processed Meat Intake and Cancer Risk: A Follow-up on the International Agency for Research on Cancer Evaluation of 2015. *Chimia (Aarau*). 2018 Oct 31;72(10):718-724. doi: 10.2533/chimia.2018.718. PMID: 30376922; PMCID: PMC6294997.
38. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen*. 2004;44(1):44-55. doi: 10.1002/em.20030. PMID: 15199546.
39. Hopkins BD, Goncalves MD, Cantley LC. Obesity and Cancer Mechanisms: Cancer Metabolism. *J Clin Oncol*. 2016 Dec 10;34(35):4277-4283. doi: 10.1200/JCO.2016.67.9712. Epub 2016 Nov 7. PMID: 27903152; PMCID: PMC5562429.
40. Luo W, Cao Y, Liao C, Gao F. Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. *Colorectal Dis.* 2012 Nov;14(11):1307-12. doi: 10.1111/j.1463-1318.2012.02875.x. PMID: 23046351.
41. Sonnenberg A, Genta RM. Helicobacter pylori is a risk factor for colonic neoplasms. *Am J Gastroenterol*. 2013 Feb;108(2):208-15. doi: 10.1038/ajg.2012.407. Epub 2012 Dec 4. PMID: 23208272.
42. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, Chan FKL, Kristiansen K, Sung JJY, Wong SH, Yu J. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome*. 2018 Apr 11;6(1):70. doi: 10.1186/s40168-018-0451-2. PMID: 29642940; PMCID: PMC5896039.
43. Boleij A, van Gelder MM, Swinkels DW, Tjalsma H. Clinical Importance of Streptococcus gallolyticus infection among colorectal cancer patients: systematic review and meta-analysis. *Clin Infect Dis*. 2011 Nov;53(9):870-8. doi: 10.1093/cid/cir609. Epub 2011 Sep 29. PMID: 21960713.
44. Park HC, Shin A, Kim BW, Jung KW, Won YJ, Oh JH, Jeong SY, Yu CS, Lee BH. Data on the characteristics and the survival of korean patients with colorectal cancer from the Korea central cancer registry. *Ann Coloproctol*. 2013 Aug;29(4):144-9. doi: 10.3393/ac.2013.29.4.144. Epub 2013 Aug 29. PMID: 24032114; PMCID: PMC3767863.
45. De Rosa M, Pace U, Rega D, Costabile V, Duraturo F, Izzo P, Delrio P. Genetics, diagnosis and management of colorectal cancer (Review). *Oncol Rep*. 2015

Sep;34(3):1087-96. doi: 10.3892/or.2015.4108. Epub 2015 Jul 3. PMID: 26151224; PMCID: PMC4530899.

46. Bonadona V, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C, Noguès C, Lejeune-Dumoulin S, Olivier-Faivre L, Polycarpe-Osaer F, Nguyen TD, Desseigne F, Saurin JC, Berthet P, Leroux D, Duffour J, Manouvrier S, Frébourg T, Sobol H, Lasset C, Bonaïti-Pellié C; French Cancer Genetics Network. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA*. 2011 Jun 8;305(22):2304-10. doi: 10.1001/jama.2011.743. PMID: 21642682.
47. Ghafari M, Mohammadian M, Valipour AA, Mohammadian-Hafshejani A. The Future Prospects of Testis Cancer in the World*. Iran J Public Health.* 2017 Dec;46(12):1750-1751. PMID: 29259956; PMCID: PMC5734981.
48. Campbell PT, Patel AV, Newton CC, Jacobs EJ, Gapstur SM. Associations of recreational physical activity and leisure time spent sitting with colorectal cancer survival. *J Clin Oncol*. 2013 Mar 1;31(7):876-85. doi: 10.1200/JCO.2012.45.9735. Epub 2013 Jan 22. PMID: 23341510.
49. Golshiri P, Rasooli S, Emami M, Najimi A. Effects of Physical Activity on Risk of Colorectal Cancer: A Case-control Study. *Int J Prev Med*. 2016 Feb 8;7:32. doi: 10.4103/2008-7802.175991. PMID: 26952161; PMCID: PMC4763467.
50. Quadrilatero J, Hoffman-Goetz L. Physical activity and colon cancer. A systematic review of potential mechanisms. *J Sports Med Phys Fitness*. 2003 Jun;43(2):121-38. PMID: 12853893.
51. Kruk J, Aboul-Enein HY. Physical activity in the prevention of cancer. *Asian Pac J Cancer Prev*. 2006 Jan-Mar;7(1):11-21. PMID: 16629509.
52. Ricciardiello L, Ahnen DJ, Lynch PM. Chemoprevention of hereditary colon cancers: time for new strategies. *Nat Rev Gastroenterol Hepatol*. 2016 Jun;13(6):352-61. doi: 10.1038/nrgastro.2016.56. Epub 2016 Apr 20. PMID: 27095653.
53. Lutgens MW, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm Bowel Dis*. 2013 Mar-Apr;19(4):789-99. doi: 10.1097/MIB.0b013e31828029c0. PMID: 23448792.
54. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology*. 2015 May;148(6):1244-60.e16. doi: 10.1053/j.gastro.2014.12.035. Epub 2015 Jan 6. PMID: 25575572; PMCID: PMC4409470.

55. Sengupta S, Muir JG, Gibson PR. Does butyrate protect from colorectal cancer? *J Gastroenterol Hepatol*. 2006 Jan;21(1 Pt 2):209-18. doi: 10.1111/j.1440-1746.2006.04213.x. PMID: 16460475.
56. World Cancer Research Fund, American Institute of Cancer Research. Continuous Update Project. Colorectal Cancer 2011 Report Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. London; 2011.
57. Huncharek M, Muscat J, Kupelnick B. Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies. *Nutr Cancer*. 2009;61(1):47-69. doi: 10.1080/01635580802395733. PMID: 19116875.
58. Aune D, Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, Norat T. Dairy products and colorectal cancer risk: a systematic review and meta-analysis of cohort studies. *Ann Oncol.* 2012 Jan;23(1):37-45. doi: 10.1093/annonc/mdr269. Epub 2011 May 26. PMID: 21617020.
59. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. *Eur J Clin Nutr*. 2003 Jan;57(1):1-17. doi: 10.1038/sj.ejcn.1601522. PMID: 12548291.
60. Guéguen L, Pointillart A. The bioavailability of dietary calcium. *J Am Coll Nutr*. 2000 Apr;19(2 Suppl):119S-136S. doi: 10.1080/07315724.2000.10718083. PMID: 10759138.
61. Barrubés L, Babio N, Mena-Sánchez G, Toledo E, Ramírez-Sabio JB, Estruch R, Ros E, Fitó M, Arós F, Fiol M, Santos-Lozano JM, Serra-Majem L, Pintó X, Martínez-González MÁ, Sorlí JV, Basora J, Salas-Salvadó J; PREvención con DIeta MEDiterránea Study Investigators. Dairy product consumption and risk of colorectal cancer in an older mediterranean population at high cardiovascular risk. *Int J Cancer*. 2018 Sep 15;143(6):1356-1366. doi: 10.1002/ijc.31540. Epub 2018 May 4. PMID: 29663376.
62. Larsson SC, Bergkvist L, Rutegård J, Giovannucci E, Wolk A. Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men. *Am J Clin Nutr*. 2006 Mar;83(3):667-73; quiz 728-9. doi: 10.1093/ajcn.83.3.667. PMID: 16522915.
63. Murphy N, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Olsen A, Tjønneland A, Dahm CC, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Nailler L, Kaaks R, Teucher B, Boeing H, Bergmann MM, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Pala V, Tumino R, Vineis P, Panico S, Peeters PH, Dik VK, Weiderpass E, Lund E, Garcia JR, Zamora-Ros R, Pérez MJ, Dorronsoro M, Navarro C, Ardanaz E, Manjer J, Almquist M, Johansson I, Palmqvist R, Khaw KT,

Wareham N, Key TJ, Crowe FL, Fedirko V, Gunter MJ, Riboli E. Consumption of dairy products and colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS One*. 2013 Sep 2;8(9):e72715. doi: 10.1371/journal.pone.0072715. PMID: 24023767; PMCID: PMC3759377.

64. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. *Nat Rev Cancer*. 2003 Aug;3(8):601-14. doi: 10.1038/nrc1144. PMID: 12894248.
65. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Bond JH, Greenberg ER. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med*. 1999 Jan 14;340(2):101-7. doi: 10.1056/NEJM199901143400204. PMID: 9887161.
66. Bonithon-Kopp C, Kronborg O, Giacosa A, Räth U, Faivre J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. *Lancet*. 2000 Oct 14;356(9238):1300-6. doi: 10.1016/s0140-6736(00)02813-0. PMID: 11073017.
67. Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? *Am J Clin Nutr*. 2001 Jan;73(1):1-2. doi: 10.1093/ajcn/73.1.1. PMID: 11124739.
68. Reedy J, Wirfält E, Flood A, Mitrou PN, Krebs-Smith SM, Kipnis V, Midthune D, Leitzmann M, Hollenbeck A, Schatzkin A, Subar AF. Comparing 3 dietary pattern methods--cluster analysis, factor analysis, and index analysis--With colorectal cancer risk: The NIH-AARP Diet and Health Study. *Am J Epidemiol*. 2010 Feb 15;171(4):479-87. doi: 10.1093/aje/kwp393. Epub 2009 Dec 21. PMID: 20026579; PMCID: PMC2842201.
69. Steck SE, Guinter M, Zheng J, Thomson CA. Index-based dietary patterns and colorectal cancer risk: a systematic review. *Adv Nutr*. 2015 Nov 13;6(6):763-73. doi: 10.3945/an.115.009746. PMID: 26567200; PMCID: PMC4642428.
70. Reedy J, Mitrou PN, Krebs-Smith SM, Wirfält E, Flood A, Kipnis V, Leitzmann M, Mouw T, Hollenbeck A, Schatzkin A, Subar AF. Index-based dietary patterns and risk of colorectal cancer: the NIH-AARP Diet and Health Study. *Am J Epidemiol*. 2008 Jul 1;168(1):38-48. doi: 10.1093/aje/kwn097. Epub 2008 Jun 4. PMID: 18525082; PMCID: PMC6287243.
71. Miller PE, Cross AJ, Subar AF, Krebs-Smith SM, Park Y, Powell-Wiley T, Hollenbeck A, Reedy J. Comparison of 4 established DASH diet indexes: examining associations of index scores and colorectal cancer. *Am J Clin Nutr*. 2013

Sep;98(3):794-803. doi: 10.3945/ajcn.113.063602. Epub 2013 Jul 17. PMID: 23864539; PMCID: PMC3743737.

72. Kontou N, Psaltopoulou T, Soupos N, Polychronopoulos E, Xinopoulos D, Linos A, Panagiotakos DB. Metabolic syndrome and colorectal cancer: the protective role of Mediterranean diet--a case-control study. *Angiology*. 2012 Jul;63(5):390-6. doi: 10.1177/0003319711421164. Epub 2012 Jan 20. PMID: 22267847.
73. Grosso G, Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S, Drago F, Basile F. Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. *Nutr Cancer*. 2014;66(4):558-65. doi: 10.1080/01635581.2014.902975. Epub 2014 Apr 22. PMID: 24754383.
74. Rosato V, Guercio V, Bosetti C, Negri E, Serraino D, Giacosa A, Montella M, La Vecchia C, Tavani A. Mediterranean diet and colorectal cancer risk: a pooled analysis of three Italian case-control studies. *Br J Cancer*. 2016 Sep 27;115(7):862-5. doi: 10.1038/bjc.2016.245. Epub 2016 Aug 18. PMID: 27537381; PMCID: PMC5046203.
75. Agnoli C, Grioni S, Sieri S, Palli D, Masala G, Sacerdote C, Vineis P, Tumino R, Giurdanella MC, Pala V, Berrino F, Mattiello A, Panico S, Krogh V. Italian Mediterranean Index and risk of colorectal cancer in the Italian section of the EPIC cohort. *Int J Cancer*. 2013 Mar 15;132(6):1404-11. doi: 10.1002/ijc.27740. Epub 2012 Aug 7. PMID: 22821300.
76. Donovan MG, Selmin OI, Doetschman TC, Romagnolo DF. Mediterranean Diet: Prevention of Colorectal Cancer. *Front Nutr*. 2017 Dec 5;4:59. doi: 10.3389/fnut.2017.00059. PMID: 29259973; PMCID: PMC5723389.
77. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available from: http://health.gov/dietaryguidelines/2015/guidelines/. The U.S. Department of Health and Human Services (HHS) prohibits discrimination on the basis of race, color, nation (accessed on 05 june 2020)
78. Kantor ED, Giovannucci EL. Gene-diet interactions and their impact on colorectal cancer risk. *Curr Nutr Rep*. 2015 Mar;4(1):13-21. doi: 10.1007/s13668-014-0114-2. PMID: 25844273; PMCID: PMC4383391.
79. Butterworth AS, Higgins JP, Pharoah P. Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. Eur J Cancer. 2006 Jan;42(2):216-27. doi: 10.1016/j.ejca.2005.09.023. Epub 2005 Dec 9. PMID: 16338133.

80. Castaño-Milla C, Chaparro M, Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. Aliment Pharmacol Ther. 2014 Apr;39(7):645-59. doi: 10.1111/apt.12651. PMID: 24612141.

81. Nottage K, McFarlane J, Krasin MJ, Li C, Srivastava D, Robison LL, Hudson MM. Secondary colorectal carcinoma after childhood cancer. *J Clin Oncol*. 2012 Jul 10;30(20):2552-8. doi: 10.1200/JCO.2011.37.8760. Epub 2012 Jun 4. PMID: 22665546.

82. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med.* 2000 Jul 13;343(2):78-85. doi: 10.1056/NEJM200007133430201. PMID: 10891514.

83. Bost B, de Vienne D, Hospital F, Moreau L, Dillmann C. Genetic and nongenetic bases for the L-shaped distribution of quantitative trait loci effects. *Genetics*. 2001 Apr;157(4):1773-87. PMID: 11290730; PMCID: PMC1461615.

84. Valle L. Genetic predisposition to colorectal cancer: where we stand and future perspectives. *World J Gastroenterol*. 2014 Aug 7;20(29):9828-49. doi: 10.3748/wjg.v20.i29.9828. PMID: 25110415; PMCID: PMC4123366.

85. Jaeger E, Webb E, Howarth K, Carvajal-Carmona L, Rowan A, Broderick P, Walther A, Spain S, Pittman A, Kemp Z, Sullivan K, Heinimann K, Lubbe S, Domingo E, Barclay E, Martin L, Gorman M, Chandler I, Vijayakrishnan J, Wood W, Papaemmanuil E, Penegar S, Qureshi M; CORGI Consortium, Farrington S, Tenesa A, Cazier JB, Kerr D, Gray R, Peto J, Dunlop M, Campbell H, Thomas H, Houlston R, Tomlinson I. Common genetic variants at the CRAC1 (HMPS) locus on chromosome 15q13.3 influence colorectal cancer risk. *Nat Genet*. 2008 Jan;40(1):26-8. doi: 10.1038/ng.2007.41. Epub 2007 Dec 16. PMID: 18084292.

86. Broderick P, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, Rowan A, Lubbe S, Spain S, Sullivan K, Fielding S, Jaeger E, Vijayakrishnan J, Kemp Z, Gorman M, Chandler I, Papaemmanuil E, Penegar S, Wood W, Sellick G, Qureshi M, Teixeira A, Domingo E, Barclay E, Martin L, Sieber O; CORGI Consortium, Kerr D, Gray R, Peto J, Cazier JB, Tomlinson I, Houlston RS. A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. *Nat Genet*. 2007 Nov;39(11):1315-7. doi: 10.1038/ng.2007.18. Epub 2007 Oct 14. PMID: 17934461.

87. Haiman CA, Le Marchand L, Yamamato J, Stram DO, Sheng X, Kolonel LN, Wu AH, Reich D, Henderson BE. A common genetic risk factor for colorectal and prostate

cancer. *Nat Genet*. 2007 Aug;39(8):954-6. doi: 10.1038/ng2098. Epub 2007 Jul 8. PMID: 17618282; PMCID: PMC2391283.

88. Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, Prendergast J, Olschwang S, Chiang T, Crowdy E, Ferretti V, Laflamme P, Sundararajan S, Roumy S, Olivier JF, Robidoux F, Sladek R, Montpetit A, Campbell P, Bezieau S, O'Shea AM, Zogopoulos G, Cotterchio M, Newcomb P, McLaughlin J, Younghusband B, Green R, Green J, Porteous ME, Campbell H, Blanche H, Sahbatou M, Tubacher E, Bonaiti-Pellié C, Buecher B, Riboli E, Kury S, Chanock SJ, Potter J, Thomas G, Gallinger S, Hudson TJ, Dunlop MG. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. *Nat Genet.* 2007 Aug;39(8):989-94. doi: 10.1038/ng2089. Epub 2007 Jul 8. PMID: 17618283.

89. Tenesa A, Farrington SM, Prendergast JG, Porteous ME, Walker M, Haq N, Barnetson RA, Theodoratou E, Cetnarskyj R, Cartwright N, Semple C, Clark AJ, Reid FJ, Smith LA, Kavoussanakis K, Koessler T, Pharoah PD, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Schmidt CO, Hampe J, Chang-Claude J, Hoffmeister M, Brenner H, Wilkening S, Canzian F, Capella G, Moreno V, Deary IJ, Starr JM, Tomlinson IP, Kemp Z, Howarth K, Carvajal-Carmona L, Webb E, Broderick P, Vijayakrishnan J, Houlston RS, Rennert G, Ballinger D, Rozek L, Gruber SB, Matsuda K, Kidokoro T, Nakamura Y, Zanke BW, Greenwood CM, Rangrej J, Kustra R, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. *Nat Genet*. 2008 May;40(5):631-7. doi: 10.1038/ng.133. Epub 2008 Mar 30. PMID: 18372901; PMCID: PMC2778004.

90. Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, Penegar S, Chandler I, Gorman M, Wood W, Barclay E, Lubbe S, Martin L, Sellick G, Jaeger E, Hubner R, Wild R, Rowan A, Fielding S, Howarth K; CORGI Consortium, Silver A, Atkin W, Muir K, Logan R, Kerr D, Johnstone E, Sieber O, Gray R, Thomas H, Peto J, Cazier JB, Houlston R. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21*. Nat Genet.* 2007 Aug;39(8):984-8. doi: 10.1038/ng2085. Epub 2007 Jul 8. PMID: 17618284.

91. Tomlinson IP, Webb E, Carvajal-Carmona L, Broderick P, Howarth K, Pittman AM, Spain S, Lubbe S, Walther A, Sullivan K, Jaeger E, Fielding S, Rowan A, Vijayakrishnan J, Domingo E, Chandler I, Kemp Z, Qureshi M, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Penegar S, Barclay E, Wood W, Martin L, Gorman M, Thomas H, Peto J, Bishop DT, Gray R, Maher ER, Lucassen A, Kerr

D, Evans DG; CORGI Consortium, Schafmayer C, Buch S, Völzke H, Hampe J, Schreiber S, John U, Koessler T, Pharoah P, van Wezel T, Morreau H, Wijnen JT, Hopper JL, Southey MC, Giles GG, Severi G, Castellví-Bel S, Ruiz-Ponte C, Carracedo A, Castells A; EPICOLON Consortium, Försti A, Hemminki K, Vodicka P, Naccarati A, Lipton L, Ho JW, Cheng KK, Sham PC, Luk J, Agúndez JA, Ladero JM, de la Hoya M, Caldés T, Niittymäki I, Tuupanen S, Karhu A, Aaltonen L, Cazier JB, Campbell H, Dunlop MG, Houlston RS. A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. *Nat Genet*. 2008 May;40(5):623-30. doi: 10.1038/ng.111. Epub 2008 Mar 30. PMID: 18372905.

92. Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *JAMA*. 2008 May 28;299(20):2423-36. doi: 10.1001/jama.299.20.2423. PMID: 18505952; PMCID: PMC2772197.
93. Castellví-Bel S, Abulí A, Castells A. Meta-analysis of several GWAS sets yields additional genetic susceptibility variants for colorectal cancer: first X-linked component identified. *Gastroenterology*. 2012 Dec;143(6):1684-5. doi: 10.1053/j.gastro.2012.10.008. Epub 2012 Oct 13. PMID: 23073136.
94. Yang CY, Lu RH, Lin CH, Jen CH, Tung CY, Yang SH, Lin JK, Jiang JK, Lin CH. Single nucleotide polymorphisms associated with colorectal cancer susceptibility and loss of heterozygosity in a Taiwanese population. *PLoS One*. 2014 Jun 26;9(6):e100060. doi: 10.1371/journal.pone.0100060. PMID: 24968322; PMCID: PMC4072675.
95. Song M, Giovannucci E. Preventable Incidence and Mortality of Carcinoma Associated With Lifestyle Factors Among White Adults in the United States. *JAMA Oncol*. 2016 Sep 1;2(9):1154-61. doi: 10.1001/jamaoncol.2016.0843. PMID: 27196525; PMCID: PMC5016199.
96. Crosara Teixeira M, Braghiroli MI, Sabbaga J, Hoff PM. Primary prevention of colorectal cancer: myth or reality? *World J Gastroenterol*. 2014 Nov 7;20(41):15060-9. doi: 10.3748/wjg.v20.i41.15060. PMID: 25386054; PMCID: PMC4223239.
97. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. 2014 Apr 26;383(9927):1490-1502. doi: 10.1016/S0140-6736(13)61649-9. Epub 2013 Nov 11. PMID: 24225001.
98. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014 Apr 26;383(9927):1490-1502. doi: 10.1016/S0140-6736(13)61649-9. Epub 2013 Nov 11. PMID: 24225001.
99. Bénard F, Barkun AN, Martel M, von Renteln D. Systematic review of colorectal cancer screening guidelines for average-risk adults: Summarizing the current global

recommendations. *World J Gastroenterol*. 2018 Jan 7;24(1):124-138. doi: 10.3748/wjg.v24.i1.124. PMID: 29358889; PMCID: PMC5757117.

100. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med*. 1993 May 13;328(19):1365-71. doi: 10.1056/NEJM199305133281901. Erratum in*: N Engl J Med* 1993 Aug 26;329(9):672. PMID: 8474513.
101. Knudsen AB, Zauber AG, Rutter CM, Naber SK, Doria-Rose VP, Pabiniak C, Johanson C, Fischer SE, Lansdorp-Vogelaar I, Kuntz KM. Estimation of Benefits, Burden, and Harms of Colorectal Cancer Screening Strategies: Modeling Study for the US Preventive Services Task Force. *JAMA*. 2016 Jun 21;315(23):2595-609. doi: 10.1001/jama.2016.6828. PMID: 27305518; PMCID: PMC5493310.
102. Goede SL, Rabeneck L, van Ballegooijen M, Zauber AG, Paszat LF, Hoch JS, Yong JH, Kroep S, Tinmouth J, Lansdorp-Vogelaar I. Harms, benefits and costs of fecal immunochemical testing versus guaiac fecal occult blood testing for colorectal cancer screening. *PLoS One*. 2017 Mar 15;12(3):e0172864. doi: 10.1371/journal.pone.0172864. PMID: 28296927; PMCID: PMC5351837.
103. Guittet L, Bouvier V, Mariotte N, Vallee JP, Levillain R, Tichet J, Launoy G. Comparison of a guaiac and an immunochemical faecal occult blood test for the detection of colonic lesions according to lesion type and location. *Br J Cancer*. 2009 Apr 21;100(8):1230-5. doi: 10.1038/sj.bjc.6604996. Epub 2009 Mar 31. PMID: 19337253; PMCID: PMC2676539.
104. Santaballa A, Pinto Á, Balanyà RP, Ramírez Merino N, Martín IR, Grau SS, Fombella JPB, Cano JM, González CH, Bayo J. SEOM clinical guideline for secondary prevention (2019). *Clin Transl Oncol*. 2020 Feb;22(2):187-192. doi: 10.1007/s12094-020-02302-0. Epub 2020 Jan 31. PMID: 32006339.
105. Idigoras I, Arrospide A, Portillo I, Arana-Arri E, Martínez-Indart L, Mar J, de Koning HJ, Lastra R, Soto-Gordoa M, van der Meulen M, Lansdorp-Vogelaar I. Evaluation of the colorectal cancer screening Programme in the Basque Country (Spain) and its effectiveness based on the Miscan-colon model. BMC Public Health. 2017 Aug 1;18(1):78. doi: 10.1186/s12889-017-4639-3. Erratum in: *BMC Public Health*. 2017 Sep 22;17 (1):736. PMID: 28764731; PMCID: PMC5540568.
106. Portillo I, Idígoras I, Ojembarrena E, Arana E, Luis Hurtado J, Basurko R, Tapia M, Luz Peña M. Lesions detected in a colorectal cancer screening program in the Basque Country: first round (2009-2011). *Gastroenterol Hepatol.* 2013 May;36(5):301-8. Spanish. doi: 10.1016/j.gastrohep.2013.02.004. Epub 2013 Apr 22. PMID: 23618538.

107. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. *Br J Cancer*. 2018 Oct;119(7):785-792. doi: 10.1038/s41416-018-0264-x. Epub 2018 Oct 4. PMID: 30287914; PMCID: PMC6189126.

108. Li P, Wu H, Zhang H, Shi Y, Xu J, Ye Y, Xia D, Yang J, Cai J, Wu Y. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival: a meta-analysis. *Gut*. 2015 Sep;64(9):1419-25. doi: 10.1136/gutjnl-2014-308260. Epub 2014 Sep 19. PMID: 25239119.

109. Zell JA, Ziogas A, Bernstein L, Clarke CA, Deapen D, Largent JA, Neuhausen SL, Stram DO, Ursin G, Anton-Culver H. Meat consumption, nonsteroidal anti-inflammatory drug use, and mortality among colorectal cancer patients in the California Teachers Study. *Cancer Prev Res (Phila).* 2010 Jul;3(7):865-75. doi: 10.1158/1940-6207.CAPR-09-0262. Epub 2010 Jun 15. PMID: 20551290; PMCID: PMC2931369.

110. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med*. 2012 Oct 25;367(17):1596-606. doi: 10.1056/NEJMoa1207756. PMID: 23094721; PMCID: PMC3532946.

111. Sostres C, Gargallo CJ, Lanas A. Aspirin, cyclooxygenase inhibition and colorectal cancer. *World J Gastrointest Pharmacol Ther*. 2014 Feb 6;5(1):40-9. doi: 10.4292/wjgpt.v5.i1.40. PMID: 24605250; PMCID: PMC3944468.

112. Frouws MA, van Herk-Sukel MPP, Maas HA, Van de Velde CJH, Portielje JEA, Liefers GJ, Bastiaannet E. The mortality reducing effect of aspirin in colorectal cancer patients: Interpreting the evidence. *Cancer Treat Rev*. 2017 Apr;55:120-127. doi: 10.1016/j.ctrv.2016.12.008. Epub 2017 Feb 20. PMID: 28359968.

113. Walter V, Jansen L, Hoffmeister M, Ulrich A, Chang-Claude J, Brenner H. Smoking and survival of colorectal cancer patients: population-based study from Germany. *Int J Cancer*. 2015 Sep 15;137(6):1433-45. doi: 10.1002/ijc.29511. Epub 2015 May 29. PMID: 25758762.

114. Wei PL, Kuo LJ, Huang MT, Ting WC, Ho YS, Wang W, An J, Chang YJ. Nicotine enhances colon cancer cell migration by induction of fibronectin. *Ann Surg Oncol*. 2011 Jun;18(6):1782-90. doi: 10.1245/s10434-010-1504-3. Epub 2011 Jan 6. PMID: 21210228.

115. Sharma A, Deeb AP, Iannuzzi JC, Rickles AS, Monson JR, Fleming FJ. Tobacco smoking and postoperative outcomes after colorectal surgery. *Ann Surg.* 2013 Aug;258(2):296-300. doi: 10.1097/SLA.0b013e3182708cc5. PMID: 23059503.

116. Vincenzi B, Santini D, Loupakis F, Addeo R, Rojas Llimpe FL, Baldi GG, Di Fabio F, Del Prete S, Pinto C, Falcone A, Tonini G. Cigarettes smoking habit may reduce benefit from cetuximab-based treatment in advanced colorectal cancer patients. *Expert Opin Biol Ther*. 2009 Aug;9(8):945-9. doi: 10.1517/14712590903081694. PMID: 19534585.

117. Walter V, Jansen L, Knebel P, Chang-Claude J, Hoffmeister M, Brenner H. Physical activity and survival of colorectal cancer patients: Population-based study from Germany. *Int J Cancer*. 2017 May 1;140(9):1985-1997. doi: 10.1002/ijc.30619. Epub 2017 Feb 10. PMID: 28120416.

118. Mishra SI, Scherer RW, Geigle PM, Berlanstein DR, Topaloglu O, Gotay CC, Snyder C. Exercise interventions on health-related quality of life for cancer survivors. *Cochrane Database Syst Rev*. 2012 Aug 15;(8):CD007566. doi: 10.1002/14651858.CD007566.pub2. PMID: 22895961.

119. Koelwyn GJ, Wennerberg E, Demaria S, Jones LW. Exercise in Regulation of Inflammation-Immune Axis Function in Cancer Initiation and Progression. *Oncology (Williston Park)*. 2015 Dec;29(12):908-20, 922. PMID: 26676894; PMCID: PMC4909049.

120. Dieli-Conwright CM, Lee K, Kiwata JL. Reducing the Risk of Breast Cancer Recurrence: an Evaluation of the Effects and Mechanisms of Diet and Exercise. *Curr Breast Cancer Rep*. 2016;8(3):139-150. doi: 10.1007/s12609-016-0218-3. Epub 2016 Jun 27. PMID: 27909546; PMCID: PMC5112289.

121. Friedenreich CM, Shaw E, Neilson HK, Brenner DR. Epidemiology and biology of physical activity and cancer recurrence. *J Mol Med (Berl)*. 2017 Oct;95(10):1029-1041. doi: 10.1007/s00109-017-1558-9. Epub 2017 Jun 15. PMID: 28620703; PMCID: PMC5613065.

122. van Zutphen M, Kampman E, Giovannucci EL, van Duijnhoven FJB. Lifestyle after Colorectal Cancer Diagnosis in Relation to Survival and Recurrence: A Review of the Literature. *Curr Colorectal Cancer Rep*. 2017;13(5):370-401. doi: 10.1007/s11888-017-0386-1. Epub 2017 Sep 14. PMID: 29104517; PMCID: PMC5658451.

123. Van Blarigan EL, Fuchs CS, Niedzwiecki D, Zhang S, Saltz LB, Mayer RJ, Mowat RB, Whittom R, Hantel A, Benson A, Atienza D, Messino M, Kindler H, Venook A, Ogino S, Giovannucci EL, Ng K, Meyerhardt JA. Association of Survival With Adherence to the American Cancer Society Nutrition and Physical Activity Guidelines for Cancer Survivors After Colon Cancer Diagnosis: The CALGB 89803/Alliance Trial. *JAMA Oncol*. 2018 Jun 1;4(6):783-790. doi: 10.1001/jamaoncol.2018.0126.

2. HYPOTHESES AND OBJECTIVES

2.1. Hypotheses

The hypotheses of this doctoral thesis, concerning participants in the CRCSP of the Osakidetza/Basque Health Service, are as follows:

1. The diet of CRC patients postsurgery is inadequate in many respects, including nutrients and food intakes, and this dietetic and nutritional inadequacy is associated with certain health determinants such as age, weight status, lifestyles and socioeconomic conditions.
2. There are significant differences between cases and controls with regard to lifestyles (including diet), weight status, use of drugs related to decreasing CRC risk, socioeconomic level, health status, quality of life and stress level. These factors are more favourable in controls than in cases, that is, cases have healthy lifestyles, a healthy weight, a better health status, higher socioeconomic level, better life quality and lower stress level and use drugs related to decreasing CRC risk.
3. The consumption of certain foods (such as red meat, processed meat and alcoholic drinks, among others) are associated with an increased risk of CRC. Whereas other foods (for example, fibre-containing foods) and dietary patterns such as the MD pattern are associated with decreasing risk of CRC. These associations vary depending on tumour location.
4. Some of the previously reported CRC-related SNPs are associated with CRC susceptibility in the case-control sample under study.

2.2. Objectives

Main objective

To date, case-control studies have revealed inconsistent evidence on the influence of dietary and genetic factors on CRC risk. In order to better elucidate the role of some of these factors in the aetiology of CRC, the main objective of this study was to analyse dietary and genetic factors in a sample of cases and controls from the population-based CRCSP of the Osakidetza/Basque Health Service. In addition, taking into account that an unhealthy diet is associated with the risk of tumour recurrence, metastasis and death, the other aim of this thesis was to assess the adequacy of nutrients consumed and diet quality in a group of CRC patients postsurgery. This doctoral thesis is part of a line of research on the impact of gene-diet interactions on the risk of CRC in the Basque Country.

Specific objectives

To achieve this general objective, the following specific objectives were set:

1. To assess the adequacy of nutrients consumed and diet quality, and to identify possible associations between nutritional adequacy and diet quality and certain health determinants (such as age, weight status, lifestyles and socioeconomic conditions), in a group of postsurgery CRC patients who participated in the CRCSP of the Osakidetza/Basque Health Service. **Study 1.**
2. To analyse lifestyle (including diet from the nutritional perspective), weight status, health status, socioeconomic level, quality of life, stress level and use of drugs related to decreasing CRC risk, in a sample of cases and controls from the population-based CRCSP of the Osakidetza/Basque Health Service. **Study 2.**
3. To assess the relationships between food group consumption, diet quality and CRC risk, and identify possible differences in consumption depending on tumour location, in the sample of cases and controls mentioned in objective 2. **Study 3.**
4. To investigate possible associations between susceptibility SNPs and development of sporadic CRC in the sample of cases and controls mentioned in objective 2. **Study 4.**

3. PARTICIPANTS, METHODS AND RESULTS

3.1. STUDY 1: "Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Post-Surgery: A Pilot Study"

NUTRITIONAL ADEQUACY AND DIET QUALITY IN COLORECTAL CANCER PATIENTS POST-SURGERY: A PILOT STUDY

Iker Alegria-Lertxundi[a], Maider Alvarez[a], Ana M Rocandio[ac], Marian M. de Pancorbo[bc], Marta Arroyo-Izaga[ac]

[a]Department of Pharmacy and Food Sciences, University of the Basque Country UPV/EHU, Spain

[b]Department of Zoology and Cellular Biology Animal, University of the Basque Country UPV/EHU, Spain

[c]BIOMICs Research Group, Lascaray Research Center, University of the Basque Country UPV/EHU, Spain

Citation text: Alegria-Lertxundi I, Alvarez M, Rocandio AM, de Pancorbo MM, Arroyo-Izaga M. Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Postsurgery: A Pilot Study. *Nutr Cancer*. 2016 May-Jun;68(4):577-88. doi: 10.1080/01635581.2016.1158299. Epub 2016 May 4. PMID: 27144653.

ABSTRACT

Recent evidence has shown that an unhealthy diet is associated with a higher risk of tumor recurrence, metastasis and death among patients with colorectal cancer (CRC). The aims of this study were to assess nutritional adequacy and diet quality in a group of CRC patients post-surgery and to identify possible associations between dietary and nutritional aspects and environmental factors and weight status. This was an observational study conducted on a random sample of 74 patients, aged 50-69 years. Dietary intake was evaluated utilizing a validated frequency questionnaire, and diet quality was evaluated utilizing the Healthy Eating Index for Spanish diet and the MedDietScore. Data regarding socio-economic, demographic, lifestyles, dietary supplements use and body mass index were collected. Participants followed a diet characterized by a low carbohydrate intake (94% of the cases), excessive protein (48%) and high fat intake (67%), and some micronutrient deficiencies. The inadequacy of some nutrients was associated with male gender, overweight/obesity, smoking and low educational level, and low adherence to the MedDiet was identified in those with a low educational level (adjusted OR=4.16, $P<0.05$). Therefore, such patients should be an important target group when applying educational programs and giving individualized nutritional advice to improve their life quality.

Keywords: Colorectal cancer, Diet, Body mass/BMI, Micronutrients

INTRODUCTION

Colorectal cancer (CRC) is a major public health challenge worldwide. In Europe, it is the leading malignancy in terms of incidence and the second in terms of mortality in both genders (1). Diet contributes to 50-90% of colon cancer cases (2). Epidemiological studies have established a strong link between some dietary factors, such as fiber (inversely) and red/processed meat (increased risk), and the risk of developing CRC (3). Furthermore, an overall assessment of diet is an important alternative to traditional methods that have focused only on single nutrients or foods (4) given that people consume a variety of foods with complex combinations of micro- and macronutrients rather than single nutrients or foods. Because foods and nutrients act synergistically rather than in isolation (5-6), recent research has investigated the role of diet quality in cancer incidence (7-8) and survival (9).

Some dietary patterns, such as the Mediterranean Diet (MD), have been widely associated with human health. Specifically, several studies have associated adherence to the MD with a reduction in the risk of some types of cancer, specifically colorectal cancer as well as other neoplasms (16,17). The MD is characterized by a nutritional model consisting mainly of olive oil, cereals, fresh or dried fruits and vegetables as well as a moderate amount of fish, dairy and meat, accompanied by wine or infusions. However, it should be noted that the MD varies from country to country, even from region to region, throughout the Mediterranean basin due to the influence of various cultural and environmental factors (12).

There is scientific evidence of the relationship between the MD pattern and CRC risk (13-15); however, little is known about dietary intake, nutrient intake as well as diet quality, in CRC patients post-surgery, especially in the medium term post-operatively (16,17). In a recent study with respect to CRC recurrence and survival, an unhealthy diet (the processed meat dietary pattern) was associated with the risk of tumor recurrence, metastasis and death (16). The consumption of an adequate diet, supplying energy and nutrients in amounts sufficient to meet needs, should improve nutritional status and may enhance disease resistance, thereby prolonging lifespan.

Thus, the aim of this work was to assess the adequacy of nutrients consumed and diet quality in a group of CRC patients post-surgery. In addition, the study identified possible associations between nutritional adequacy and diet quality and environmental factors

and weight status. To the best of our knowledge, this nutritional study is the first one dedicated to nutritional adequacy and diet quality in CRC patient's post-surgery.

METHODS

Study participants

This study is part of a project that aims to analyze diet/genetic interactions on the risk of CRC in a population group in the Basque Country. Recruitment and data collection for the present pilot study were conducted between 2013 and 2014. A sample size of 142 participants was randomly selected from the list of confirmed CRC diagnosis and subjected to a surgical resection between January 1, 2009 and December 31, 2012 at some hospitals that participate in the CRC screening program in the Basque Country (Osakidetza/The Basque Health Service) (n=191). Participants were randomized using computer generated random numbers by SPSS version 22.0. (SPSS Inc., Chicago, IL). The sample size for this pilot study represents the 10% of the sample required for the full research, such as it is recommended for pilot studies (18), and was estimated taking into account the expected participation rate (50%).

The sample was stratified according to the age and gender distribution of the target population. The participation rate was 55.6%, and the low number of participants (n=5) excluded due to missing information supports the high quality of the data. Finally, 74 subjects (66.2% males) consented to participate in the survey and complete all questionnaires. CRC stage at diagnosis was stage 1 (62.2%), stage 2a (14.9%), stage 3a (4.1%) and stage 3b (18.9%).

This research was approved by the Clinical Research Ethics Committee of the Basque Country (Reference number PI2011006 and PI2014042). To be eligible for this CRC screening program, patients had to be between the ages of 50 and 69 years, asymptomatic with respect to colorectal symptoms, and registered with the services of the Osakidetza/The Basque Health Service. Subjects with symptoms suggestive of CRC or known risk groups for CRC such as familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer are managed outside this program and are not included in this analysis. The CRC screening program in the Basque Country employed the immunochemical fecal occult blood test (FOBT), and patients with a positive result were invited to undergo colonoscopy with sedation (19).

Participants in the present study who had tested positive in their FOBT results and had positive colonoscopy results had undergone surgical resection of CRC, and some of them had undergone adjuvant treatments, radiotherapy and/or chemotherapy. The percentages of participants according to the type of surgery procedure were the following: 50% sigmoidectomy, 18.9% right hemicolectomy resection, 13.5% left hemicolectomy resection, 9.5% right hemicolectomy resection + rectal anterior resection, 4.1% low anterior resection and 4.1% rectum amputation. The percentage who received chemotherapy was 33.8%, and chemotherapy and radiation 14.9%. Subjects were invited to take part in this survey at least 1 year after their operation (median, 2.0 years; range, 1.0-2.8 years). Written informed consent to assess their medical records was required from study participants. Consenting participants completed and returned a detailed Food Frequency Questionnaire (FFQ) and one General Questionnaire (GQ). All questionnaires were self-completed. Assistance from study staff was available to help with understanding items on the questionnaires.

Dietary intake and adequacy of nutrients intake

Diet was assessed using a FFQ that is a modified version of the Rodríguez *et al.* questionnaire (20). This adaptation was validated utilizing multiple 24-h recalls in a subsample of participants. It consists of 67 items and requires participants to recall the number of times each food item was consumed either per week or per month. The respondents might also record consumption of other foods not included on the food list. Moreover, data regarding dietary supplements and foods with added dietary ingredients were recorded.

Average portion sizes were employed to convert FFQ consumptions (21). For items that included several foods, each food's contribution was estimated with weighting coefficients obtained from usual consumption data (22). All food items consumed were entered into DIAL 2.12 (2011 ALCE INGENIERIA), a dietary assessment program. Initial energy and nutrient intakes were reported as mean and standard deviation (SD). The macronutrients were expressed as a percentage of total energy intake (TEI) and were compared to acceptable macronutrient distribution ranges (AMDR), ranges of intakes associated with reduced risk of chronic disease and which provide adequate intakes of essential nutrients (23). The approach employed to evaluate nutrient adequacy was the estimated average requirements (EARs) (24). Results of micronutrient and protein intakes were expressed as a percentage of the EARs. The EAR is the mean daily intake value which is estimated to meet the requirement of half the healthy individuals in a life-stage and gender group for that nutrient (25). Nutrient data were also compared with

tolerable upper intake levels (ULs) (26). Caffeine consumption was compared to reference values without any adverse effects (27).

Diet quality assessment

Adherence to the dietary guidelines was evaluated utilizing the Healthy Eating Index for Spanish Diet (HEISD) (28) and the MedDietScore (MDS) (29). The first one is a rapid and cheap method to estimate the quality of the diet in the population because it utilizes secondary data taken from the National Health Questionnaire and from feeding guidelines. The HEISD included 10 items: cereals and derivatives, vegetables, fruits, milk and dairy products, meats, legumes, cold meats, sweets, soft-drinks with added sugar, and diet variety. The items are equally weighted; thus, each item can contribute 10 points to the total score, and the theoretical range is 0-100.

MDS is an index that estimates the adherence level to the MD and is associated with biomarkers of cardiovascular disease risk (30). This diet score has 11 main components: non-refined cereals, fruits, vegetables, potatoes, legumes, olive oil, fish, red meat and products, poultry, full fat dairy products and alcohol beverages. Each component was scored separately. For the consumption of items presumed to be close to the MD pattern, scores 0, 1, 2, 3, 4, and 5 were assigned when a participant reported no consumption, rare, frequent, very frequent, weekly and daily, respectively. For the consumption of foods presumed to be away from this diet pattern, the scores were assigned on a reverse scale (scores 5 to 0). Reverse scale was applied to four components of the MDS (red meat and products, poultry, full fat dairy products and alcohol beverages). Especially for alcohol, score 5 was assigned for the consumption of less 300 ml per day, score 0 for consumption of more than 700 ml per day or no consumption, and scores 4 to 1 for consumption of 300-400, 400-500, 500-600, and 600-700 ml per day, respectively (100 ml = 12 g ethanol). The total score (sum) is between 0 and 55. Higher values of this score indicate greater adherence to the MD pattern. A score equal to 55 represents 100% adherence to the MD pattern; then, a score equal to *k* represents (*k*/55) x 100% agreement to this pattern.

Covariates

The GQ measured weight status (self-reported weight and height) and environmental factors (socioeconomic status: educational attainment, economic activity and last work activity; demographic factors: age and gender; and lifestyle information: physical activity in free time, smoking and alcohol consumption). These questions were taken from the Spanish Health Questionnaire (31). Body mass index (BMI) estimated from self-reported

height and weight was classified according to the WHO criteria (32). Additionally, life quality in general and stress level during the last month were analyzed utilizing a continuous scale with a range from 0 to 100, with higher scores indicating a better life quality and a higher stress level.

Statistical analysis

Data were analyzed utilizing SPSS version 22.0. (SPSS Inc., Chicago, IL) and reported as mean (standard deviation, SD), 95% confidence interval (CI) and frequencies. Symmetry in the distribution of continuous variables was determined by Kolmogorov–Smirnov–Lilliefors test for sample sizes greater than 30 and by Shapiro-Wilk test for sample size fewer than 30.

Differences between variables were calculated employing the Student´s *t* test and ANOVA one way (in the case of normally distributed data) or Mann-Whitney U test and Wilcoxon W test (if the variables were not normally distributed). In addition, the effect size was performed employing the statistical Cohen's *d* and *f* for mean comparisons and *g* for proportions (G-power 3.1.7 software) to estimate the magnitude and significance of the result. Cohen´s guidelines for the interpretation of effect size were employed (33). The categorical variables were analyzed using the Chi-square test (or the Fisher test when applicable), and the relationships between these categories were analyzed utilizing adjusted residuals.

Logistic regression analysis was performed for adjusted odds ratio (OR) based on weight status (BMI) and environmental factors to identify possible associations between these variables and inadequate intake of nutrients and inadequate diet quality. For simplicity and descriptive purposes, the variables *educational attainment*, *economic activity* and *last work* were regrouped according to the criteria of the Spanish Health Questionnaire (31). Continuous variable age was split at the sample median, thereby defining high and low groups of this variable in question. In the same sense, the dependent variable, adherence to the MD pattern, was dichotomized into less than or equal to 67.3%, and higher than 67.3%, utilizing the median split to define low and high adherence to the MD. All reported *P* values are two tailed ($P<0.05$; $P<0.01$; $P<0.001$). Analyses were conducted separately for men and women, because of the differences in dietary intake (34). In addition, differences in dietary intake were analyzed by cancer stage and type of surgery. These variables were regrouped to simplify the analyses of results: cancer stage (stage 1; stage 2 or greater) and type of surgery (hemicolectomy; sigmoidectomy or rectal resection). Those participants who have undergone two types of surgery, that it is

mean, hemicolectomy and rectal resection, were excluded for these comparisons, since the number of these cases was small (n=7).

RESULTS

General characteristics of the studied sample

General characteristics of the sample are shown in Table 1. BMI of the total sample was 27.2(3.5) kg/m^2 (95% CI 26.3-28.0). For men, BMI was 27.2(3.5) kg/m^2 (95% CI 26.2-28.2) and for women, was 27.1(3.5) kg/m^2 (95% CI 25.6-28.6). There was no difference in BMI between genders (P=0.912; d=0.03). Moreover, 69.4% of men and 60.0% of women had excess weight (overweight/obesity), and none of the sample reported underweight.

In the total sample, the score for life quality was 71.3(14.8) to 100 and for the stress level 41.2(28.1) to 100. There were no differences in these variables by gender (P=0.474 for life quality and P=0.248 for stress level), being the effect size for mean comparisons between low and moderate (d=0.21 for quality life and d=0.35 for stress level).

Dietary intake and adequacy of nutrients intake

The average daily intakes of energy, macro- and micronutrients are shown in Table 2. Significant gender differences were found only in polyunsaturated fatty acids (PUFA) and alcohol consumption (P<0.05). The effect sizes of the gender differences were between low and moderate for lipids (d=0.41), saturated fatty acids (SFA) (d=0.20) and fiber (d=0.37). Without allowing for gender, more than 94% participants had an inadequate carbohydrate intake by default; over 48% of them had excessive protein intake; and more than 67% of the sample had an excessive fat intake.

Dietary nutrient density is presented in Table 3. Over one half of the participants had intakes below EARs for folic acid (66.2%), vitamins A (56.8%), D (100%) and E (85.1%), calcium (66.2%), magnesium (75.7%) and iodine (59.5%). Although gender differences were not found in the dietary nutrient density, the effect sizes of some micronutrients were between small and moderate: riboflavin (d=0.39), vitamins B_6 (d=0.33), B_{12} (d=0.23), C (d=0.31) and E (d=0.38), folic acid (d=0.21), Ca (d=0.44) and Fe (d=0.24). And males were more likely to have inadequate intakes of thiamine (55.1% of men had intakes below EARs vs. 24.0% of women, P=0.011), vitamin A (69.4% of men vs. 32.0% of women, P=0.002), magnesium (95.9% of men vs. 76.0% of women, P<0.001) and zinc (59.2% of men vs. 8.0% of women, P<0.001). Some of these results were also

observed in logistic regression analysis, which revealed the following adjusted ORs, 95%CIs and significance level for men: inadequate intake of thiamine (OR=4.16, 1.06-16.40, *P*=0.041) and vitamin A (OR=5.37, 1.44-20.12, *P*=0.013), compared with women.

According to the cancer stage and type of surgery, no significant differences were found for energy and nutrients intake, nor for %EARs. However, the effect size for α-linolenic acid (g/d) was large (*d*=0.89), being higher the intake in stage 1 (12.7(5.5)) than in the other ones (8.9(2.5)). And according to the type of surgery, the effect size for thiamine was large (*d*=1.26), those who had undergone hemicolectomy had lower %EARs (96.3(21.3)) than who had undergone sigmoidectomy or rectal resection (147.2(52.9)). Participants who had undergone hemicolectomy were more likely to have inadequate intakes of thiamine (60.0% had intakes below EARs) than those who had undergone sigmoidectomy or rectal resection (15.4% had intakes below EARs) (Chi-square=3.58; *P*=0.058; effect size *g*=0.45).

Furthermore, participants who were overweight/obese had a higher inadequate intake of folic acid (OR=4.99, 1.37-18.22, *P*=0.015), vitamin A (OR=4.95, 1.34-18.30, *P*=0.016) and zinc (OR=7.05, 1.66-29.87, *P*=0.008) than those of normal weight. Smokers had a high inadequacy of folic acid intake (OR=4.40, 1.08-17.88, *P*=0.038), and those without studies or primary education had a higher inadequacy of thiamine (OR=5.42, 1.31-22.40, *P*=0.020). Logistic regression was not estimated for all nutrients because of their not meeting the criteria for this analysis (minimum ratio of valid cases to independent variables: 10 to 1).

However, there was a large proportion of participants with sodium intake exceeding the tolerable upper intake level (UL: 2300 mg/day), 57.1% of males and 48.0% of females (*P*=0.011). Furthermore, the median intake of caffeine was 63.0 mg/day; there were no cases with caffeine intakes higher than 300 mg/day (consumption that can cause adverse effects).

Diet quality

Table 4 presents the scores for components of the HEISD and the percentage of participants who did not meet the recommendations. No significant differences by gender were found, either for total score or for the components scores. Nevertheless, the effect sizes were small to medium for vegetables (*d*=0.35), fruits (*d*=0.37), legumes (*d*=0.21) and soft-drinks (*d*=0.22). According to the classification of HEISD, 14.9% had a "healthy diet" and 85.1% were in the category of "not healthy/need changes" (*P*<0.001). The

HEISD components with higher cases of non-compliance of HEISD recommendations were meats (100%), cold meats (89.2%), sweets (87.8%) and diet variety (100%); no differences were found by gender ($P>0.05$).

The scores of adequacies for components of the MDS for the total sample and by gender are presented in Table 5. Significant gender differences were found for non-refined cereals, the highest score for this component being found in women rather than in men ($P<0.01$). And the effect sizes for gender differences were small to medium for fruits ($d=0.44$), legumes ($d=0.31$), poultry ($d=0.33$), olive oil ($d=0.20$) and MDS total ($d=0.35$). The percentage of adherence to the MD was 66.6% (5.5) and there were no gender differences ($P>0.05$). No statistically significant correlation was found between HEISD and MDS ($P>0.05$).

No significant differences were found in HEISD and MDS, neither in components´ scores, nor in the total scores, by cancer stage or by type of surgery. However, the effect size for potatoes group was large ($d=0.88$), being higher the score for participants who had undergone hemicolectomy (2.5(1.5)) than sigmoidectomy or rectal resection (3.7(1.2)). Table 6 presents the HEISD score and the adherence to the MD pattern by lifestyle and socio-economic and demographic factors. Participants who were 61 years or older obtained higher HEISD scores than subjects younger than 61 ($P<0.05$). None of the other variables considered in this study (weight status and environmental factors) were significantly associated with HEISD score. Nevertheless, the following data had the most significance for the HEISD: schooling ($f=0.44$), smoking status ($f=1.12$), physical activity during free time ($d=0.80$) and most recent job ($f=1.41$). Subjects who registered higher HEISD scores were those that had secondary educational level, were ex-smokers or had never smoked, had engaged in physical activity, and had worked as a businessman/women in the past. Logistic regression analyses did not show significant associations between HEISD and environmental factors and weight status.

Regarding MDS, although no significant differences were found for the adherence to the MD pattern between the categories according the socio-economic, demographic and lifestyle characteristics, the greatest size effect occurred in the variables schooling ($f=0.62$) and smoking status ($f=0.68$); subjects having secondary education and who are non-smokers exhibited a higher adherence to the MD (Table 6). According to results of logistic regression analysis, educational level, without studies or primary education, was associated with a low adherence to the MD (adherence $\leq$ 67.3%) (adjusted OR= 4.16, 1.15-15.03, $P=0.029$). In addition, the association between health behavior (alcohol,

tobacco use and physical activity) and adherence to the MD was analyzed; however, there was no relationship.

Table 1. General characteristics of the studied sample: colorectal patients post-surgery

	Total sample (n= 74)
Gender, male, %	66.2
Age, Mean(SD)	60.2(5.5)
Schooling, %	
Without studies	6.8
Primary education	35.1
Secondary education	44.6
University degree	13.5
Smoking status, %	
Never	33.8
Past	40.5
Current	25.7
BMI classification, overweight /obesity, %	66.2
Physical activity during free time, yes, %	74.3
Economic activity (multiple answer), %	
Working	21.6
Unemployed	6.8
Retired	62.2
Housework	9.5
Last work, %	
Employer	5.4
Businessman/women	8.1
Family help	6.8
Steady salaried employee	70.3
Temporary salaried employee	4.1
Member of a cooperative	5.4

SD, standard deviation

Table 2. Intakes of energy, macronutrients, cholesterol, fiber, water and alcohol in the studied sample: colorectal patients post-surgery

	Total (n= 74)	Men (n=49)	Women (n=25)	*P*[a]
	Mean(SD)			
Energy, kcal/d	1,993.1(586.5)	1,976.4(600.3)	2,025.9(569.2)	0.631
Protein, %TEI (AMDR, 10-15%TEI)	15.0(2.5)	15.0(2.7)	15.1(2.2)	0.914
Carbohydrate, %TEI (AMDR, 50-60%TEI)	41.5(6.3)	41.6(6.4)	41.3(6.0)	0.858
Lipids, %TEI (AMDR, <30-35%TEI)	38.3(5.0)	37.6(5.0)	39.6(4.8)	0.102
SFA, %TEI (AMDR, <7-8%TEI)	10.9(2.1)	10.7(2.0)	11.1(2.1)	0.398
MUFA, %TEI (AMDR, 20%TEI)	17.4(2.9)	17.3(3.0)	17.5(2.6)	0.841
PUFA, %TEI (AMDR, 5%TEI)	7.0(2.6)	6.5(2.3)	8.0(2.8)	**0.017**
Linoleic acid, g/d	12.4(6.2)	12.3(6.0)	12.7(6.6)	1.000
α-linolenic acid, g/d	1.8(0.7)	1.8(0.7)	1.8(0.7)	0.918
Cholesterol, mg/d	295.5(148.6)	293.8(161.8)	298.8(121.6)	0.797
Fiber, g/d	20.4(5.4)	19.7(4.7)	21.8(6.6)	0.116
Water[b], g/d	1,062.6(248.1)	1,047.0(252.4)	1,092.2(241.6)	0.467
Alcohol, g/d	8.6(8.2)	10.4(8.9)	5.1(5.0)	**0.001**

AMDR, acceptable macronutrient distribution ranges; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid; TEI, total energy intake; [a]Differences by gender; [b]Intake of water from foods, not from drinking water.

Table 3. Dietary nutrient density (per 1000 kcal/day) in the studied sample: colorectal patients' post-surgery

Nutrient density (/1000 kcal/day)	Total (n=74)		Men (n=49)	Women (n=25)	*P*[a]
	Estimated intake	%EAR	Estimated intake		
	Mean(SD)				
Proteins, g	37.5(6.3)	78.7(25.8)	37.5(6.7)	37.6(5.5)	0.914
Vitamins					
Thiamine, mg	0.6(0.2)	104.5(42.6)	0.5(0.2)	0.6(0.2)	0.053
Riboflavin, mg	0.8(0.3)	123.1(43.5)	0.8(0.2)	0.9(0.3)	0.070
Niacin, mg	15.3(3.5)	255.7(70.0)	15.2(3.6)	15.5(3.3)	0.677
B_6, mg	1.0(0.3)	140.5(55.5)	1.0(0.3)	1.1(0.3)	0.301
Folic acid, mcg	152.2(42.2)	97.6(26.3)	149.1(38.7)	158.4(48.6)	0.779
B_{12}, mcg	2.9(0.9)	284.9(117.5)	3.0(1.0)	2.8(0.7)	0.344
C, mg	89.5(38.6)	283.4(116.6)	85.2(33.8)	97.9(46.1)	0.195
A, mcg	297.0(80.9)	87.0(27.6)	296.8(85.7)	297.5(72.4)	0.779
D, mcg	1.0(0.6)	34.2(25.1)	1.0(0.6)	1.1(0.6)	0.148
E, mg	4.5(3.9)	58.9(28.6)	4.2(1.6)	5.0(2.5)	0.199
Minerals					
Ca, mg	425.9(107.0)	82.5(21.0)	410.1(104.7)	456.9(106.7)	0.075
P, mg	701.3(119.4)	195.2(49.1)	694.0(131.0)	715.5(93.4)	0.334
Mg, mg	143.1(27.6)	83.0(21.0)	141.8(29.3)	145.5(24.4)	0.586
Fe, mg	7.4(2.0)	123.9(21.0)	7.2(1.9)	7.7(2.3)	0.370
Zn, mg	4.8(1.1)	114.3(38.4)	4.8(1.1)	5.0(1.0)	0.176
Cu, mg	0.6(0.07)	101.0(27.6)	0.6(0.07)	0.6(0.07)	0.862
I, mcg	47.7(9.9)	62.1(15.9)	47.3(10.2)	48.6(9.3)	0.603
Se, mcg	59.4(15.2)	212.2(70.7)	60.2(16.6)	57.9(12.3)	0.555

EARs, estimated average requirements; SD, standard deviation; [a]differences by gender.

Table 4. Healthy Eating Index for Spanish Diet (HEISD): scores and percentage of participants who did not meet the recommendations in the studied sample: colorectal patients' post-surgery

HEISD components	Scores[a], Mean(SD)				Participants who did not meet the recommendations[b], %			
	Total (n=74)	Men (n=49)	Women (n=25)	*P*[c]	Total (n=74)	Men (n=49)	Women (n=25)	*P*[c]
Cereals	9.9(0.5)	9.9(0.5)	9.9(0.5)	0.987	4.1	4.1	4.0	1.000
Vegetables	9.2(1.4)	9.4(1.2)	8.9(1.6)	0.186	27.0	22.4	36.0	0.214
Fruits	9.5(1.2)	9.3(1.3)	9.7(0.8)	0.260	18.9	22.4	12.0	0.358
Milk and dairies	9.8(0.8)	9.8(0.9)	9.7(0.8)	0.405	8.1	6.1	12.0	0.400
Legumes	9.1(1.8)	9.2(1.5)	8.8(2.3)	0.657	29.7	28.6	32.0	0.760
Meats	3.2(1.8)	3.3(1.9)	3.1(1.7)	0.623	100	100	100	-
Cold meats	4.0(2.6)	4.0(2.8)	3.9(2.4)	0.937	89.2	87.8	92.0	0.578
Sweets	1.8(3.1)	1.6(3.0)	2.2(3.3)	0.395	87.8	87.8	88.0	0.976
Soft-drinks	9.1(2.3)	8.9(2.3)	9.4(2.2)	0.152	14.9	18.4	8.0	0.314
Diet variety	7.5(1.6)	7.6(1.6)	7.4(1.6)	0.512	100	100	100	-
Total	73.0(7.1)	73.0(7.2)	73.0(7.0)	0.991	85.1	83.7	88.0	0.621

HEISD, Healthy Eating Index for Spanish Diet; SD, standard deviation; [a]Each component can contribute 10 points to the total score and the theoretical range is 0-100; [b]Percentage of participants who did not meet the recommendations of the HEISD; [c]Score differences by gender.

Table 5. Scores for components of the MedDietScore (MDS) in the studied sample: colorectal patients' post-surgery

	Scores[a], Mean(SD)			
MDS components	Total (n=74)	Men (n=49)	Women (n=25)	*P*[b]
Non-refined cereals	0.7(1.0)	0.5(0.9)	1.2(1.1)	**0.004**
Potatoes	2.4(1.2)	2.4(1.3)	2.4(1.2)	0.727
Fruits	2.7(0.9)	2.5(0.9)	2.9(0.9)	0.051
Vegetables	3.1(0.9)	3.1(0.8)	3.1(1.0)	1.000
Legumes	2.3(0.6)	2.3(0.6)	2.1(0.7)	0.344
Fish	3.1(1.0)	3.1(1.1)	3.3(0.6)	0.194
Red meat and products	2.8(1.3)	2.8(1.3)	3.0(1.3)	0.505
Poultry	4.8(0.6)	4.8(0.6)	4.6(0.6)	0.061
Full fat dairy	4.8(0.4)	4.8(0.4)	4.8(0.5)	0.824
Olive oil	4.9(0.5)	4.9(0.4)	4.8(0.6)	0.743
Alcoholic beverages	4.9(0.5)	4.9(0.6)	4.9(0.6)	0.475
MDS total	36.6(3.03)	36.2(2.7)	37.3(3.5)	0.104

MDS, MedDietScore; SD, standard deviation; [a]Each component can contribute 5 points to the total score and the theoretical range is 0-55; [b]differences by gender.

Table 6. Healthy Eating Index for Spanish Diet (HEISD) score and the adherence to MedDietScore (MDS) by lifestyle and socio-economic and demographic factors and body weight status in the studied sample: colorectal patients post-surgery

	HEISD		Adherence to MDS	
	Score[a], Mean(SD)	*P*	Means(SD)	*P*
Age[b]				
<62 y	72.2(6.4)	0.046	66.7(5.7)	0.923
≥62 y	74.6(6.6)		66.0(5.2)	
Schooling[c]				
Without studies and primary education	72.2(7.0)	0.837	65.7(4.9)	0.870
Secondary education	74.3(6.8)		67.0(6.1)	
University education	72.1(3.8)		66.9(5.1)	
Smoking status				
Current	71.2(9.3)	0.292	66.4(5.2)	0.493
Never	73.2(6.1)		67.8(5.3)	
Past	74.0(6.3)		65.8(5.9)	
BMI				
Overweight/obese	72.0(7.2)	0.373	68.0(5.3)	0.360
Non overweight/obese	73.5(6.3)		65.8(5.5)	
Physical activity during free time				
Yes	73.3(6.9)	0.805	66.8(5.5)	0.943
No	72.5(5.5)		66.6(5.6)	
Economic activity				
Working	71.6(4.3)	0.370	66.1(5.9)	0.629
Unemployed, retired, housework	73.6(7.0)		66.5(5.4)	
Last work				
Employer and businessman/women	76.7(5.4)	0.837	66.0(5.0)	0.730
Family help				
Steady or temporary salaried	75.3(4.4)		66.4(6.0)	
employee, member of a cooperative	72.3(6.7)		66.5(5.6)	

HEISD, Healthy Eating Index for Spanish Diet; MDS, MedDietScore, [a]Theoretical range is 0-100; [b]Age variable was split at the sample median, thereby defining high and low groups on this variable in question; [c]The answers of the variables *Schooling*, *Economic activity* and *Last work* were regrouped to simplified the analyses and presentation of results.

DISCUSSION

Research on the adequacy of nutrient intakes and the diet has recognized the greater vulnerability of certain population subgroups, such as the elderly (35) and cancer patients (36). Nevertheless, dietary intake data in CRC patient's post-surgery, especially in the medium term post-operatively, are limited (16,17). The present pilot study is, therefore,

an important seed contribution to the current literature on the dietary status of patients with CRC.

The current study reports dietary intakes from food, dietary supplements and foods with added dietary ingredients that are inadequate with respect to the recommended levels of a number of nutrients. In relation to macronutrients, diet was characterized by inadequate carbohydrate intake by default and by excessive protein and fat intakes, these results agree with those from general population surveys (22). Scientific evidence demonstrates that this type of diet has a causative link to colon cancer; however, mechanisms of action are not fully elucidated (2).

Regarding micronutrient intakes, a significant proportion of subjects did not meet daily requirements for folic acid, vitamins A, D and E, calcium, magnesium and iodine. Inadequate intakes of these nutrients were also noted by other authors in cancer patients (16,17) and in the general population (37). The inadequacy of dietary intake seems common in people of the age group of the present study (38-40). Similarly, Johnson *et al.* (41) reported that elderly persons were consuming more than the recommended amount of protein, but the average intakes of many vitamins and minerals were less than optimal based on the average intakes.

It should be noted that the prevalence of inadequate folate intakes in the present study (66.2%) was lower than that reported by Gómez *et al.* (17). In any case, there is some evidence that folic acid, calcium, and vitamin D reduce the risk of CRC. In particular, recent research indicates that calcium and vitamin D might act together, rather than separately, to reduce the risk of colorectal adenomas (42). However, the evidence is not completely consistent (43).

By comparing the inadequacy for nutrient intakes by gender, men had significantly less adequate intakes for thiamine, vitamin A, magnesium and zinc than women ($P<0.05$). Consistent with our results, Lim *et al.* (36) reported more dietary habit problems and poor nutritional balance in males with gastric cancer than those of females. And significant gender differences in nutrient intakes were also observed in the general population (44). Additionally, in the present study, patients who were overweight/obese presented higher inadequate intake of folic acid, vitamin A and zinc than those whose weight was normal ($P<0.05$). It should be noted that more than one half of the participants had excess weight. In the literature about CRC patients, data on prevalence of overweight/obesity do not present consistent results; thus, some studies detected a high prevalence of

overweight/obesity in patients with CRC (45), whereas others had associated malnutrition with this form of cancer (46,47).

In relation to the diet quality, we applied two scores, HEISD and MDS. The evaluation of the overall diet from a global perspective by these types of scores is widely used in nutritional studies (48-50). In particular, MDS has a beneficial effect on the risk of CRC (13-15,51) and on the risk of tumor recurrence, metastasis and death (16,52). In the current study, the mean percentage for adherence to the MD was of 66.6%, and a significant proportion of subjects did not meet food groups recommendations; 85.1% had "no healthy diet or need changes" according to the HEISD. The percentage of subjects classified as "no healthy diet or need changes" was similar to the general population of the Basque Country (28), and the quality of their diet was characterized by a low score for non-refined cereals, especially in males. To our knowledge, there are not published data on gender differences in whole grain intake in Spanish population, however, for British adults, no significant gender differences were found (53). There is convincing evidence that whole grains help reduce the risk of CRC. In addition to the high content of bioactive compounds, whole grain also represents a source of high-quality carbohydrate, as assessed by a low glycemic index, because of its slow digestion and absorption (54). Whole grain may exert beneficial effects on colorectal carcinogenesis by decreasing insulin (55). Nevertheless, in the present study no significant correlation was found between both scores (HEISD and MDS), most likely due to methodological differences, because items and reference criteria are different.

According to cancer stage and type of surgery, no significant differences were found neither for energy and nutrients intake nor for diet quality, probably due to the time spent after the diagnosis and treatment. To our knowledge, it is unknown whether the variance in adherence to dietary recommendations is consistent across all stages of CRC, or treatment settings. Although CRC patients admitted that they changed to a healthy diet after being diagnosed with CRC (56), these patients often either receive no dietary information or dietary advice is scarce (57,58). This fact could influence dietary adequacy and quality. In fact, evidence indicates that nutritional advice and education about diet can be effective in improving nutritional intake and status, quality of life (59) and long-term prognosis in CRC (60).

In our case, the HEISD components with more cases of non-compliance of the recommendations were meats, cold meats, sweets and diet variety, and the MDS components with a higher percentage of participants who did not comply with the

recommendations were non-refined cereals, legumes and potatoes. It should be noted that CRC patients can have less tolerance of high-fiber foods (such as legumes and non-refined cereals). With respect to meat consumption, Zhu *et al.* (16) found that a high level of conformity with the processed meat pattern is significantly associated with an increased risk of all-cause mortality and recurrence in CRC; however, the prudent vegetable or the high-sugar patterns present no association with disease-free survival.

Furthermore, participants who were 61 years of age or older obtained higher HEISD scores than subjects younger than 61 ($P<0.05$); this result agrees with the findings of other studies (28). Other factors related to diet quality scores (HEISD and/or MDS) were educational attainment, most recent job, smoking status and physical activity participation; nevertheless, no significant association was identified; the size effect was considerable. Moreover, regression analyses demonstrated that those without studies or primary education had a low adherence to the MD and greater thiamine inadequacy ($P<0.05$). Results concerning educational level and MDS agreed with the findings of other authors (61). However, care must be taken in the interpretation of our analysis of logistic regression due to sample size.

In relation to lifestyle factors, the greatest size effect occurred in the variables of smoking status for both diet quality indices. In our study, tobacco consumption was associated with a greater inadequacy in the intake of folic acid ($P<0.05$) and could be associated with a low diet quality, even if the *P*-value were not significant. Other authors have observed relationships between smoking and dietary intake (62). Furthermore, significant interactions observed between smoking and CRC, suggesting a potential mediating effect of the MD (13,15). As in previous studies, physical activity and diet quality were associated (63). It should be noted that a quarter of the participants in the present study reported no physical activity in their free time. This proportion is higher than that of previous studies (64), and this is an unfavorable result, given that the known risks of sedentary behavior for survivors include the development of comorbid conditions and cancer recurrence (65,66).

LIMITATIONS

Several limitations to this study should be recognized. First, the sample size was small. As a result, we are increasing the sample size to be able to analyze more precisely dietary adequacy of this population group. Second, the data on dietary intake are self-reported, which is assumed to be related to some degree of under- or over-reporting, especially in specific groups of the populations defined by weight status or gender

(67,68). This fact could make it difficult to estimate actual micronutrient intake and produces some bias that can be avoided utilizing biomarkers (69). Anyway, FFQ can provide valid information on intake for a large number of micronutrients (69-71). Finally, the lack of control of some possible confounders such as comorbidities and other conditions that could affect food consumption and the capacity to absorb and use of nutrients should be noted.

CONCLUSIONS

In conclusion, our results demonstrated that the diet of the studied group is inadequate in many respects, including nutrients and food intakes. The inadequacy of some nutrients was associated with male gender, excess of weight, smoking and low educational level, and the low adherence to the MD was pronounced in those with a low educational level. Therefore, these patients should be an important target group for the application of educational programs and individualized nutritional counseling sessions to improve their quality of life and reduce the risk of CRC mortality. Further studies are needed to confirm the determinants of the poor dietary habits and the inadequate nutrient intakes, keeping in mind surgical treatment and radiotherapy and/or chemotherapy as adjuvant treatments, and to know the diet changes after treatment for CRC.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by two projects (The Department of Health and Consumer Affairs, Basque Government, 2011111153; Saiotek, S-PE12UN058). We gratefully acknowledge the contributions of Isabel Portillo, PhD, manager of the CRC screening program of the Osakidetza/The Basque Country Health Service, and participants of the present study.

REFERENCES

1. Altobelli E, Lattanzi A, Paduano R, Varassi G, and di Orio F: Colorectal cancer prevention in Europe: burden of disease and status of screening programs. *Prev Med* **62**,132-141, 2014.
2. Kasdagly M, Radhakrishnan S, Reddivari L, Rao Veeramachaneni DN, and Vanamala J: Colon carcinogenesis: influence of Western diet-induced obesity and targeting stem cells using dietary bioactive compounds. *Nutrition* **30**, 1242-1256, 2014.

3. Norat T, Chan D, Lau R, Aune D, and Vieira R: *WCRF/AICR Systematic Literature Review Continuous Update Project Report. The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer*. Imperial College London, 2010. Available at: http://www.wcrf.org/PDFs/Colorectal-cancer-CUP-report-2010.pdf (accessed 23 May 2014).
4. Jacobson HN, and Stanton JL: Pattern analysis in nutrition. *Clin Nutr* **5**, 249–53, 1986.
5. Dixon LB, Balder HF, Virtanen MJ, Rashidkhani B, Männistö S, *et al.*: Dietary patterns associated with colon and rectal cancer: results from the Dietary Patterns and Cancer (DIETSCAN) Project. *Am J Clin Nutr* **80**, 1003-1011, 2004.
6. Kim MK, Sasaki S, Otani T, and Tsugane S; Japan Public Health Center-based Prospective Study Group: Dietary patterns and subsequent colorectal cancer risk by subsite: a prospective cohort study. *Int J Cancer* **115**, 790-798, 2005.
7. Mai V, Kant AK, Flood A, Lacey JV, Schairer C, and Schatzkin A: Diet quality and subsequent cancer incidence and mortality in a prospective cohort of women. *Int J Epidemiol* **34**, 54-60, 2005.
8. Fitzgerald AL, Dewar RA, and Veugelers PJ: Diet quality and cancer incidence in Nova Scotia, Canada. *Nutr Cancer* **43**, 127-132, 2002.
9. Izano MA, Fung TT, Chiuve SS, Hu FB, and Holmes MD: Are diet quality scores after breast cancer diagnosis associated with improved breast cancer survival? *Nutr Cancer* **65**, 820-6, 2013.
10. Tyrovolas S and Panagiotakos DB: The role of Mediterranean type of diet on the development of cancer and cardiovascular disease, in the elderly: a systematic review. *Maturitas* **65**, 122-130, 2010.
11. Kontou N, Psaltopoulou T, Panagiotakos D, Dimopoulos MA, and Linos A: The Mediterranean diet in cancer prevention: a review. *J Med Food* **14**, 1065-1078, 2011.
12. United Nations Educational, Scientific and Cultural Organization: *Representative list of the intangible cultural heritage of humanity*, 2010. Available from: www.unesco.org/culture/ich/index.php?lg=en&pg =00011&RL=00394.
13. Grosso G, Biondi A, Galvano F, Mistretta A, Marventano S, *et al.*: Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. *Nutr Cancer* **66**, 558-565, 2014.
14. Bamia C, Lagiou P, Buckland G, Grioni S, Agnoli C, *et al.*: Mediterranean diet and colorectal cancer risk: result from a European Cohort. *Eur J Epidemiol* **28**, 317-328, 2013.

15. Kontou N, Psaltopoulou T, Soupos N, Polychronopoulos E, Xinopoulos D, *et al.*: The mediating effect of Mediterranean diet on the relation between smoking and colorectal cancer: a case-control study. *Eur J Public Health* **23**, 742-746, 2013.
16. Zhu Y, Wu H, Wang PP, Savas S, Woodrow J, *et al.* Dietary patterns and colorectal cancer recurrence and survival: a cohort study. *BMJ Open* **3**, e002270, 2013.
17. Gómez Valiente da Silva H, Fonseca de Andrade C, and Bello Moreira AS: Dietary intake and nutritional status in cancer patients: comparing adults and older adults. *Nutr Hosp* **29**, 907-912, 2014.
18. Hertzog MA: Considerations in determining sample size for pilot studies. *Res Nurs Health* **31**, 180-191, 2008.
19. Portillo I, Idígoras I, Ojembarrena E, Arana E, Luis Hurtado J, *et al.*: Lesions detected in a colorectal cancer screening program in the Basque Country: first round (2009-2011). *Gastroenterol Hepatol* **36**, 301-308, 2013.
20. Rodríguez IT, Ballart F, Cucó G, Biarnés E, and Arija V: Validación de un cuestionario de frecuencia de consumo alimentario corto: reproducibilidad y validez. *Nutr Hosp* **23**, 4247-4252, 2008.
21. Carbajal A, and Sánchez-Muniz FJ: Guía de prácticas. En: *Nutrición y dietética*. MT García-Arias, MC García-Fernández (Eds). Secretariado de Publicaciones y Medios Audiovisuales. Universidad de León, 2003, 1a-130a.
22. Elika: *Estudio cuantitativo del consumo de alimentos en la CAPV*. Vitoria, Spain: Dpto. Agricultura, Pesca y Alimentación, Gobierno Vasco, 2008.
23. Serra L, and Aranceta J: Objetivos nutricionales para la población española: consenso de la Sociedad Española de Nutrición Comunitaria. *Rev Esp Nutr Com* **17**, 178-199, 2011.
24. Institute of Medicine of the National Academies: *Dietary Reference Intakes. The Essential Guide to Nutrient Requirement*. JJ Otten, JP Hellwig and LD Meyers, editors. Washington, DC: The National Academy Press, 2006; World Health Organization, Food and Agriculture Organization of the United Nations: *Vitamin and Mineral Requirements in Human Nutrition*, *2nd ed*. Geneva: World Health Organization and Rome/Food and Agriculture Organization of the United Nations, 2004. Available at: http://whqlibdoc.who.int/publications/2004/9241546123.pdf (accessed 4 June 2014).
25. Velho S, Marques-Vidal P, Baptista F, and Camilo ME: Dietary intake adequacy and cognitive function in free-living active elderly: a cross-sectional and short-term prospective study. *Clin Nutr* **27**, 77-86, 2008.

26. Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamine E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Calcium and Vitamin D (2011). Copyright 2001 by The National Academies. These reports may be accessed via www.nap.edu (accessed 10 June 2014).
27. Heckman MA, Weil J, and De Mejia EG: Caffeine (1, 3, 7-trimethylxanthine) in Foods: A Comprehensive Review on Consumption, Functionality, Safety, and Regulatory Matters. *Journal of Food Science* **75**, R77–R87, 2010.
28. Norte AI, and Ortiz R: Spanish diet quality according to the Healthy eating index. *Nutr Hosp* **26**, 330-336, 2011.
29. Panagiotakos DB, Milias GA, Pitsavos C, and Stefanadis C: MedDietScore: A computer program that evaluates the adherence to the Mediterranean dietary pattern and its relation to cardiovascular disease risk. *Comput Methods and Programs in Biomed* **83**, 73-77, 2006.
30. Panagiotakos DB, Pitsavos C, and Stefanadis C: Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. *Nutr Metab Cardiovasc Dis* **16**, 559-568, 2006.
31. Ministerio de Sanidad, Servicios Sociales e Igualdad. *Encuesta Nacional de Salud 2011/12*. Available at: http://www.ine.es/jaxi/menu.do?type=pcaxis&path=/t15/p419&file=inebase (accessed 7 January 2013).
32. World Health Organization, WHO: *Obesity: preventing and managing the global epidemic. Report of a WHO Consultation*. Geneva: WHO, 2000.
33. Cohen J. *Statistical power analysis for the behavioural sciences, 2nd ed*. Hillsdale, NJ: Lawrence Erlbaum Associates, 1988.
34. Touvier M, Me´jean C, Kesse-Guyot E, Vergnaud AC, Hercberg S, *et al.*: Sociodemographic and economic characteristics associated with dairy intake vary across genders. *J Hum Nutr Diet* **24**, 74–85, 2011.
35. Sharkey JR, Branch LG, Zohoori N, Giuliani C, Busby-Whitehead J, et al: Inadequate nutrient intakes among homebound elderly and their correlation with individual characteristics and health-related factors. *Am J Clin Nutr* **76**, 1435–1445, 2002.

36. Lim H, Cho G, and Kim S: Evaluation of nutrient intake and diet quality of gastric cancer patients in Korea. *Nutr Res Pract* **6**, 213-220, 2012.
37. Mensink GB, Fletcher R, Gurinovic M, Huybrechts I, Lafay L, et al: Mapping low intake of micronutrients across Europe. *Br J Nutr* **110**, 755-773, 2013.
38. Zhu K, Devine A, Suleska A, Tan CY, Toh CZ, *et al.*: Adequacy and change in nutrient and food intakes with aging in a seven-year cohort study in elderly women. *J Nutr Health Aging* **14**, 723–729, 2010.
39. Baumgartner MR: Vitamin-responsive disorders: cobalamin, folate, biotin, vitamins B1 and E. *Handb Clin Neurol* **113**, 1799–1810, 2013.
40. Ahmadieh H, and Arabi A: Vitamins and bone health: beyond calcium and vitamin D. *Nutr Rev* **69**, 584–598, 2011.
41. Johnson CS, and Begum MN: Adequacy of nutrient intake among elderly persons receiving home care. *J Nutr Elder* **27**, 65–82, 2008.
42. Ryan-Harshman M, and Aldoori W: Diet and colorectal cancer. *Can Fam Physician* **53**, 1913-1920, 2007.
43. World Cancer Research Fund / American Institute for Cancer Research: *Continuous Update Project Interim Report Summary. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer*. 2011. Available at: http://www.wcrf.org/PDFs/Colorectal%20cancer%20report%20summary%202011.pdf (accessed 12 March 2014).
44. Bates CJ, Prentice A, and Finch S: Gender differences in food and nutrient intakes and status indices from the National Diet and Nutrition Survey of people aged 65 years and over. *Eur J Clin Nutr* **53**, 694-9, 1999.
45. Lopes JP, de Castro Cardoso Pereira PM, dos Reis Baltazar Vicente AF, Bernardo A, de Mesquita MF: Nutritional status assessment in colorectal cancer patients. *Nutr Hosp* **28**, 412-418, 2013.
46. Stratton RJ, Green CJ, and Elia M: *Disease-related Malnutrition: An Evidence-based Approach to Treatment*. Wallingford: CABI Publishing, 2003.
47. Garth AK, Newsome CM, Simmance N, and Crowe TC: Nutritional Status, Nutrition Practices and Post-operative Complications in Patients with Gastrointestinal Cancer. *J Hum Nutr Diet* **23**, 393-401, 2010.
48. Bach A, Serra-Majem L, Carrasco JL, Roman B, Ngo J, *et al.*: The use of indexes evaluating the adherence to the Mediterranean diet in epidemiological studies: a review. *Public Health Nutr* **9**, 132–146, 2006.
49. Gerber M: Qualitative methods to evaluate Mediterranean diet in adults. *Public Health Nutr* **9**, 147–151, 2006.

50. Kant AK: Dietary patterns and health outcomes. *J Am Diet Assoc* **104**, 615–635, 2004.
51. Dixon LB, Subar AF, Peters U, Weissfeld JL, Bresalier RS, *et al.*: Adherence to the USDA Food Guide, DASH Eating Plan, and Mediterranean dietary pattern reduces risk of colorectal adenoma. *J Nutr* **137**, 2443–2450, 2007.
52. Sofi F, Cesari F, Abbate R, Gensini GF, and Casini A: Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* **337**, a1344, 2008.
53. Thane CW, Jones AR, Stephen AM, Seal CJ, and Jebb SA: Comparative whole-grain intake of British adults in 1986-7 and 2000-1. *Br J Nutr* **97**, 987-992, 2007.
54. Jenkins DJ, Wesson V, Wolever TM, Jenkins AL, Kalmusky J, Guidici S, *et al.*: Whole meal versus wholegrain breads: proportion of whole or cracker grain and the glycaemic response. *BMJ* **297**, 958-960, 1988.
55. Ortega R, Aparicio A, Jimenez A, and Rodriguez E: Wholegrain cereals and sanitary benefits. *Nutr Hosp* **32**(Supl. 1), 25-31, 2015.
56. Yusof AS, Isa ZM, Shah SA: Perceptions of Malaysian colorectal cancer patients regarding dietary intake: a qualitative exploration. *Asian Pac J Cancer Prev* **14**, 1151-4, 2013.
57. Cha R, Murray MJ, Thompson J, Wall CR, Hill A, *et al.*: Dietary patterns and information needs of colorectal cancer patients post-surgery in Auckland. *N Z Med* **125**, 38-46, 2012.
58. Geirsdottir OG, and Thorsdottir I: Nutritional status of cancer patients in chemotherapy; dietary intake, nitrogen balance and screening. *Food Nutr Res* **52**, 10.3402/fnr.v52i0.1856, 2008.
59. Ravasco P, Monteiro-Grillo I, Vidal PM, and Camilo ME: Dietary counselling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *J Clin Oncol* **23**, 1431-1438, 2005.
60. Ravasco P, Monteiro-Grillo I, and Camilo M: Individualized nutrition intervention is of major benefit to colorectal cancer patients: long-term follow-up of a randomized controlled trial of nutritional therapy. *Am J Clin Nutr* **96**, 1346-1343, 2012.
61. Hu EA, Toledo E, Diez-Espino J, Estruch R, Corella D, *et al.*: Lifestyles and risk factors associated with adherence to the Mediterranean diet: a baseline assessment of the PREDIMED trial. *PloS One* **8**, e60166, 2013.
62. Ma J, Betts NM, and Hampl JS: Clustering of Lifestyle Behaviors: The Relationship Between Cigarette Smoking, Alcohol Consumption, and Dietary Intake. *Am J Health Promot* **15**, 107-117, 2000.

63. Gillman MW, Pinto BM, Tennstedt S, Glanz K, Marcus B, and Friedman RH: Relationships of physical activity with dietary behaviours among adults. *Prev Med* **32**, 295-301, 2001.
64. Stephenson LE, Bebb DG, Reimer RA, and Culos-Reed SN: Physical activity and diet behaviour in colorectal cancer patients receiving chemotherapy: associations with quality of life. *BMC Gastroenterology* **9**, 60, 2009.
65. Irwin ML: Physical activity interventions for cancer survivors. *Br J Sports Med* **43**, 32-38, 2009.
66. Courneya KS, and Friedenreich CM: Physical activity and cancer control. *Semin Oncol Nurs* **23**, 242-252, 2007.
67. Trabulsi J, and Schoeller DA: Evaluation of dietary assessment instruments against doubly labelled water, a biomarker of habitual energy intake. *Am J Physiol Endocrinol Metab* **281**, E891-899, 2001.
68. Gunnare NA, Silliman K, and Morris MN: Accuracy of self-reported weight and role of gender, body mass index, weight satisfaction, weighing behaviour, and physical activity among rural college students. *Body Image* **10**, 406-410, 2013.
69. Jacques PF, Sulsky SI, Sadowski JA, Phillips JC, Rush D, *et al.*: Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* **57**, 182-189, 1993.
70. Pirouzpanah S, Taleban FA, Mehdipour P, Atri M, Hooshyareh-rad A, *et al.*: The biomarker-based validity of a food frequency questionnaire to assess the intake status of folate, pyridoxine and cobalamin among Iranian primary breast cancer patients. *Eur J Clin Nutr* **68**, 316-323, 2014.
71. Fayet F, Flood V, Petocz P, and Samman S: Relative and biomarker-based validity of a food frequency questionnaire that measures the intakes of vitamin B(12), folate, iron, and zinc in young women. *Nutr Res* **31**, 14-20, 2011.

3.2. STUDY 2: "Gene-diet interactions in colorectal cancer: survey design, instruments, participants and descriptive data of a case-control study in the Basque Country"

Gene-diet interactions in colorectal cancer: survey design, instruments, participants and descriptive data of a case-control study in the Basque Country

Iker Alegria-Lertxundi[1], Carmelo Aguirre[2], Luis Bujanda[3], Francisco Javier Fernández[4], Francisco Polo[5], José Mª Ordovás[6], Mª Carmen Etxezarraga[7], Iñaki Zabalza[8], Mikel Larzabal[9], Isabel Portillo[10], Marian M. de Pancorbo[11], Leire Palencia-Madrid[11],Koldo Garcia-Etxebarria[12], Ana Mª Rocandio[1,11], Marta Arroyo-Izaga[1]

[1]Department of Pharmacy and Food Sciences. Faculty of Pharmacy, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain

[2]Pharmacovigilance Unit, Galdakao-Usansolo University Hospital, Osakidetza, Spain

[3]Department of Gastroenterology, Donostia University Hospital / Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 20014 San Sebastian, Spain

[4]Department of Gastroenterology, Galdakao-Usansolo University Hospital, Osakidetza, Galdakao, Spain

[5]Department of Gastroenterology, Basurto University Hospital, Osakidetza, Bilbao, Spain

[6]Nutrition and Genomics Laboratory, Jean Mayer Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts, United States of America; IMDEA Food, Madrid, Spain

[7]Department of Pathology, Basurto University Hospital, Osakidetza, Bilbao, Spain. Department of Physician and Surgeon Specialities, University of the Basque Country, Leioa, UPV/EHU, Spain

[8]Department of Pathology, Galdakao-Usansolo University Hospital, Osakidetza, Galdakao, Spain

[9]Department of Pathology, Donostia Hospital / Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 20014 San Sebastian, Spain

[10]Colorectal cancer screening programme, Osakidetza, Bilbao, Spain

[11]BIOMICs Research Group, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain

Citation text: Alegria-Lertxundi I, Aguirre C, Bujanda L, Fernández FJ, Polo F, Ordovás JM, Etxezarraga MC, Zabalza I, Larzabal M, Portillo I, de Pancorbo MM, Palencia-Madrid L, Garcia-Etxebarria K, Rocandio AM, Arroyo-Izaga M. Gene-diet interactions in colorectal cancer: survey design, instruments, participants and descriptive data of a case-control study in the Basque Country has been sent to *Nutrients*.

Abstract: Epidemiologic studies have revealed inconsistent evidence of gene-diet interaction in relation to colorectal cancer (CRC). In order to better elucidate the role of these types of interactions in the etiology of CRC, the aim of this study was to analyze them in a sample of cases and controls from the population-based screening programme for CRC of the Osakidetza/Basque Health Service. This case-control study analyzed dietetic, genetic, demographic, socioeconomic factors and lifestyles (smoking, alcohol or Physical activity (PA). The participants were 308 patients diagnosed with CRC and 308 age- and sex-matched subjects as controls. Cases were more likely than controls to have overweight/obesity (67.5 vs. 58.1%, P<0.05), a lower intake of vitamin B2 (0.86(0.23) vs. 0.92(0.23) mg/1,000 kcal, P<0.01) and Ca/P ratio (0.62(0.12) vs. 0.65(0.13), P<0.01). Moreover a higher proportion of cases than controls “always” or “often” used salt added to cooking (76.8 vs. 69.7%, P<0.05) and did not meet the Nutritional Objectives for saturated fatty acids (85.7 vs. 67.5%, P<0.001) or cholesterol (35.4 vs. 25.0%, P<0.01). Moreover, the results presented in this manuscript allow us to conclude that some environmental factors, such as weight status and dietary components, could have an influence on the etiology of CRC in this population.

Keywords: Colorectal cancer, Diet, Genetic factors, Gene-diet interactions, Risk-factors, Case-control study.

1. Introduction

CRC is already the third leading cause of cancer death in the world, and its incidence is steadily rising in western countries [1]. According to GLOBOCAN 2018 data, CRCs are the third most commonly diagnosed form of cancer globally, comprising 11% of all cancer diagnoses [2]. Incidence varies geographically, more-developed regions have a higher incidence than less-developed ones [3]. Europe is among the seven world regions ranked according to increasing age-standardized incidence rate (ASRi) with an ASRi of 30.0 per 100,000 [4] . In particular, in Spain, the ASRi for CRC is 33.4 per 100,000 (21.1 per 100,000 in males and 12.4 per 100,000 in females) [5]. In the Basque Country, one of the autonomous regions of the North of Spain, this pathology is the most frequent type of cancer (taking into account the combined incidence both sexes) [6].

During the recent years, mortality rates for CRC have been decreasing due to early screening programs [7,8] and better treatment options [9]. However, the etiology of CRC is complex and still not fully understood. Both genetic and environmental factors play an important role in the etiology of this disease [10]. Large population studies with varying strength of evidence have found CRC protective factors such as diet (fruits and vegetables, fibre, resistant starch, fish), vitamin supplements (folate, vitamin B_6, vitamin D, calcium, magnesium), garlic and coffee, PA, and drugs (aspirin, non-steroidal anti-inflammatory drugs, hormonal replacement therapy in postmenopausal); and CRC risk factors such as obesity, red/processed meat, tobacco and alcohol among others [11].

In any case, protective and/or risk factors are not present in isolation, but coexist and interact with each other and with other factors, for example, both dietary and genetic factors affect CRC risk, in an interactive manner [12]. The recognition of these gene-diet interactions as a driver in CRC may open up new areas of research in disease epidemiology, risk assessment, and treatments. To date, epidemiologic studies have revealed inconsistent evidence of gene-diet interaction in relation to CRC. In order to better elucidate the role of these types of interactions in the etiology of CRC, the aim of this study was to analyze them in a sample of cases and controls from the population-based screening programme for CRC of the Osakidetza/Basque Health Service. The main advantage of the present study compared to other similar researches [13-15] is that we confirmed that controls were free of the disease through colonoscopy. Colonoscopy was used as the diagnosis criteria to identify the cases in order to avoid false positives and negatives.

In particular, in this paper, we present the survey design, instruments, measurements and related quality management; this detailed information will allow its replication in other populations for a comparison of the results. In addition, we analyze differences between cases and controls in some data, especially those related to diet, but also in demographic data, weight status, lifestyle (different from diet), quality of life and stress level and use of drugs related with decreasing CRC risk.

2. Materials and Methods

Overall, this epidemiologic study is an observational analytic case-control study designed to address possible gene-diet interaction in relation to CRC.

Sampling and study subjects

Participants in this study were recruited from among patients attending any of the three hospitals of the Osakidetza/Basque Health Service (Basurto, Galdakao and Donostia) members of the Basque Country'*s* CRC screening programme (CRCSP). To be eligible for this CRCSP, the patients had to be aged between 50 and 69, asymptomatic for colorectal symptoms and registered with the Osakidetza/Basque Health Service [16]. These inclusion criteria were applied to both case and control group, that is, controls fulfilled the same eligibility criteria defined for the cases, with the exception of the disease (outcome). Recruitment and data collection through questionnaires were conducted between 2014 and 2016.

All the patients who were newly diagnosed with CRC (n=601) were invited to participate in this study. Of those, 283 refused to participate in the study, and 10 were excluded due to missing information. Ultimately, 308 subjects (66.2% men) consented to participate in the survey and completed all the questionnaires. In addition, for each case, three age- (±9.0 years) and sex-matched control patients were randomly sought from the list of CRC-free subjects (n=1,836) who participated in the CRCSP during the same period as the cases. The matched controls were patients with positive results (abnormal) for immunochemical fecal occult blood test (iFOBT) and negative colonoscopy results (normal). The participation rate of the controls was 37.6%, and 17 subjects were excluded due to missing information. Finally, the matched case-to-control ratio was 1:1, and the final data set included 308 cases who were diagnosed with CRC and 308 age- and sex-matched controls. Further details on recruitment and data collection have been described elsewhere [17] .

The characteristics of the cases (pathological staging, location of the cancer, tumor grade and treatments) have been also described before (World Journal of Gastroenterology, in review process). Briefly, 72% were diagnosed with early-stage (I/II) CRC, 76% had distal location of the cancer, 80.5% of tumors were well/moderately differentiated and 73.7% had undergone surgical resection. The cases were invited to take part in this survey at least one month after finishing their last treatment (surgery, chemotherapy or radiotherapy) (median, 1.3 years; range, 0.1 to 4.2 years). No statistically significant differences were found in the time elapsed between participation in the CRCSP and collaboration in this survey, between cases and controls (cases, 1.8(1.0) years, controls 1.6 (1.5) years; p = 0.119). All these clinical data were obtained from the Basque Country's population-based CRCSP database, which links patient medical records and clinical databases and were reviewed by expert staff.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the Clinical Research Ethics Committee of the Basque Country (reference numbers PI2011006 and PI2014042). Written informed consent was obtained from all the study participants.

Procedures and survey modules

The modules in this study were initially selected to cover the assessment dietary intake, lifestyle, demographic and socioeconomic determinants and genetic factors. Table 1 provides an overview of the major study components. Consenting participants self-completed and returned one general questionnaire (GQ) and a short Food Frequency Questionnaire (SFFQ). The questions referred to behaviours before participating in the CRCSP. Assistance from the study staff was available to help the patients to understand the items on the questionnaires.

General questionnaire

The GQ was used to gather information on weight status (self-reported weight and height), environmental factors (demographic and socio-economic factors: age and sex, marital status and children, birthplace, place of residence, total number of co-residents per household, total number of rooms (excluding the kitchen and bathrooms), educational attainment, economic activity and last work; and lifestyle information: PA, physical exercise (PE) and smoking consumption). These questions were taken from the Spanish Health Questionnaire [15]. Body mass index (BMI), estimated from self-reported height and weight was classified according to the WHO criteria for those under 65 years

of age [18] and according to the criteria proposed by Silva Rodríguez *et al.* for those 65 years and older [19].

The GQ included as well information about perceived quality of life (QoL) and stress, and the use of drugs related with decreasing CRC risk (antiplatelet (including non-steroidal anti-inflammatory drugs), anticoagulants and hormone replacement therapy in the case of women) [20-23]. To assess the QoL and perceived stress an analog linear scale with a range from 0 to 100 was used [24].

The differences in general characteristics (age, BMI, educational attainment, economic activity, last employment, PE and smoking habit) between cases and controls were previously described (World Journal of Gastroenterology, in review process). Briefly, significant differences between cases and controls were found for educational level, smoking, and weight status; with a higher percentage of cases with low-medium educational level, past or current smoking status and with overweight/obesity compared to controls ($P<0.01$).

Dietary habits questionnaire

Diets were assessed using a self-reported SFFQ that was a modified version of the Rodríguez *et al.* [25] questionnaire. This adaptation was validated with multiple 24-h recalls in the Basque general population [26] and in CRC diagnosed patients in a pilot of the present study [27]. It consisted of 67 items and requires the subjects to recall the number of times each food item was consumed either per week or per month. This SFFQ included specific questions about frequency of intake of alcoholic beverages. Moreover, the respondents could also record the consumption of other foods that were not included on the food list, as well as the use of dietetic products and nutritional supplements (name –generic and brand-, dose and frequency).

The SFFQ included additional items to ask about the consumption of fried foods, grilled or roast meat, added salt (cooking and at the table), as well as the average weekly consumption of some food types (cooked vegetables, salads, fruit/fruit products, fish/fish products, and meat/meat products/meat dishes). These last questions were taken from the EPIC-Norfolk Food Frequency Questionnaire [28-29]. Once the completed SFFQ was received back, it was reviewed by a dietitian. Consumption frequencies were standardized to “per day” and multiplied by standard serving sizes (grams) [30]. For items that included several foods, each food’s

contribution was estimated with weighting coefficients that were obtained from the usual consumption data [31].

Food items were then regrouped according to nutritional characteristics [32] and considering the potential contribution of food to the pathogenesis of CRC [33,34]. All food items that were consumed were entered into DIAL 2.12 (2011 ALCE INGENIERIA) [35] a type of dietary assessment software, to estimate energy intake (kilocalories/day, kcal/d), nutrients and dietary compounds intakes that were expressed in absolute values, as a percentage of the total energy intake (TEI) and as daily consumption per 1,000 kcal. Some nutrients and dietary compounds intakes were estimated by other food composition databases as detailed below. Methyl donor compounds (methionine, choline, and betaine), fatty acids (arachidonic, eicosapentaenoic and docosahexaenoic) and dietary antioxidants (pro-vitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin), lutein and lycopene) were estimated using the US nutrients database [36]. And flavonoids and glucosinolate intakes were calculated using Phenol-Explorer [37].

Mean daily energy intake was compared with energy requirements derived from basal metabolic rate that was estimated using the Harris-Benedict formula [38] multiplied by activity factors [39]. The activity factor was based on self-reported main daily activity. Nutrient intake data (from diet and dietetic products and supplements) were compared with the Nutritional Objectives for the Spanish Population (NOSP) [40], the estimated energy requirements (EER), the Estimated Average Requirements (EAR) or Adequate Intakes (AI) [41,42]. The EAR is the mean daily intake value which is estimated to meet the requirement of half of healthy individuals in a life-stage and sex group for that nutrient, and the AI is established when there is insufficient scientific evidence to determine an EAR [41,42]. Results of energy and micronutrients were expressed as a percentage of the EER and EAR or AI, respectively. Micronutrient data were also compared with Tolerable Upper Intake Levels (UL) [44-47]. And caffeine consumption was compared to the Denmark and the UK's recommended limit for caffeine intake [48].

Additionally, in this survey, we also calculated a mineral score, which is a modified version of the score proposed by Swaminath *et al.* [49] who have associated this score with CRC risk. In the present study, this mineral score included six minerals with possible colon anti-carcinogenic effects (calcium, magnesium, zinc, selenium, potassium and iodine) and four with pro-carcinogenic properties (iron, copper, phosphorus and sodium). We did not include intakes of manganese, even though it

was part of the original score, due to the lack of data on this mineral in the food composition database used in the present study. Mineral intake was expressed as daily consumption per 1,000 kcal, and then the intakes of each mineral were categorized into tertiles based on the distribution within the controls group (taking into account sex differences when they were significantly different). We applied a similar score methodology to that developed by Swaminath *et al.* [49].

That is, for each mineral hypothesized to reduce CRC risk, each participant was assigned a value equal to their tertile rank (i.e., a value of 1–3, with lower ranks indicating lower mineral intakes and higher ranks indicating higher mineral intakes). For each mineral hypothesized to have predominantly pro-carcinogenic properties in the colon, the values assigned to the rankings were reversed (i.e., values of 3–1, with lower ranks indicating higher mineral intakes and higher ranks indicating lower mineral intakes). Finally, each participant's values for each mineral were summed to represent his/her mineral score; thus, the range of possible scores was 10–35.

Regarding alcohol consumption, the SFFQ used in this study included specific questions about the frequency of intake of the following five major types of alcoholic beverages: beer, wine, cider, aperitif with alcohol and liquor. The alcohol consumption data are expressed as grams of alcohol and standard drink units (SDU) per week [50]. We used the SDU defined for Spain (one SDU is the equivalent to 10 g of alcohol). With this information, the participants were categorized into those who did and did not meet the recommendations [51]. Finally, adherence to the dietary recommendations was evaluated utilizing the Healthy Eating Index for Spanish Diet (HEISD) [52] and the MedDietScore (MDS) [53], as previously explained [27]. The theoretical range of the HEISD is 0-100 and of the MDS 0-55, higher values of these scores indicate greater adherence to the dietary recommendations for the Spanish population and the Mediterranean diet pattern, respectively.

Adherence to guidelines for CRC prevention

Adherence to guidelines for CRC prevention was assessed using a modified version of the World Cancer Research Fund/ American Institute for Cancer Research (WCRF/AICR) score [54]. Of the 7 recommendations (components) (6 in men) included in the original version of this score, we selected 5 with convincing evidence of an association with CRC risk [11]: PA/PE, dietary fiber, red meat and processed meat, alcoholic beverages and body fatness (as BMI). The recommendation on abdominal

fatness and factors that lead to greater adult attained height were not included in this score because sufficient data were not available.

The score assigned for each component was 1 when the recommendation was met. An intermediate category (0.5 points) was created to appraise a higher proportion of the variability in the population. All other individuals received 0 points. The score was constructed using quantitative criteria laid down in the recommendations as cut-off points WCRF/AICR, and therefore, higher scores indicate a greater concordance with the recommendations of the WCRF/AICR [11]. Moreover, each component of the score was added to calculate a total score for each study participant. The theoretical range was between 0-5 points for both sexes.

Lifestyle changes after participating in the CRCSP

A questionnaire developed ad hoc, that included items related to the recommendations with convincing evidence of an association with CRC risk [11], was used to assess lifestyle changes after participating in the CRCSP. This tool was applied to a subsample of 102 matched case-control pairs, randomly selected from the study sample, through phone interviews by previously trained health professionals.

The questionnaire included the following items: PA/PE, food consumption (vegetables, fruit, whole cereals, red meat, processed meat, alcoholic drinks) and smoking habits. Possible answers were: “increase”, “decrease”, “the same” or “do not consume/practice”. If the answer was “increase” or “decrease”, additionally, subjects were asked about the reason/s for the change. Answers about the reasons were open and were analyzed manually and categorized as follows: “health promotion”, “food preference”, “changes in work, family or personal life”, “diagnostic and/or side effects of CRC treatment/s”, “other pathologies”, and “do not know or missing”.

Data obtained from clinical databases

Data about diagnosis and treatment of cases, as previously mentioned (pathological staging, location of the cancer, tumor grade and treatments), were obtained from clinical databases. Additionally, in both cases and controls, socio-economic level and health status (specifically health resource consumption) data were assessed with two indices that were obtained from the clinical databases developed by the Health Department of the Basque Government, namely the socioeconomic deprivation index (DI) and predictive risk modelling (PRM), respectively. The first one was estimated using the MEDEA project criteria [55] , as has been described elsewhere [17] and was

divided into quintiles (Q), with the first being the least disadvantaged and the fifth being the most disadvantaged. The DI was successfully assigned to 80.2% of participants, while the quality of the registered information did not permit the linking of the remaining 19.8%.

The PRM is an index that is based on Adjusted Clinical Groups (ACG) [56], Diagnostic Cost Groups/Hierarchical Condition Categories (DCG-HCC) [57] and Clinical Risk Groups (CRG) [58]. This index combines information about diagnoses, prescriptions, previous costs and the use of specific procedures. It is capable of predicting the use of health resources [59] (), and it was stratified into four levels (L); the first included participants with a risk of high health resource consumption and the fourth included those with low health resource consumption. The PRM was successfully assigned to 95.1% of participants, while the quality of the registered information did not permit the linking of the remaining 4.9%.

The differences in these two indices (DI and PRM) between cases and controls were previously described (World Journal of Gastroenterology, in review process). Briefly, significant differences between the cases and the controls were found for DI and PRM, with a higher percentage of controls than cases in Q1-3 (the least disadvantaged) for DI, and a higher percentage of cases than controls in L1-2 (these levels included those with a risk of high health resource consumption) for PRM ($P<0.001$).

Biological samples and genotyping

In this survey, healthy tissues or saliva samples of 230 CRC patients and 230 controls were collected and genotyped. Samples were provided by the Basque Biobank for Research-OEHUN www.biobancovasco.org and were processed following standard operation procedures with appropriate ethical approval. DNA was extracted using AllPrep DNA/RNA kit (Qiagen) for paraffin-embedded tissue samples and AutoGenFlex Tissue DNA Extraction kit (Autogen) for mouthwash saliva samples, and then was analyzed with NanoDrop™ Spectrophotometer (ThermoFisher).

Double-stranded DNA was quantified by fluorometry using theQuant-iT™ PicoGreen1 dsDNA Assay Kit (Invitrogen, CA) on a DTX 880 Multimode Detector (Beckman Coulter) to normalize DNA concentration. SNPs were selected for analysis on the basis of published studies concerning: (1) SNPs associated with susceptibility for development of CRC [60,61]; and (2) associations between SNPs and food groups or dietary factors as well as gene-diet interactions in CRC. SNPs were organized in the context of the gene(s) at or near locus and chromosome locus. The allelic

discrimination was assessed using the MassARRAY1 System (Agena Bioscience) on CeGen-PRB2-ISCII (Nodo USC) following the procedure provided by the manufacturer. Quality control samples were included in the genotyping assays.

Regarding the susceptibility SNPs for CRC, after an updated summary of the published SNPs [60,61]; 48 previously reported CRC-susceptibility SNPs were selected and analyzed. The results of these SNPs were described in the manuscript of Alegria-Lertxundi *et al.* [17] . In summary, we have confirmed a CRC susceptibility locus and the existence of associations between modifiable factors and the rs6687758 SNP; moreover, the GRS was associated with CRC. In relation to the SNPs associated with food groups or dietary factors as well as gene-diet interactions in CRC, after a bibliographic review on the topic, 82 SNPs were selected and analyzed, results are currently under review.

Quality management

We applied a similar methodology of those used in the IDEFICS study [62]. A unique subject identification number was attached to each recording sheet, questionnaire, and sample, as in other researches. The identification number had to be entered twice before the document could be entered into its respective database. All data were entered twice independently, and deviating entries were corrected. Inconsistencies that were identified by additional plausibility checks were rectified.

Timeline

Consenting participants self-completed and returned questionnaires between 2014 and 2016. Interviews to obtain data about lifestyle changes after participating in the CRCSP were carried out between 2015 and 2016, both in cases and controls after returning self-reported questionnaires. The collection of data from clinical databases, as well as the obtaining of biological samples were made after receiving and reviewing the questionnaires.

Statistical analysis

Statistical analyses for the present paper were performed using SPP 22.0 (SPSS Inc, Chicago, USA). Categorical variables are shown as a percentage, and continuous variables are shown as the means and standard deviation (SD). Normality was checked using the Kolmogorov-Smirnov-Lilliefors test. Paired t-test or the Wilcoxon rank-sum test was used to two related means comparison. The categorical variables

were analyzed using the $\chi 2$ test or the Fisher exact test. All tests were two-sided, and *P* values less than 0.05 were considered statistically significant.

3. Results

Table 2 provides information regarding the demographic data, weight status, lifestyle (such as main daily activity, alcohol consumption, dietetic products and supplement use), drugs use, and quality of life and stress level. It should be noted that cases had a significantly higher prevalence of overweight/obesity than controls ($P<0.05$). Although the percentage of controls whose main daily activity was sedentary (sitting or standing) was higher compared to cases, these differences were not statistically significant. In addition, the use of dietary products and dietary or nutritional supplements was similar in cases and controls; being vegetable drinks the most common dietary product used and mineral supplements the most common nutritional supplement used. On the other hand, the use of antiplatelet agents was more frequent in cases than controls ($P<0.05$).

With respect to the weekly consumption of some food types, there were no significant differences between cases and controls in intakes of cooked vegetables, salads, fruit/fruit products, fish/fish products, and meat/meat products/meat dishes; neither in the consumption of fried foods nor grilled or roast meat nor in the frequency of use of added in table. However, a higher proportion of cases “always” or “often” used salt for cooking (76.8%) compared to controls (69.7%) ($P=0.036$). In any case, the average intake of Na from SFFQ was similar in cases and controls (Table 3).

Table 3 shows the daily energy and nutrients intake from diet and dietary products and supplements. Both in cases and controls, the average intake of protein and fat, especially SFA, expressed in percentage of the TEI was higher than NOSP; while, the average consumption of carbohydrate and dietary fiber was lower than NOSP. The cholesterol intake was lower than the NOSP. In particular, protein intake was higher than NOSP in 60.7 % of the sample, fat intake in 94.2%; and carbohydrate intake was lower than NOSP in 99.0%, and dietary fiber in 93.0%; there were no significant differences in any of these variables between cases and controls. Nevertheless, the percentage of cases whose consumption of SFA and cholesterol did not comply with NOSP was higher compared to controls (SFA: 85.7% vs. 67.5%, $P<0.001$; cholesterol: 35.4% vs. 25.0%, $P=0.009$).

The average intake of vitamin B_1, B_6/protein, cholesterol and caffeine were higher in cases than controls ($P<0.05$); while vitamin B_2 and the Ca/P ratio were higher in controls than cases ($P<0.01$). The percentage of cases whose vitamin B_2 intake did not

comply with NOSP was higher than controls (7.1% vs. 2.9%, *P*=0.029). Concerning the average consumption of caffeine, this was lower than the recommended limit in both cases and controls (400 mg/d).

On the other hand, the percentage of EAR (from diet and dietetic products and supplements) for Ca, Fe and vitamin B_2 was lower in cases than controls (*P*<0.05); while, the percentage of EAR for vitamin B_1 and B_6 was lower in controls than cases (P<0.05) (Table 4). The nutrients with the lowest proportions of subjects with intakes below EAR were: vitamin B_3 (4.8%), Fe (9.7%), vitamin B_{12} (21.4%) and P (23.7%); there were no significant differences between cases and controls in these percentages. The percentage of the total sample (cases and controls) that exceeded the UL was: 18.8% for Mg (of which 1% used supplements and/or dietetic products), 37.4% for Na and 47.7% for vitamin B_3 (of which 1.6% used supplements and/or dietetic products); there were no significant differences between cases and controls. In cases group, 0.9% exceeded the UL value for the vitamin B_6.

Finally, the average mineral score was 20.2(3.4) with a range between 14 and 28, without significant differences between cases and controls. The distribution in tertiles of the mineral score showed a greater proportion of controls (76%) than cases (24%) in the second tertile (*P*<0.001), and a greater proportion of cases than controls in the third tertile (59.6% vs. 40.0%, *P*< 0.001; respectively).

Table 1. Overview of the measurements and variables collected in this survey

Method	Measure of interest	Variables*
Self-reported data	Weight status	**Self-reported weight (kg) and height (cm).** ***BMI***
	Demographic data	**Age, sex.**
	Socio-economic data	Educational attainment, economic activity, last employment, total number of co-residents per household and total number of rooms, excluding the kitchen and bathrooms. *HCI.*
	Lifestyle	**PA** (main daily activity), PE *(at least 20 minutes* per session), smoking habit (yes or no, age at start, number of cigarettes per day, years without smoking), and **alcohol consumption** (frequency and type of alcohol, these items were included in the SFFQ). **Dietary habits: SFFQ, dietetic products and nutrient supplements consumption**. ***Energy intake, macro/micronutrients*** *(based on food composition tables)****, adequacy of energy and nutrients intake*** *(percentage of the EER, NOSP, AI and UL)****, mineral score*** *and diet quality index (HEISD and MDS). Adherence to guidelines for CRC prevention* (PA/PE, dietary fiber, red meat and processed meat, alcoholic beverages and BMI) (subsample randomly selected).
	Quality of life and stress	**Perceived quality of life and stress.**
	Drugs	**Antiplatelet, anticoagulants and HRT (females).**
Clinical databases	Date of participation in the *CRCSP*	Date of the iFOBT *Time spent between iFOBT and participation in this survey.*
	Diagnosis and treatment (cases)	Pathological staging, location of the cancer, differentiation, tumor grade and treatments (type and data of surgery, radiation therapy and/or chemotherapy). *Time spent between treatments and participation in this survey.*
	Socio-economic level	*DI*
	Health status (specifically health resource consumption)	*PRM*
Phone interviews	Lifestyle changes after participating in the CRCSP (subsample)	PA/PE, food consumption (vegetables, fruits, whole cereals, red meat, processed meat, alcoholic drinks) and smoking habits.
Genotyping	48 SNPs of susceptibility 82 SNPs that could be associated with food groups or dietary factors and gene-diet interactions	

To be continued in the next page.

Continuation of Table 1

AI: adequate intakes; BMI: body mass index; CRCSP: colorectal cancer screening program; DI: deprivation index; EER: estimated energy requirements; HCI: Household crowding index; HEISD: Healthy Eating Index for Spanish Diet; HRT: hormone replacement therapy; iFOBT: immunochemical fecal occult blood test; MDS, MedDietScore; NOSP: Nutritional objectives for the Spanish population; PA: physical activity; PE: physical exercise; PRM: predictive risk modelling; SFFQ: short food frequency questionnaire; SNP: single nucleotide polymorphism; UL: daily tolerable upper limits.

*Text in italics corresponds to data derived from direct measurements.

Results presented in this manuscript are highlighted in bold

Table 2. Demographic data, weight status, lifestyle, drugs use, quality of life and stress level in cases and controls studied

Variables, % or mean SD	Cases (n=308)		Controls (n=308)		*P*[a]
Sex, men	66.2		66.2		
Age, y	61.5	5.2	61.1	5.5	0.093
BMI, kg/m^2	27.5	4.4	26.8	4.4	**0.049**
Overweight/obesity	67.5		58.1		**0.015**
Main daily activity					
Sitting	31.8		40.1		
Standing	29.8		29.9		
Walking	34.1		28.6		
Activities that demanded great *physical effort*	4.3		1.3		0.079
Alcohol consumption, SDU	0.9	1.0	0.8	0.9	0.682
Non-compliance with recommendations, %	13.0		12.0		0.715
Dietetic products and supplement use[b]	9.7		13.0		0.306
Among those who consumed dietetic products and/or supplements[c]					
Dietetic products use	26.1		21.7		0.249
Milk/dairy[d]	28.6		16.7		
Vegetable drinks[e]	71.4		66.6		
Iodized salt	-		16.7		1.000
Nutrient supplements	73.9		13.0		0.306
Vitamins	19.4		15.9		
Minerals	29.0		27.3		

To be continued in the next page.

Continuation of Table 2

Variables, % or mean SD	Cases (n=308)		Controls (n=308)		*P*[a]
Complex vitamin-mineral products	12.9		13.6		
n-3 PUFA	16.1		22.7		
n-6 PUFA	6.5		-		
Fiber	16.1		20.5		0.701
Drugs					
Antiplatelet	18.5		13.7		**0.038**
Anticoagulants	1.3		1.3		0.359
HRT (females)[f]	16.0		16.7		0.585
QoL[g]	71.5	14.0	69.8	15.2	0.141
Stress level[g]	40.4	26.4	41.1	24.8	0.846

BMI: body mass index; HRT: hormone replacement therapy; PUFA: polyunsaturated fatty acids; QoL: quality of life; SD: standard deviation; SDU: standard drink unit.

[a]Differences between cases and controls. Significant results are highlighted in bold.

[b]Percentage calculated on the number of cases and controls.

[c]This question presented multiple answers. Percentage based on the number of answers obtained in each item.

[d]Enriched with calcium, omega-3, lactose-free milk

[e]Rice, oat or soy drinks.

[f]Percentage of females

[g]To assess the QoL and perceived stress an analog linear scale with a range from 0 to 100 was used

Table 3. Daily energy and nutrients intake (from diet and dietetic products and supplements) in cases and control studied

Daily intake from diet and dietetic products and supplements	Cases (n=308)		Controls (n=308)		P[a]
	Mean	SD	Mean	SD	
Energy, kcal/d	1774.3	388.0	1743.1	390.9	0.205
Macronutrients					
Protein, % TEI	15.7[b]	2.3	16.1	7.1	0.681
NOSP, 10–15% TEI					
Carbohydrates,% TEI	36.2	4.9	36.7	5.6	0.277
NOSP, 50-60% TEI					
Fat, %TEI	42.5	4.5	42.1	5.1	0.256

To be continued in the next page.

Continuation of Table 3

Daily intake from diet and dietetic products and supplements	Cases (n=308)		Controls (n=308)		*P*[a]
	Mean	SD	Mean	SD	
NOSP, <30-35% TEI					
SFA, % TEI	12.7	2.5	12.4	2.8	0.179
NOSP, <7–8% TEI					
MUFA, % TEI	19.6	2.7	19.7	2.8	0.631
NOSP, 20% TEI					
PUFA, % TEI	6.6	2.0	6.3	1.7	0.183
NOSP, 5% TEI					
Linoleic acid, % TEI	5.1	1.9	4.8	1.5	0.118
NOSP, 3% TEI					
α-linolenic acid, % TEI	0.7	0.2	0.7	0.2	0.422
NOSP, 1-2% TEI					
EPA, mg	334.7	192.8	329.9	209.9	0.542
DHA, mg	582.4	321.7	563.3	316.9	0.380
NOSP, 300 mg					
Minerals and electrolytes					
Ca, mg	759.4	238.0	780.6	227.0	0.225
NOSP, 800-1,000 mg					
P, mg	1220.2	314.5	1207.9	297.6	0.648
Ca/P[c]	0.6	0.1	0.6	0.1	**0.009**[b]
NOSP, 1,3/1					
Fe, mg	14.1	3.9	14.4	4.2	0.765
Mg, mg	263.1[b]	73.7	263.8	63.1	0.760
K, mg	2616.0	615.3	2627.7	610.2	0.954
I, μg	88.8	39.9	87.3	24.7	0.961
NOSP, 150 μg					
Na[d], mg	1950.8	1041.4	1820.1	1004.3	0.081
NOSP, <2,000 mg/d					
Se, μg	88.3	24.3	87.3	24.4	0.682
Cu, mg	1.0	0.3	1.0	0.3	0.568
Zn, mg	9.4	2.8	9.3	2.9	0.375
Vitamins					

To be continued in the next page.

Continuation of Table 3

Daily intake from diet and dietetic products and supplements	Cases (n=308)		Controls (n=308)		*P*[a]
	Mean	SD	Mean	SD	
Vitamin B_1[e], mg/1,000 kcal	1.0	7.6	0.6	0.2	**0.003**
NOSP, 0,4 mg/1,000 kcal					
Vitamin B_2[f], mg/1,000 kcal	0.9	0.2	0.9	0.2	**0.002**
NOSP, 0,6 mg/1,000 kcal					
Vitamin B_3, mg/1,000 kcal	17.1	3.3	17.2	3.3	0.117
NOSP, 6,6 mg/1,000 kcal					
Vitamin B_6 (mg)/protein (g)	0.04	0.2	0.03	0.01	**0.020**
NOSP, > 0.02 vitamin B_6 (mg)/protein (g)					
Folate, µg	267.2	80.1	273.3	76.5	0.406
NOSP, >300-400 µg					
Vitamin B_{12}[e], µg	6.7	28.5	4.9	1.7	0.094
Vitamin C, mg	149.3	66.0	147.8	59.9	0.611
Vitamin A, µg	532.4	206.9	522.0	181.4	0.420
Vitamin D, µg	2.1	1.0	2.3	1.9	0.799
NOSP (>50 y old), 10 µg					
Vitamin E, mg	0.6	0.1	0.6	0.1	0.139
NOSP, > 0.4 vitamin E (mg)/PUFA (g)					
Others					
Cholesterol, mg	274.7[b]	96.7	256.2	95.3	**0.019**
Fiber, g	19.9	6.5	20.1	6.0	0.459
Caffeine, mg	15.1	9.7	13.8	11.7	**0.025**
Water[g], ml	1062.3[b]	264.8	1069.9	261.2	0.769

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; NOSP, Nutritional objectives for the Spanish population; PUFA: polyunsaturated fatty acids; SD, standard deviation; SFA: saturated fatty acids; TEI: total energy intake.

[a]Differences between cases and controls. Significant results are highlighted in bold.

[b]This variable followed a normal distribution.

[c]The Ca/P ratio was 0.62(0.12) in cases and 0.65(0.13) in controls.

[d]Sodium from foods, does not include the amount of salt added

[e]This high SD is due to the use of nutritional supplements by one of the cases.

[f]Vitamin B_2 intake per 1,000 kcal was 0.86(0.23) in cases and 0.92(0.23) in controls.

[g]Intake of water from foods, but not from water from beverages.

Table 4. Energy and nutrients intake (from diet and dietetic products and supplements) expressed as a percentage of the estimated energy requirement (EER), the Estimated Average Requirements (EAR) or Adequate Intakes (AI) in cases and controls studied

% of energy requirement estimated, EARs or AI	Cases (n=308)		Controls (n=308)		P[a]
	Mean	SD	Mean	SD	
Energy	114.4	32.7	107.0	31.3	0.056
Minerals and electrolytes					
Ca	49.7[b]	11.1	52.7	13.4	**0.025**
P	118.9	18.1	120.1	18.5	0.469
Fe	142.5	35.7	147.5	36.4	**0.044**
Mg	47.5	13.2	48.1	12.0	0.094
K[c]	55.7	13.1	55.9	13.0	0.081
I	53.2	22.9	53.0	10.5	0.236
Na[c]	150.1	80.1	140.0	77.3	0.954
Se	111.9	24.6	112.3	24.6	0.598
Cu	80.6	13.4	81.1	13.1	0.360
Zn	64.0	19.5	64.1	18.7	0.938
Vitamins					
Vitamin B_1[d]	110.7	847.4	66.5	19.4	**0.003**
Vitamin B_2	84.6	25.5	89.9	24.8	**0.002**
Vitamin B_3	147.4	30.5	150.5	29.0.	0.121
Vitamin B_6	110.8	73.6	81.2	19.6	**0.010**
Folate	47.8	13.7	49.8	13.1	0.060
Vitamin B_{12}[d]	185.2	763.1	139.2	36.5	0.350
Vitamin C	124.0	55.5	124.5	51.7	0.865
Vitamin A	52.5	19.9	52.4	17.5	0.943
Vitamin D	11.9	5.7	13.1	11.9	0.956
Vitamin E	38.3	16.3	35.7	12.2	0.053

AI : adequate intakes ; EAR : estimated average requirements ; SD : standard deviation

[a]Differences between cases and controls. Significant results are highlighted in bold.

[b]This variable followed a normal distribution.

[c]Nutrient intake expressed as a percentage of the AI (the rest of the nutrients were expressed as a percentage of the EAR).

[d]This high SD is due to the use of nutritional supplements by one of the cases.

4. Discussion

This study was successful in obtaining a sample of cases and controls from the population-based CRCSP of the Basque Health Service (Osakidetza), all of whom agreed to participate in the full study protocol (self-reported data, clinical databases and genotyping). More men than women participated (1.96:1.0), and they were mostly elderly people (average age in cases= 61.5; and in controls=61.1 years), which was consistent with previous literature on CRCSP of the Osakidetza/Basque Health Service [63]. Although the average participation rate in this CRCSP was higher in women than men (70.9% vs. 65.6%), the proportion of CRC diagnosed was higher in men than in women (4.8% vs. 2.1%) [63].

With regard to the characteristics of the sample studied the prevalence of overweight/obesity was higher in cases than controls. This result is in agreement with previous studies [64-66] that have confirmed that obesity is associated with an increased risk of CRC. Even though the biological mechanisms underlying the association between body-fat in excess and CRC remain unclear [67], evidence seems to support the important role of metabolic syndrome, insulin resistance [68], systemic inflammation and immunity [69], microbial dysbiosis [70] , as well as certain genetic factors especially in early-onset CRC [71,72]. Elucidating the mediating role of these factors in obesity-induced CRC should be very useful in the prevention and treatment of this type of cancer. In addition to the direct contribution of obesity to CRC risk, body-fat in excess, in turn, could be associated to other risk factors for CRC, such as unhealthy diet and sedentary lifestyle [73,74] (). Notably, we also observed a slightly higher proportion of controls whose main daily activity was sedentary compared to cases, but this result could be influenced by a greater awareness of the associations between diseases and lifestyle factors among cases.

Regarding the diet, no significant differences were found for the food group studied, except for the frequency of use of salt added to cooking that was significantly higher in cases than controls. In other case-control studies, a positive association between sodium intake and CRC was also observed [75]. In any case, our average intake of Na from SFFQ was similar in cases than controls, probably due to the difficulty to estimate this intake from self-reported data on salt added [76].

From the nutritional point of view, the diet of participants, both cases and controls, was characterized by high intakes of protein, fat, SFA, and low intakes of carbohydrates and dietary fibre; thus, it was a western diet pattern. This dietary pattern has been associated

before with an elevated CRC incidence [77,78]. Moreover, the percentage of cases whose consumption of SFA and cholesterol did not comply with NOSP was higher than controls. This result is in agreement with those reported by other authors that have observed a higher CRC risk among subjects with high intake of both SFA and cholesterol (highest vs. lowest) [79]. Arafaet *et al.* [80] also reported a higher intake of saturated fats and cholesterol among CRC diagnosed subjects as compared to controls. The mechanisms involved in the influence of fat on the colorectal carcinogenesis is complex and appear to be related with its effect on the insulin-signal pathway and the c-Hun N-terminal kinase pathway that promote the colonic cell proliferation [81].

On the other hand, in the present study, we have not found a higher intake in controls than in cases of protective factors associated with a decrease in CRC risk according to the scientific literature, such as, for example, Ca, Mg, fiber diet, vitamin D, B_6 or regular use of certain drugs [9]. However, the average intake of vitamin B_2 and the Ca/P ratio was higher in controls than cases. Some studies have indicated before that vitamin B_2 intake is inversely associated with CRC risk [82]. Although this vitamin has received less attention than other ones, as protective factor of epithelial cancers (including CRC), the interest in vitamin B_2 is increasing due the role of flavins in folate metabolism and the possible synergistic protective effect between these two vitamins for cancer [83]. With respect to Ca/P ratio, Botron *et al.* [84] reported a case-control study in which they analyzed the possible association between this ratio and colorectal carcinogenesis, and found positive associations, but they did not observe any modulation by P intake of the association between dietary Ca intake and CRC.

Finally, on the contrary, the hypothesis that higher intakes of minerals with colon anti-carcinogenic effects, combined with lower intakes of those minerals with pro-carcinogenic effects may be associated with lower CRC risk was not supported. A greater proportion of controls than cases positioned in the second tertile of the minerals score, in contrast to what has been observed in the third tertile.

Strengths and limitations

The main strength is the fact that information is provided based on a standardised protocol including not only dietary and genetic factors, but also other possible determinants of CRC such as health determinants and weight status, among others. Another strength of this study compared to others [13-15] is that colonoscopy was used as diagnosis criteria to identify the cases in order to avoid false positives and negatives. However, there are some limitations that should be mentioned. First, recall bias inherent

in a case-control study design cannot be ruled out. Second, self-reported data could be subject to measurement errors and the problem of food omissions due to memory failure and under-reporting of unhealthy habits among disease subjects. However, previous validation studies indicate that the self-reported dietary information is reported with sufficient accuracy for use in epidemiology analysis [85]; and it should be noted that dietary changes are usually modest after participating in the CRCSP due to a lack of information and personalized advice [86,87]. Finally, to avoid selection bias of controls, we obtained controls from the same CRCSP and in the same period as cases, thus, it was confirmed that they did not suffer from CRC by colonoscopy.

5. Conclusions

This study provides valuable data for analyzing the complexity of gene-diet interaction in relation to CRC in a sample from a screening programme. These data include not only lifestyle and genetic determinants of CRC risk but also demographic, socio-economic data, weight status, perceived quality of life and stress, use of drugs related with decreasing CRC risk and lifestyle changes after participating in the CRCSP. Thus, this research provides valuable data for analyzing the determinants of this pathology and for designing prevention strategies. The authors hope that this report could help other researchers replicate this survey in other populations in order to easily and accurately compare their results. However, it is worth noting that some questionnaires include culturally sensitive topics, such as dietary habits and should be adapted to and validated in the population of interest. In addition, the results presented in this manuscript allow us to conclude that cases were more likely than controls to have overweight/obesity, a higher frequency of consumption of salt added for cooking, a lower intake of vitamin B_2 and Ca/P ratio, and not to have adequate intakes of SFA and cholesterol. Thus, some environmental factors, such as weight status and dietary components, could influence on the etiology of CRC in this population.

Funding: by two projects (from the Department of Health and Consumer Affairs, Basque Government 2011111153; and Saiotek, Basque Government S-PE12UN058), by a pre-doctoral grant from the Basque Government (PRE_2015_2_0084) and by the U.S. Department of Agriculture-Agricultural Research Service (ARS), under Agreement No. 58-1950-4-003. The Basque Government had no role in the design, analysis or writing of this article. CIBERehd is funded by the Instituto de Salud Carlos III.

Acknowledgments: The authors want to particularly acknowledge the patients who enrolled in this study for their participation and the Basque Biobank for Research-

OEHUN for its collaboration. The genotyping service was carried out at CEGEN-PRB2-ISCIII; it is supported by grant PT13/0001, ISCIII-SGEFI / FEDER.

Footnotes

Institutional review board statement: This study was approved by the Clinical Research Ethics Committee of the Basque Country (reference numbers PI2011006 and PI2014042).

Informed consent statement: Written informed consent was obtained from all the study participants.

Conflict-of-interest statement: The authors declare no conflict of interest.

References

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol.* **2019**;*14*:89-103. doi:10.5114/pg.2018.81072
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. **2018**;*68*:394-424. doi:10.3322/caac.21492
3. Ferlay J, Ervik M, Lam F, *et al.* Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer; Available from: https://gco.iarc.fr/today (Accessed on 02 November 2018)
4. Globocan 2018 at https://gco.iarc.fr/today/online-analysis-table?v=2018&mode=cancer&mode_population=continents&population=900&populations=908&key=asr&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&group_cancer=1&include_nmsc=1&include_nmsc_other=1#collapse-group-1-4-0 (accesed 02 November 2018)
5. Globocan 2018 at https://gco.iarc.fr/today/data/factsheets/populations/724-spain-fact-sheets.pdf) (accessed on 02 November 2018).
6. Lopez de Munain A, Audicana C. Cáncer en la Comunidad Autónoma de Euskadi 2001-2017. Departamento de Salud de Gobierno Vasco/Eusko Jaurlaritza. 2019. https://www.euskadi.eus/contenidos/informacion/registros_cancer/es_def/adjuntos/Cancer-CAE-2001-2017.pdf (accessed on 02 November 2018)

7. Roquette R, Painho M, Nunes B. Geographical patterns of the incidence and mortality of colorectal cancer in mainland Portugal municipalities (2007-2011). *BMC Cancer*. **2019**;*19*:512. doi:10.1186/s12885-019-5719-9
8. Bosetti C, Levi F, Rosato V, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Recent trends in colorectal cancer mortality in Europe. *Int J Cancer*. **2011**;*129*:180-91. doi:10.1002/ijc.25653
9. Thanikachalam K, Khan G. Colorectal Cancer and Nutrition. *Nutrients*. **2019**;11:164
10. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ, Watanabe T. Colorectal cancer. *Nat Rev Dis Primers*. **2015**;*1*:15065. doi:10.1038/nrdp.2015.65
11. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. 2017. Available from: https://www.wcrf.org/sites/default/files/Colorectal-cancer-report.pdf (accessed on 1 June 2019)
12. Kantor ED, Giovannucci EL. Gene-diet interactions and their impact on colorectal cancer risk. *Curr Nutr Rep*. **2015**;*4*:13-21. doi:10.1007/s13668-014-0114-2
13. Banqué M, Raidó B, Masuet C, Ramon JM. Food groups and nutrient intake and risk of colorectal cancer: a hospital-based case-control study in Spain. *Nutr Cancer*. **2012**;*64*:386-392. doi:10.1080/01635581.2012.657334.
14. Castelló A, Amiano P, Fernández de Larrea N, Martín V, Alonso MH, Castaño-Vinyals G, Pérez-Gómez B, Olmedo-Requena R, Guevara M, Fernandez-Tardon G, Dierssen-Sotos T, Llorens-Ivorra C, Huerta JM, Capelo R, Fernández-Villa T, Díez-Villanueva A, Urtiaga C, Castilla J, Jiménez-Moleón JJ, Moreno V, Dávila-Batista V, Kogevinas M, Aragonés N, Pollán M; MCC-Spain researchers. Low adherence to the western and high adherence to the mediterranean dietary patterns could prevent colorectal cancer. *Eur J Nutr*. **2019**;*58*:1495-1505. doi:10.1007/s00394-018-1674-5
15. Rosato V, Guercio V, Bosetti C, Negri E, Serraino D, Giacosa A, Montella M, La Vecchia C, Tavani A. Mediterranean diet and colorectal cancer risk: a pooled analysis of three Italian case-control studies. *Br J Cancer*. **2016**;*115*:862-5. doi:10.1038/bjc.2016.245
16. Idigoras I, Arrospide A, Portillo I, Arana-Arri E, Martínez-Indart L, Mar J, de Koning HJ, Lastra R, Soto-Gordoa M, van der Meulen M, Lansdorp-Vogelaar I. Evaluation of the colorectal cancer screening Programme in the Basque Country

(Spain) and its effectiveness based on the Miscan-colon model. *BMC Public Health*. **2017**;*18*:78. doi: 10.1186/s12889-017-4639-3.

17. Alegria-Lertxundi I, Aguirre C, Bujanda L, Fernández FJ, Polo F, Ordovás JM, Etxezarraga MC, Zabalza I, Larzabal M, Portillo I, de Pancorbo MM, Palencia-Madrid L, Rocandio AM, Arroyo-Izaga M. Single nucleotide polymorphisms associated with susceptibility for development of colorectal cancer: Case-control study in a Basque population. *PLoS One.* **2019**;*14*:e0225779. doi: 10.1371/journal.pone.0225779.
18. World Health Organization (WHO). Report of WHO consultation: Obesity: preventing and managing the-global epidemic.Geneva;2000
19. Silva Rodrigues RA, Martinez Espinosa M, Duarte Melo C, Rodrigues Perracini M, Rezende Fett WC, Fett CA. New values anthropometry for classification of nutritional status in the elderly. *J Nutr Health Aging*. **2014**;*18*:655-61. doi: 10.1007/s12603-014-0451-2.
20. Iles-Shih L, Collins JF, Holub JL, Lieberman DA. Prevalence of significant neoplasia in FOBT-positive patients on warfarin compared with those not on warfarin. *Am J Gastroenterol*. **2010**;*105*:2030-2035. doi:10.1038/ajg.2010.264.
21. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *JAMA*. **2005**;*294*:47-55. doi: 10.1001/jama.294.1.47.
22. Serebruany VL. Aggressive chronic platelet inhibition with prasugrel and increased cancer risks: revising oral antiplatelet regimens?. *Fundam Clin Pharmacol*. **2009**;*23*:411-417. doi:10.1111/j.1472-8206.2009.00710.x.
23. Johnson JR, Lacey JV Jr, Lazovich D, Geller MA, Schairer C, Schatzkin A, Flood A. Menopausal hormone therapy and risk of colorectal cancer. Cancer Epidemiol *Biomarkers Prev*. **2009**;*18*:196-203. doi: 10.1158/1055-9965.EPI-08-0596.
24. Zamora P, Espinosa E, Castelo B. *Valoración nutricional del paciente oncológico*. In: González Barron M, Lacasta MA, Ordoñez A Eds. Valoración clínica del paciente con cáncer. 1st edition; Médica Panamericana: Madrid, Spain, 2006; pp 83.
25. Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. [Validation of a short questionnaire on frequency of dietary intake: reproducibility and validity]. *Nutr Hosp*. **2008**;*23*:242-252.
26. Telleria-Aramburu N, Alegria-Lertxundi, Arroyo-Izaga M. Adaptation, validation and reproducibility of a short food frequency questionnaire to assess food group

intake in the population resident in the Basque Country (Spain). *Public Health Nutrition* **2020**; In press.

27. Alegria-Lertxundi I, Alvarez M, Rocandio AM, de Pancorbo MM, Arroyo-Izaga M. Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Postsurgery: A Pilot Study. *Nutr Cancer* **2016**; *68*: 577-88.
28. Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, Oakes S, Khaw KT, Wareham N, Day NE. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr*. **2001**;*4*:847-58. doi: 10.1079/phn2000102.
29. Mulligan AA, Luben RN, Bhaniani A, Parry-Smith DJ, O'Connor L, Khawaja AP, Forouhi NG, Khaw KT; EPIC-Norfolk FFQ Study. A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open*. **2014**;*4*:e004503. doi: 10.1136/bmjopen-2013-004503.
30. Carbajal A, Sánchez-Muniz FJ. Guía de prácticas. In: García-Arias, MC García-Fernández *Nutrición y dietética*. León: Secretariado de Publicaciones y Medios Audiovisuales, Universidad de León, Spain, 2003; pp 1-3.
31. Elika. *Estudio cuantitativo del consumo de alimentos en la CAPV*. Dpto. Agricultura, Pesca y Alimentación, Gobierno Vasco:Vitoria-Gasteiz, Spain, 2008; pp 1-111.
32. Dapchich V, Salador Castell G, Ribas Barba L, Pérez Rodrigo C, Aranceta Bartrina J, Serra Majem L. Guía de alimentación saludable. 1st ed..; Sociedad Española de Nutrición Comunitaria: Madrid; Spain, 2004; pp. 1-106.
33. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology*. **2015**;*148*:1244-60.e16. doi:10.1053/j.gastro.2014.12.035.
34. Schwingshackl L, Hoffmann G. Does a Mediterranean-Type Diet Reduce Cancer Risk?. *Curr Nutr Rep*. **2016**;*5*:9-17. doi:10.1007/s13668-015-0141-7.
35. Ortega RM, López-Sobaler AM, Andrés P, Requejo AM, Aparicio A, Molinero LM.DIAL software for assessing diets and food calculations (for Windows, version 8). Department of Nutrition (UCM) &Alce Ingeniería, S.L. Madrid, Spain, 2016 Available from: http://www.alceingenieria.net/nutricion/descarga.htm. Last access: [accesed: 20/10/2016]
36. U.S. Department of Agriculture, Agricultural Research Service. 2018. *USDA Food and Nutrient Database for Dietary Studies 2015-2016.* Food Surveys Research Group Home Page, http://www.ars.usda.gov/nea/bhnrc/fsrg (accessed on 15 December 2019).

37. Neveu V, Perez-Jimenez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford): bap024. www.phenol-explorer.eu (accessed on 15 December 2019).
38. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A*. **1918**;*4*:370-373. doi:10.1073/pnas.4.12.370.
39. Dwyer JT, Melanson KJ, Sriprachy-anunt U, Cross P, Wilson M. Dietary Treatment of Obesity. 2015 Feb 28. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Perreault L, Purnell J, Rebar R, Singer F, Trence DL, Vinik A, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000–. PMID: 25905223.Avalaible from: https://www.ncbi.nlm.nih.gov/books/NBK278991/ (accessed on 1 May 2020).
40. Carbajal A. Manual de Nutrición y Dietética. Universidad Complutense de Madrid, Spain, 2013. Available from: https://www.ucm.es/nutricioncarbajal/manual-de-nutricion [accessed on 1 May 2020].
41. Institute of Medicine of the National Academies: Dietary Reference Intakes. The Essential Guide to Nutrient Requirement. JJ Otten, JP Hellwig and LD Meyers, Eds. Washington, DC:The National Academy Press, 2006. Available from: http://www.nap.edu/catalog/11537/dietary-reference-intakes-the-essential-guide-to-nutrient-requirements (accessed on 15 March 2014)
42. World Health Organization, Food and Agriculture Organization of the United Nations: Vitamin and Mineral Requirements in Human Nutrition, 2nd ed. Geneva: World Health Organization and Rome/Food and Agriculture Organization of the United Nations, 2004. Available from: http://whqlibdoc.who.int/publications/2004/9241546123.pdf (accessed on 4 June 2014]
43. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington (DC): National Academies Press (US); 1997.
44. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington (DC): National Academies Press (US); 1998.

45. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington (DC): National Academies Press (US); 2000.
46. Institute of Medicine (US) Panel on Micronutrients. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington (DC): National Academies Press (US); 2001.
47. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US); 2011.
48. Reyes CM, Cornelis MC. Caffeine in the Diet: Country-Level Consumption and Guidelines. *Nutrients*. **2018**;*10*:1772. doi:10.3390/nu10111772
49. Swaminath S, Um CY, Prizment AE, Lazovich D, Bostick RM. Combined Mineral Intakes and Risk of Colorectal Cancer in Postmenopausal Women. *Cancer Epidemiol Biomarkers Prev*. **2019**;*28*:392-399. doi:10.1158/1055-9965.EPI-18-0412
50. Ministerio de Sanidad y Consumo (2017). *Informes de la Comisión Clínica*. Alcohol (Reports of the Clinical Commission. Alcohol). Available from: http://www.pnsd.msc.es/Categoria2/publica/pdf/InformeAlcohol.pdf (accessed July 2017 (accessed on 10 December 2019).
51. Aranceta J, Serra Majem L, Arija V, Gil A, Martínez de Vitoria E, Ortega R, *et al.* Objetivos nutricionales para la población Española Consenso de la Sociedad Española de Nutrición Comunitaria 2011. *Rev Esp Nutr Comunitaria*. **2011**;*17*:178–199.
52. Norte Navarro AI, Ortiz Moncada R. [Spanish diet quality according to the healthy eating index]. *Nutr Hosp*. **2011**;*26*:330-336. doi:10.1590/S0212-1611201100020001.
53. Panagiotakos DB, Milias GA, Pitsavos C, Stefanadis C. MedDietScore: a computer programme that evaluates the adherence to the Mediterranean dietary pattern and its relation to cardiovascular disease risk. *Comput Methods Programs Biomed*. **2006**;*83*:73-77. doi:10.1016/j.cmpb.2006.05.003.
54. Romaguera D, Vergnaud AC, Peeters PH, van Gils CH, Chan DS, Ferrari P, Romieu I, Jenab M, Slimani N, Clavel-Chapelon F, Fagherazzi G, Perquier F, Kaaks R, Teucher B, Boeing H, von Rüsten A, Tjønneland A, Olsen A, Dahm CC, Overvad K, Quirós JR, Gonzalez CA, Sánchez MJ, Navarro C, Barricarte A, Dorronsoro M, Khaw KT, Wareham NJ, Crowe FL, Key TJ, Trichopoulou A,

Lagiou P, Bamia C, Masala G, Vineis P, Tumino R, Sieri S, Panico S, May AM, Bueno-de-Mesquita HB, Büchner FL, Wirfält E, Manjer J, Johansson I, Hallmans G, Skeie G, Benjaminsen Borch K, Parr CL, Riboli E, Norat T. Is concordance with World Cancer Research Fund/American Institute for Cancer Research guidelines for cancer prevention related to subsequent risk of cancer? Results from the EPIC study. *Am J Clin Nutr.* **2012**;96:150-63. doi: 10.3945/ajcn.111.031674.

55. Domínguez-Berjón MF, Borrell C, Cano-Serral G, Esnaola S, Nolasco A, Pasarín MI, Ramis R, Saurina C, Escolar-Pujolar A. [Constructing a deprivation index based on census data in large Spanish cities (the MEDEA project)]. *Gac Sanit.* **2008**;*22*:179-87. doi: 10.1157/13123961. PMID: 18579042.
56. Johns Hopkins University, School of Public Health. The Johns Hopkins University ACG Case-Mix System. Available from: http://www.acg.jhsph.org/index.php?option=com_content&view=article&id=46&Itemid=61 (accessed on 10 August 2012)
57. Verisk Health's DxCGDCG_Methodology. Available from: http://www.dxcg.com/resources/library (accessed on 10 August 2012)
58. 3M™ Clinical Risk Grouping Software. Available from: http://solutions.3m.com/wps/portal/3M/en_US/3M_Health_Information_Systems/HIS/Products/CRG/ (accesed on 10 August 2012)
59. Orueta JF, Nuño-Solinis R, Mateos M, Vergara I, Grandes G, Esnaola S. Predictive risk modelling in the Spanish population: a cross-sectional study. *BMC Health Serv Res*. **2013**;*13*:269. doi: 10.1186/1472-6963-13-269.
60. Carethers JM, Jung BH. Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer. *Gastroenterology*. **2015**;*149*:1177-1190.e3. doi:10.1053/j.gastro.2015.06.047.
61. Real LM, Ruiz A, Gayán J, González-Pérez A, Sáez ME, Ramírez-Lorca R, Morón FJ, Velasco J, Marginet-Flinch R, Musulén E, Carrasco JM, Moreno-Rey C, Vázquez E, Chaves-Conde M, Moreno-Nogueira JA, Hidalgo-Pascual M, Ferrero-Herrero E, Castellví-Bel S, Castells A, Fernandez-Rozadilla C, Ruiz-Ponte C, Carracedo A, González B, Alonso S, Perucho M. A colorectal cancer susceptibility new variant at 4q26 in the Spanish population identified by genome-wide association analysis. *PLoS One*. **2014** *30*;9:e101178. doi: 10.1371/journal.pone.0101178.
62. Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, Hebestreit A, Krogh V, Lissner L, Mårild S, Molnár D, Moreno LA, Pitsiladis YP, Reisch L, Tornaritis M, Veidebaum T, Pigeot I; IDEFICS Consortium. The

IDEFICS cohort: design, characteristics and participation in the baseline survey. *Int J Obes (Lond)*. **2011**;*35* Suppl 1:S3-15. doi: 10.1038/ijo.2011.30.

63. Arana-Arri E, Idigoras I, Uranga B, Pérez R, Irurzun A, Gutiérrez-Ibarluzea I, Fraser CG, Portillo I; EUSKOLON Group. Population-based colorectal cancer screening programmes using a faecal immunochemical test: should faecal haemoglobin cut-offs differ by age and sex? *BMC Cancer*. **2017**;*17*:577. doi: 10.1186/s12885-017-3555-3.
64. Alsheridah N, Akhtar S. Diet, obesity and colorectal carcinoma risk: results from a national cancer registry-based middle-eastern study. *BMC Cancer*. **2018**;*18*:1227. doi:10.1186/s12885-018-5132-9.
65. Ulaganathan V, Kandiah M, Shariff ZM. A case-control study on the association of abdominal obesity and hypercholesterolemia with the risk of colorectal cancer. *J Carcinog*. **2018**;*17*:4. doi:10.4103/jcar.JCar_2_18.
66. Liu PH, Wu K, Ng K, Zauber AG, Nguyen LH, Song M, He X, Fuchs CS, Ogino S, Willett WC, Chan AT, Giovannucci EL, Cao Y. Association of Obesity With Risk of Early-Onset Colorectal Cancer Among Women. Version 2. *JAMA Oncol*. **2019**;*5*:37-44. doi: 10.1001/jamaoncol.2018.4280. Erratum in: JAMA Oncol. 2019 Apr 1;5(4):579.
67. Bardou M, Barkun AN, Martel M. Obesity and colorectal cancer. *Gut*. **2013**;*62*:933-947. doi:10.1136/gutjnl-2013-304701
68. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. **2006**;*444*:840-846. doi:10.1038/nature05482
69. Hanyuda A, Cao Y, Hamada T, Nowak JA, Qian ZR, Masugi Y, da Silva A, Liu L, Kosumi K, Soong TR, Jhun I, Wu K, Zhang X, Song M, Meyerhardt JA, Chan AT, Fuchs CS, Giovannucci EL, Ogino S, Nishihara R. Body mass index and risk of colorectal carcinoma subtypes classified by tumor differentiation status. *Eur J Epidemiol*. **2017**;*32*:393-407. doi: 10.1007/s10654-017-0254-y.
70. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z, Su L, Li X, Li X, Li J, Xiao L, Huber-Schönauer U, Niederseer D, Xu X, Al-Aama JY, Yang H, Wang J, Kristiansen K, Arumugam M, Tilg H, Datz C, Wang J. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun*. **2015**;*6*:6528. doi: 10.1038/ncomms7528.
71. Kirzin S, Marisa L, Guimbaud R, De Reynies A, Legrain M, Laurent-Puig P, Cordelier P, Pradère B, Bonnet D, Meggetto F, Portier G, Brousset P, Selves J. Sporadic early-onset colorectal cancer is a specific sub-type of cancer: a

morphological, molecular and genetics study. *PLoS One*. **2014**;*9*:e103159. doi: 10.1371/journal.pone.0103159.

72. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, Goldberg RM, Paskett E, Shields PG, Freudenheim JL, Stanich PP, Lattimer I, Arnold M, Liyanarachchi S, Kalady M, Heald B, Greenwood C, Paquette I, Prues M, Draper DJ, Lindeman C, Kuebler JP, Reynolds K, Brell JM, Shaper AA, Mahesh S, Buie N, Weeman K, Shine K, Haut M, Edwards J, Bastola S, Wickham K, Khanduja KS, Zacks R, Pritchard CC, Shirts BH, Jacobson A, Allen B, de la Chapelle A, Hampel H; Ohio Colorectal Cancer Prevention Initiative Study Group. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. *JAMA Oncol*. **2017**;*3*:464-471. doi: 10.1001/jamaoncol.2016.5194.
73. Kerr J, Anderson C, Lippman SM. Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. *Lancet Oncol*. **2017**;*18*:e457-e471. doi:10.1016/S1470-2045(17)30411-4
74. Keum N, Cao Y, Oh H, Smith-Warner SA, Orav J, Wu K, Fuchs CS, Cho E, Giovannucci EL. Sedentary behaviors and light-intensity activities in relation to colorectal cancer risk. *Int J Cancer*. **2016**;*138*:2109-2117. doi:10.1002/ijc.29953
75. Yakoob MY, Baig-ansari N. Dietary Sodium (salt) Intake and Risk of Colorectal Cancer: A Systematic Review (P05-039-19). *Curr Dev Nutr*. **2019**;*3*:nzz030.P05-039-19. doi: 10.1093/cdn/nzz030.P05-039-19. Corrigendum: Dietary Sodium (salt) Intake and Risk of Colorectal Cancer: A Systematic Review (P05-039-19). *Curr Dev Nutr*. **2019**;*3*:nzz098. doi:10.1093/cdn/nzz098.
76. Quader ZS, Zhao L, Harnack LJ, Gardner CD, Shikany JM, Steffen LM, Gillespie C, Moshfegh A, Cogswell ME. Self-Reported Measures of Discretionary Salt Use Accurately Estimated Sodium Intake Overall but not in Certain Subgroups of US Adults from 3 Geographic Regions in the Salt Sources Study. *J Nutr*. **2019**;*149*:1623-1632. doi: 10.1093/jn/nxz110.
77. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC. Major dietary patterns and the risk of colorectal cancer in women. *Arch Intern Med*. **2003**;*163*:309-14. doi: 10.1001/archinte.163.3.309.
78. Park Y, Lee J, Oh JH, Shin A, Kim J. Dietary patterns and colorectal cancer risk in a Korean population: A case-control study. *Medicine (Baltimore)*. **2016**;*95*:e3759. doi:10.1097/MD.0000000000003759
79. Tayyem RF, Bawadi HA, Shehadah IN, Abu-Mweis SS, Agraib LM, Bani-Hani KE, Al-Jaberi T, Al-Nusairr M, Heath DD. Macro- and micronutrients consumption

and the risk for colorectal cancer among Jordanians. *Nutrients*. **2015**;*7*:1769-86. doi: 10.3390/nu7031769.

80. Arafa MA, Waly MI, Jriesat S, Al Khafajei A, Sallam S. Dietary and lifestyle characteristics of colorectal cancer in Jordan: a case-control study. *Asian Pac J Cancer Prev*. **2011**;*12*:1931-1936.
81. Endo H, Hosono K, Fujisawa T, Takahashi H, Sugiyama M, Yoneda K, Nozaki Y, Fujita K, Yoneda M, Inamori M, Wada K, Nakagama H, Nakajima A. Involvement of JNK pathway in the promotion of the early stage of colorectal carcinogenesis under high-fat dietary conditions. *Gut*. **2009**;*58*:1637-43. doi: 10.1136/gut.2009.183624.
82. Ben S, Du M, Ma G, Qu J, Zhu L, Chu H, Zhang Z, Wu Y, Gu D, Wang M. Vitamin B_2 intake reduces the risk for colorectal cancer: a dose-response analysis. *Eur J Nutr.* **2019**;*58*:1591-1602. doi: 10.1007/s00394-018-1702-5.
83. Powers HJ. Interaction among folate, riboflavin, genotype, and cancer, with reference to colorectal and cervical cancer. *J Nutr*. **2005**;*135*:2960S-2966S. doi:10.1093/jn/135.12.2960S
84. Boutron MC, Faivre J, Marteau P, Couillault C, Senesse P, Quipourt V. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case--control study. *Br J Cancer*. **1996**;*74*:145-151. doi:10.1038/bjc.1996.330
85. Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Béghin L, Manios Y, Hallström L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA; HELENA Study Group. Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. *Int J Obes (Lond)*. **2008**;*32*:S26-34. doi: 10.1038/ijo.2008.180.
86. Anderson AS, Steele R, Coyle J. Lifestyle issues for colorectal cancer survivors--perceived needs, beliefs and opportunities. *Support Care Cancer*. **2013**;*21*:35-42. doi:10.1007/s00520-012-1487-7
87. Berstad P, Løberg M, Larsen IK, Kalager M, Holme Ø, Botteri E, Bretthauer M, Hoff G. Long-term lifestyle changes after colorectal cancer screening: randomised controlled trial. *Gut*. **2015**;64:1268-76. doi: 10.1136/gutjnl-2014-307376.

3.3. STUDY 3: "Food groups, diet quality and colorectal cancer risk in the Basque Country"

Name of Journal: *World Journal of Gastroenterology*
Manuscript NO: 55021
Manuscript Type: ORIGINAL ARTICLE

Case Control Study

Food groups, diet quality and colorectal cancer risk in the Basque Country

Alegria-Lertxundi I *et al.* Food groups, diet quality and colorectal cancer risk in the Basque Country

Iker Alegria-Lertxundi, Carmelo Aguirre, Luis Bujanda, Francisco Javier Fernández, Francisco Polo, José Mª Ordovás, Mª Carmen Etxezarraga, Iñaki Zabalza, Mikel Larzabal, Isabel Portillo, Marian M. de Pancorbo, Koldo Garcia-Etxebarria, Ana Mª Rocandio, Marta Arroyo-Izaga

Iker Alegria-Lertxundi, Marta Arroyo-Izaga, Department of Pharmacy and Food Sciences. Faculty of Pharmacy, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain.

Carmelo Aguirre, Pharmacovigilance Unit, Galdakao-Usansolo University Hospital, Osakidetza, Spain.

Luis Bujanda, Department of Gastroenterology, Donostia University Hospital / Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 20014 San Sebastian, Spain.

Francisco Javier Fernández, Department of Gastroenterology, Galdakao-Usansolo University Hospital, Osakidetza, Galdakao, Spain.

Francisco Polo, Department of Gastroenterology, Basurto University Hospital, Osakidetza, Bilbao, Spain.

José Mª Ordovás, Nutrition and Genomics Laboratory, Jean Mayer Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts, United States of America; IMDEA Food, Madrid, Spain.

Mª Carmen Etxezarraga, Department of Pathology, Basurto University Hospital, Osakidetza, Bilbao, Spain. Department of Physician and Surgeon Specialities, University of the Basque Country, Leioa, UPV/EHU.

Iñaki Zabalza, Department of Pathology, Galdakao-Usansolo University Hospital, Osakidetza, Galdakao, Spain.

Mikel Larzabal, Department of Pathology, Donostia Hospital / Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 20014 San Sebastian, Spain.

Isabel Portillo, Colorectal cancer screening programme, Osakidetza, Bilbao, Spain.

Marian M. de Pancorbo, Ana Mª Rocandio, BIOMICs Research Group, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain

Koldo Garcia-Etxebarria, Biodonostia, Group of Gastrointestinal Genetics; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), 20014 San Sebastian, Spain.

Supported by two projects (from the Department of Health and Consumer Affairs, Basque Government 2011111153; and Saiotek, Basque Government S-PE12UN058), by a pre-doctoral grant from the Basque Government (PRE_2015_2_0084). Basque Government had no role in the design, analysis or writing of this article.

Abstract

BACKGROUND

The results obtained to date concerning food groups, diet quality and colorectal cancer (CRC) risk vary according to criteria used and the study populations.

AIM

To study the relationships between food groups, diet quality and CRC risk, in an adult population of the Basque Country (North of Spain).

METHODS

This observational study included 308 patients diagnosed with CRC and 308 age- and sex-matched subjects as controls. During recruitment, dietary, anthropometric, lifestyle,

socioeconomic, demographic and health status information was collected. Adherence to the dietary recommendations was evaluated utilizing the Healthy Eating Index for the Spanish Diet and the MedDietScore. Conditional logistic regressions were used to evaluate the associations of food group intakes, diet quality scores, categorized in tertiles, with CRC risk.

RESULTS

The adjusted models for potential confounding factors showed a direct association between milk/dairy products consumption, in particular high-fat cheeses (OR third tertile *vs* first tertile=1.87, 95% CI 1.11-3.16), and CRC risk. While the consumption of fiber-containing foods, especially whole grains (OR third tertile *vs* first tertile=0.62, 95% CI 0.39-0.98), and fatty fish (OR=0.53, 95% CI 0.27-0.99) was associated with a lower risk for CRC. Moreover, higher MD adherence was associated with a reduced CRC risk in adjusted models (OR=0.40, 95% CI 0.20-0.80).

CONCLUSION

Direct associations were found for high-fat cheese, whereas an inverse relation was reported for fiber-containing foods and fatty fish, as well as adherence to a Mediterranean dietary pattern.

Keywords: Colorectal cancer; Food group; Dietary quality; Mediterranean diet; Risk-factors; Case-control study

Core tip: This matched case-control study supports the role of diet in colorectal cancer (CRC) risk. The results suggest that high consumption of high-fat cheeses is associated with CRC risk, whereas, a high intake of fiber-containing foods, especially whole grains, and fatty fish, as well as adherence to the Mediterranean dietary pattern, was associated with a lower risk for CRC. Future studies are needed to better understand the influence of the dietary habits on CRC prevention in this population that can provide leads for the design and tailoring of future interventions, and guide counselling strategies for promoting a healthy lifestyle.

INTRODUCTION

Colorectal cancer (CRC) is a major public health challenge worldwide. CRC is the third-most commonly diagnosed malignancy and the fourth leading cause of cancer deaths in the world, accounting for approximately 1.8 million new cases and almost 900,000 deaths in 2018[1]. In Europe, CRC is the leading malignancy in terms of incidence and the second in mortality in both sexes[2]. CRC is linked to western lifestyles, in particular, to diet, physical inactivity, smoking, alcohol consumption, and body weight[3,4].

Epidemiological evidence suggests that dietary factors may both protect against and promote the development of CRC. A comprehensive review[5] shows robust evidence about the protective role of dietary fiber. Other foods, such as milk or garlic, also may be protective. Conversely, red meat and processed meat intake and alcoholic drinks increase CRC risk. This food group approach has the advantage of reducing some of the problems inherent to analyses of nutrient intake (*e.g.,* inaccuracy and incompleteness of food-composition tables). Furthermore, it offers an advantage from a preventive perspective since food group results are easier to transform into dietary recommendations than those of nutrients[6].

In this regard, foods are not consumed in isolation but as part of a dietary pattern; therefore, the actual effect of diet on disease risk may be observed only when all components are considered jointly[6]. For this purpose, several diet quality indexes have been developed using point systems to measure whole diet quality based on the alignment of food choices with dietary recommendations. Some of these indices have been used to begin assessing the relationships between overall diet quality and CRC risk, and the results show that high scores in these indices are associated with a lower CRC risk[7-10]. However, the results vary considerably according to the index used and other factors such as sex and age. Therefore, there is a need to further examine these relationships in diverse population studies.

The current case-control study was undertaken in the North of Spain to elucidate the relationships between food group consumption, diet quality and CRC risk, and identify possible differences in consumption depending on tumor location, in an adult population that participated in a CRC screening programme (CRCSP) in the Basque Country. To our knowledge, this is the first study in the Basque country population, in which both CRC incidence and mortality have increased in recent years[11]. There are few studies in this regard in Spain[12,13]. And both in these Spanish studies and in others carried out in

other Mediterranean countries controls were apparently healthy subjects without clinical symptoms or signs of any type of cancer[14].

MATERIALS AND METHODS

Study subjects

This is an observational, matched case-control study in a population group residing in the Basque Country (North of Spain). Participants in this study were recruited from among patients attending any of the three hospitals of the Osakidetza/Basque Health Service (Basurto, Galdakao and Donostia) members of the Basque Country CRCSP. To be eligible for this CRCSP, the patients had to be aged between 50 and 69, asymptomatic for colorectal symptoms and registered with the Osakidetza/Basque Health Service[11]. These inclusion criteria were applied to both case and control group, that is, controls fulfilled the same eligibility criteria defined for the cases, with the exception of the disease (outcome). Recruitment and data collection for the present study were conducted between 2014 and 2016.

All the patients who were newly diagnosed with CRC (n=601) were invited to participate in this study. Of those, 283 refused to participate in the study, and 10 were excluded due to missing information. Ultimately, 308 subjects (66.2% men) consented to participate in the survey and completed all the questionnaires. In addition, for each case, three age- (±9.0 years) and sex-matched control patients were randomly sought from the list of CRC-free subjects (n=1,836) who participated in the CRCSP during the same period as the cases. The matched controls were patients with positive results (abnormal) for immunochemical fecal occult blood test (iFOBT) and negative colonoscopy results (normal). The participation rate of the controls was 37.6%, and 17 subjects were excluded due to missing information. Finally, the matched case-to-control ratio was 1:1, and the final data set included 308 cases who were diagnosed with CRC and 308 age- and sex-matched controls. Further details on recruitment and data collection have been described elsewhere[15]. The main advantage of the present study compared to other above-mentioned researches[12-14] is that we confirmed that controls were free of the disease through colonoscopy. Colonoscopy was used as diagnostic criteria to identify the cases in order to avoid false positives and negatives.

The pathological staging was based on the 7th edition of the AJCC cancer staging manual[16] as follows: I (57.1%), IIA (13.6%), IIB (1.0%), IIC (0.3%), IIIA (7.5%), IIIB (14.6%), IIIC (1.9%), IVA (2.9%), and IVB (1.0%). The location of the cancer was distal in 76% and proximal (to the splenic flexure of the colon) in 24.0% of the samples.

Concerning the tumor grade classification, we adopted a two-grade classification that was divided into low grade (well or moderately differentiated) (80.5%) and high grade (poorly differentiated, anaplastic, or undifferentiated) (4.5%); the percentage of missing data for this classification was 14.9%.

Some of the cases had undergone surgical resection (73.7%) and/or adjuvant treatments, chemotherapy (34.1%), and chemotherapy and radiation (6.8%). The percentages of subjects according to the type of surgical procedure were as follows: 26.3% sigmoidectomy, 17.5% right hemicolectomy resection, 18.8% low anterior resection, 6.5% left hemicolectomy resection, 2.3% transverse colectomy, 1.0% abdominoperineal resection, 1.0% total colectomy, and 0.3% transanal endoscopic operation. The cases were invited to take part in this survey at least one month after finishing their last treatment (surgery, chemotherapy or radiotherapy) (median, 1.3 years; range, 0.1 to 4.2 years). All the clinical data were obtained from the Basque Country's population-based CRCSP database, which links patient medical records and clinical databases and reviewed by expert staff. This review allowed the monitorization of all cases from the submission of the sample through the analysis, colonoscopy, pathology and follow-up.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the Clinical Research Ethics Committee of the Basque Country (reference numbers PI2011006 and PI2014042). Written informed consent was obtained from all the study participants. Consenting participants self-completed and returned a detailed food frequency questionnaire (FFQ) and one general questionnaire (GQ). The questions referred to the behaviors before participating in the CRCSP. Assistance from the study staff was available to help the patients to understand the items on the questionnaires. The quality management applied in the present study has been described in a previous article[15].

Dietary assessment

Diets were assessed using a short FFQ that was a modified version of the Rodríguez *et al*[17] (2008) questionnaire. This adaptation was validated with multiple 24-h recalls in the Basque general population[18] and in CRC diagnosed patients in a pilot of the present study[19]. It consists of 67 items and requires the subjects to recall the number of times each food item was consumed either per week or per month. This FFQ included specific questions about the frequency of intake of alcoholic beverages. Moreover, the

respondents could also record the consumption of other foods that were not included on the food list.

Consumption frequencies were standardized to "per day" and multiplied by standard serving sizes (grams)[20]. For items that included several foods, each food's contribution was estimated with weighting coefficients that were obtained from the usual consumption data[21]. Food items were then regrouped according to nutritional characteristics[22] and considering the potential contribution of food to the pathogenesis of CRC[23,24]. Details on the items included in each food group are shown in Table 1. All food items that were consumed were entered into DIAL 2.12 (2011 ALCE INGENIERIA)[25], a type of dietary assessment software, to estimate energy intake *(kilocalories/day, kcal/d).*

Adherence to the dietary recommendations was evaluated utilizing the Healthy Eating Index for Spanish Diet (HEISD)[26] and the MedDietScore (MDS)[27], as previously described[19].The theoretical range of the HEISD is 0-100 and of the MDS 0-55, higher values of these scores indicate greater adherence to the dietary recommendations for the Spanish population and the Mediterranean diet pattern, respectively. HEISD was divided into the following categories: poor diet (<50 points), needs improvement (50-80 points) and proper diet (>80 points)[26]; and the MDS into the following ones: low adherence to MD (0-34 points) and high adherence (>35 points). The cut-off point of MDS was established taking into account that scores below 34 points were associated with a higher risk of coronary heart disease, being the relative odds $\geq$1.42[27].

General questionnaire

A general questionnaire was used to gather information on weight status (self-reported weight and height) and environmental factors (demographic factors: age and sex; and lifestyle information: physical exercise (PE) and smoking consumption). These questions were taken from the Spanish Health Questionnaire[28]. Body mass index (BMI) estimated from self-reported height and weight was classified according to the WHO criteria for those under 65 years of age[29] and according to the criteria proposed by Silva Rodríguez *et al*[30] (2014) for those 65 years and older.

Additionally, socioeconomic and health status data were assessed with two indices that were obtained from the clinical databases developed by the Health Department of the Basque Government, namely the socioeconomic deprivation index (DI) and predictive risk modelling (PRM), respectively. The first one was estimated using the MEDEA project criteria[31], as has been described elsewhere[12] and was divided into quintiles (Q), with the first being the least disadvantaged and the fifth being the most disadvantaged. The

DI was successfully assigned to 80.2% of participants, while the quality of the registered information did not permit the linking of the remaining 19.8%.

The PRM is an index that is based on Adjusted Clinical Groups (ACG)[32], Diagnostic Cost Groups/Hierarchical Condition Categories (DCG-HCC)[33] and Clinical Risk Groups (CRG)[34]. This index combines information about diagnoses, prescriptions, previous costs and the use of specific procedures. It is capable of predicting the use of health resources[35], and it was stratified into four levels (L); the first included participants with a risk of high health resource consumption and the fourth included those with low health resource consumption. The PRM was successfully assigned to 95.1% of participants, while the quality of the registered information did not permit the linking of the remaining 4.9%.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, USA) and STATA 13.0 (StataCorp LP, Texas, USA). Categorical variables are shown as a percentage, and continuous variables are shown as the means and standard deviations (SD). Normality was checked using the Kolmogorov-Smirnov-Lilliefors test. Differences between continuous variables were calculated with a Wilcoxon test, and a McNemar's test was used for categorical variables.

Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) for CRC risk according to tertiles of food group intakes and diet quality scores for unadjusted and adjusted models. Intake of all food groups and total diet quality scores were categorized into tertiles by the distribution in the control population, taking into account sex differences when they were significant. The lowest tertile was used as the reference group. Tertile cut-offs for HEISD were: 1st tertile (T_1), 69; 2nd tertile (T_2), 69-74.5 and 3rd tertile (T_3), >74.5; and for MDS: T_1, <35; T_2, 35-37 and T_3, >37.

Based on known risk factors for CRC[36,37,38], covariates in adjusted models included age, sex, weight status, energy intake, PE level, smoking status, intensity of smoking (in current and past smokers) and time not smoking (in past smokers), DI and PRM. Quantitative covariates (cigarettes/d and years not smoking) were dichotomized by mean or median, according to the normality test. We used the cut-off of Romaguera *et al.* (2012)[39] to create two PE levels expressed in *minutes/day* (min/d) of cycling/sports: sedentary-light (<15 min/d) and moderate-vigorous (≥15 min/d). Age was dichotomized using the same age ranges that were used in the sample selection process (50-59 years

old *vs* 60-69 years old). Qualitative ones, such as DI and PRM were dichotomized taking into account the distribution of frequencies to obtain similar sample sizes for each category (DI, Q_{1-3} *vs* Q_{4-5}; PRM, L_{3-4} *vs* L_{1-2}). Energy intake was included as a quantitative variable in the adjusted models. We included participants with missing data for the covariates as a separate category. The reference categories were those that, according to the literature, have a lower CRC risk. All tests were 2-sided, and *P*-values less than 0.05 were considered statistically significant.

RESULTS

Comparisons of general characteristics between the cases and the controls are presented in Table 2. Significant differences between the cases and the controls were found for educational level, smoking and weight status, with a higher percentage of cases with low-medium educational level, past or current smoking status and with overweight/obesity compared to the controls ($P<0.01$).

Table 3 shows food group intakes expressed as mean values and SD according to case-control status. No significant differences were found between the two groups for the majority of foods groups, except for a higher consumption of eggs and a lower intake of whole grains in the cases than the controls ($P<0.05$).

The ORs for CRC risk by the main food group and food subgroup intakes are presented in Table 4 and 5, respectively. The adjusted ORs for CRC risk increased with higher red and processed meat, eggs, milk/dairy products intakes; whereas it decreased with higher fiber-containing foods and nut intakes. The food group with the highest adjusted OR for CRC risk was milk/dairy products. Fish consumption showed an association with CRC risk in the unadjusted analysis but not in the adjusted analysis. For some of these food groups, specifically for red and processed meat, fish, eggs and nuts, the null value 1 was contained in the confidence interval. Concerning the food subgroup intakes, the ORs for CRC risk increased with higher high-fat cheese intakes, while it decreased with higher fatty fish, in the adjusted analysis.

Supplementary Table 1 describes food group intakes of cases according to tumor location and their matched controls. Food group intakes were not substantially different between proximal and distal cancer cases, except for fish, milk/dairy products and fat. The fish consumption was higher in both case subgroups (proximal and distal cancer cases) in comparison with their matched controls ($P<0.001$). However, the milk/dairy products intake was higher in proximal tumor cases and was lower in distal tumor cases than in their matched controls ($P<0.001$). Finally, the fat intake was higher in proximal

tumor cases in comparison with their matched controls (P<0.05). The sample sizes did not allow the assessment of food group intakes related to disease risk, stratifying according to the tumor location.

The components and total scores of the HEISD and MDS are displayed in Table 6. According to HEISD, 91.9% of the participants (cases and controls) followed a diet classified as “needs improvement”, 7.6% followed a “good diet” and 0.5 followed a “poor diet”. Significant differences were neither observed in the HEISD classification nor the components scores nor in the total score. However, the total score for this dietary quality index and the score of diet variety components were higher for cases with the proximal location of cancer than for their matched controls (P<0.05) (Supplementary Table 2). No association was found between this index and risk of CRC, in the conditional logistic regressions.

Concerning the MDS, in the total sample, 39.8% showed low adherence to the MD and the remaining percentage had high adherence, without significant differences in the MDS classification between the cases and the controls. However, the scores for whole grains and total index were lower for cases than for controls (P<0.05). This last result was confirmed using conditional logistic regressions, showing that those participants with higher MDS had a lower CRC risk than those with a lower score, in both unadjusted (model I: T_3 *vs* T_1, OR 0.57, 95% CI 0.37-0.89, *P*=0.013) and adjusted models (model II: T_3 *vs* T_1, OR 0.40, 95% CI 0.20-0.80, *P*=0.009). No significant differences were observed in total MDS between cases stratified by tumor location and their matched controls, but the total score was higher for cases with the proximal location of cancer than for those with distal location (P<0.05) (Supplementary Table 2). Moreover, the score for potatoes and whole grain components were lower for cases with the distal location of cancer than for their matched controls (P<0.05).

Table 1. Food group definitions

Food group	Food items
Red and processed meat	
Red meat	Beef, pork and lamb, minced meat, hamburgers, meatballs...
Processed meat	Ham, sausage, salami, mortadella, black pudding or blood sausage...
Egg	Egg
Fish	White fish (hake, grouper, sole, cod) and fatty fish (sardine, tuna, salmon, mackerel)
Milk/dairy products	
Non-cheese products	Whole milk, semi-skimmed milk, skimmed milk, whole yogurt, skimmed yogurt and dairy desserts
Cheese	Burgos cheese, curd, cottage and cheeses low in calories, mature, semi-mature and creamy cheese
Fiber-containing foods	
Fruits	Orange, tangerine, apple, pear, banana, peach, raisins, prunes, dried figs... natural fruit juices
Vegetables	Salads, green beans, chard, spinach... garnish vegetables (eggplant, mushrooms, peppers...), garlic, onion
Whole grains	Whole grain pasta, brown rice, whole grain cookies, whole breakfast cereals (Muesli, All-Bran)...
Nuts	Walnuts, almonds, hazelnuts, peanuts...
Fat	Vegetable oils (olive, sunflower, corn, soy), butter, margarine, mayonnaise...
Sweet and added sugar	Chocolate, breakfast cereals, cookies, muffins, donuts, honey, sugar, commercial fruit juice, soft-drinks, cakes, pies...
Alcoholic beverages	Beer, wine, hard cider, vermouth, whiskey, rum, gin, brandy, cocktails...

Table 2. General characteristics of the sample studied

Characteristics	**Cases (*n*=308)**		**Controls (*n*=308)**		***P***
Sex, men, *n*(%)	204(66.2)		204(66.2)		
Age, y, mean SD	61.5	5.2	61.1	5.5	0.093
Schooling, %					
No education/primary education	36.7		29.2		
Technical/secondary education	48.0		44.5		
University degree	15.3		26.3		0.005
Economic activity, %					
Working	27.9		32.1		
Unemployed	5.2		3.2		
Retired	58.8		56.2		
Housework	8.1		8.4		0.496
Last work, %					
Employer or businessman/women	19.2		17.9		
Steady salaried employee	75.0		71.8		
Temporary salaried employee or member of a cooperative	0.6		4.5		
Household help and other activities without salary	5.1		5.8		0.073
Smoking status, %					
Never	27.9		38.6		
Past/current	72.1		61.4		0.004
Time to quit smoking					
≥ 11 y	67.2		66.7		
< 11 y	32.8		33.3		0.931
Intensity of smoking[1]					
≤15 cigarettes/d	50.7		33.1		
>15 cigarettes/d	49.3		66.9		0.003

To be continued in the next page.

Continuation of Table 2.

Characteristics	**Cases (*n*=308)**		**Controls (*n*=308)**		***P***
Physical exercise, %					
< 15 minutes/d of cycling/sports	79.2		65.9		
≥ 15 minutes/d of cycling/sports	20.8		34.1		<0.001
BMI, %					
Underweight	6.5		7.8		
Normal weight	26.0		34.1		
Overweight/obesity	67.5		58.1		0.033
Energy intake (kcal/d), mean SD	1769.9	383.4	1736.6	388.2	0.172
DI,%[2]					
Q_{1-3}	47.1		65.6		
Q_{4-5}	18.8		29.5		<0.001
PRM,%[2]					
L_{1-2}	15.6		12.3		
L_{3-4}	83.4		79.2		<0.001

[1]Percentages were calculated excluding never smokers;

[2]Valid percentages. BMI: body mass index; d: day; DI: deprivation index (this index was successfully assigned to 80.2% of the study sample); L: level; PRM: predictive risk modelling (this index was successfully assigned to 95.1% of the study sample); Q: quintile; SD: standard deviation; y: years.

Table 3. Food group intakes of the sample studied

Food groups, g/d	Cases (n=308)		Controls (n=308)		*P*
	Mean	SD	Mean	SD	
Red and processed meat	70.9	36.6	66.0	39.7	0.064
Red meat	49.7	30.5	46.1	31.0	0.130
Processed meat	21.2	16.6	19.9	17.2	0.155
Total fish	76.8	39.2	77.6	40.9	0.540
White fish	40.9	25.2	44.1	27.4	0.055
Fatty fish	35.9	22.6	33.6	24.1	0.236
Eggs	20.8	12.7	18.7	11.5	0.038
Milk/dairy products	264.7	153.4	271.0	119.4	0.310
Non-cheese dairy products	246.0	152.4	253.8	118.5	0.203
Total cheeses	18.8	17.4	17.1	16.8	0.172
Fresh cheeses[1]	6.9	10.3	7.1	13.3	0.867
Other cheeses[2]	11.7	11.8	10.1	10.7	0.172
Fiber-containing foods	570.3	243.9	564.8	214.1	0.761
Fruits (including natural juices)	330.2	202.5	322.6	168.2	0.791
Vegetables	202.1	88.8	200.6	90.9	0.803
Whole grains	14.4	19.9	18.8	23.4	0.012
Fat	35.5	6.9	34.6	6.4	0.064
Nuts	9.1	10.1	10.9	10.5	0.055
Sweets and added sugar	108.3	95.4	110.7	116.5	0.969
Alcoholic beverages	103.4	100.7	96.8	105.9	0.269

[1]Fresh cheeses, *e.g.*, Burgos cheese and cheeses low in calories;

[2]Other cheeses, mature, semi-mature and creamy cheeses. g/d: grams/day; SD: standard deviation.

Table 4. Association between main food group in and colorectal cancer risk

Main food group intakes[1]	No. Case/Control	Model I[2]	Model II[3]	Model III[4]
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Red and processed meat				
T_1	90/102	1.00	1.00	1.00
T_2	97/103	1.06(0.72-1.57)	1.02(0.61-1.72)	1.08(0.61-1.94)
T_3	121/109	1.32(0.91-1.93)	1.65(0.99-2.75)	1.26(0.71-2.23)
P		0.314	<0.001	-
Fish				
T_1	95/105	1.00	1.00	1.00
T_2	77/103	0.82(0.53-1.25)	0.97(0.56-1.68)	0.83(0.46-1.51)
T_3	136/105	1.49(1.01-2.20)	1.06(0.62-1.79)	1.25(0.68-2.29)
P		0.008	<0.001	-
Eggs				
T_1	71/98	1.00	1.00	1.00
T_2	107/116	1.15(0.77-1.72)	1.04(0.62-1.76)	0.97(0.61-1.93)
T_3	130/104	1.55(1.03-2.33)	1.72(1.00-2.94)	1.26(0.71-2.23)
P		0.081	<0.001	
Milk/dairy products				
T_1	60/102	1.00	1.00	1.00
T_2	127/104	2.05(1.35-3.11)	2.02(1.19-3.42)	1.97(1.10-3.53)
T_3	121/102	2.00(1.31-3.05)	2.12(1.25-3.84)	1.80(0.95-3.42)
P		<0.001	<0.001	-
Fiber-containing foods				
T_1	121/102	1.00	1.00	1.00
T_2	75/101	0.60(0.39-0.92)	0.47(0.26-0.85)	0.49(0.25-0.95)
T_3	112/105	0.86(0.58-1.28)	0.63(0.36-1.11)	0.65(0.35-1.21)
P		0.048	<0.001	-
Nuts				
T_1	121/102	1.00	1.00	1.00
T_2	75/101	0.60(0.39-0.92)	0.47(0.26-0.85)	0.49(0.25-0.95)
T_3	112/105	0.86(0.58-1.28)	0.63(0.36-1.11)	0.65(0.35-1.21)

To be continued in the next page.

Continuation of Table 4.

Main food group intakes[1]	No. Case/Control	Model I[2]	Model II[3]	Model III[4]
		OR (95% CI)	OR (95% CI)	OR (95% CI)
P		0.048	<0.001	-
Fat				
T_1	86/100	1.00	1.00	1.00
T_2	101/105	1.12(0.75-1.67)	0.94(0.56-1.59)	0.83(0.45-1.51)
T_3	121/100	1.34(0.92-1.97)	1.46(0.85-2.50)	1.25(0.68-2.29)
P		0.297	<0.001	-
Sweets and added sugar				
T_1	82/120	1.00	1.00	1.00
T_2	120/103	1.47(0.99-2.20)	1.67(0.98-2.86)	1.88(1.01-3.52)
T_3	106/103	1.30(0.87-1.94)	1.63(0.92-2.89)	1.39(0.72-2.67)
P		0.159	<0.001	-
Alcoholic beverage				
T_1	90/103	1.00	1.00	1.00
T_2	107/101	1.20(0.81-1.77)	1.05(0.63-1.75)	1.10(0.63-1.92)
T_3	111/104	1.19(0.83-1.72)	0.82(0.50-1.36)	0.75(0.42-1.32)
P		0.558	<0.001	-

[1]Food groups consumption was categorized into tertiles according to the distribution in controls, and by sexes for food groups with significant differences according to sex; Tertiles of food groups: red and processed meat, T_1<47.7 grams/day, T_2 47.7-78.5, T_3>78.5; total fish, T_1<42.8, T_2 42.8-67.2, T_3>67.2; eggs, T_1<15.7, T_2 15.7-23.5, T_3>23.5; milk/dairy products, T_1<72.0, T_2 72.0-232.1, T_3>232.1;fat, T_1<30.8, T_2 30.8-34.8, T_3>34.8; nuts, T_1<2.9, T_2 2.9-12.8, T_3>12.8; sweets and added sugar, T_1<50.1, T_2 50.1-117.3, T_3>117.3; Tertiles of food groups for men: fiber-containing foods, T_1<424.3, T_2 424.3-617.8, T_3>617.8; alcoholic beverages, T_1<66.7, T_2 66.7-137.2, T_3>137.2; Tertiles of food groups for women: fiber-containing foods, T_1<537.9, T_2 537.9-723.6, T_3>723.6; T_1< 8.3; T_2 8.3-85.7; $T3_3$>85.7. [2]Model I, analyses were performed using crude conditional logistic regression, without taking into account confounding factors. [3]Model II, analyses were performed using conditional logistic regression analysis adjusted for age (50-59 years old, 60-69 years old), sex, Body Mass Index (underweight/normal weight, overweight/obesity), energy intake (kcal/day), physical exercise level (< 15 minutes/day of cycling/sports, ≥15 minutes/day), smoking status and intensity of smoking (never; past: quit smoking ≥ 11 years ago, quit < 11 years ago; smoker: ≤15 cigarettes/day,>15 cigarettes/day), Deprivation Index (quintile 1-3,quintile 4-5) and Predictive Risk Modelling (level 1-2, level 3-4), including food groups separately; participants with missing data for the confounding variables were included as a separate category for these variables. [4]Model III, model II including all the mean food groups. CI: confidence interval; No.: number; OR: odd ratio; T: tertile.

Table 5. Association between food subgroup intakes and colorectal cancer risk

Food subgroup intakes[1]	No. Case/Control	Model I[2]	Model II[3]	Model III[4]
		OR(95% CI)	OR(95% CI)	OR(95%CI)
Red meat				
T_1	88/101	1.00	1.00	1.00
T_2	103/98	1.20(0.81-1.79)	1.38(0.82-2.34)	1.10(0.62-1.96)
T_3	117/109	1.22(0.84-1.78)	1.41(0.87-2.30)	1.17(0.67-2.03)
P		0.534	<0.001	-
Processed meat				
T_1	102/103	1.00	1.00	1.00
T_2	82/99	0.84(0.57-1.24)	0.62(0.36-1.07)	0.67(0.38-1.18)
T_3	124/106	1.21(0.83-1.77)	1.54(0.91-2.60)	1.54(0.88-2.70)
P		0.206	<0.001	-
White fish				
T_1	95/105	1.00	1.00	1.00
T_2	77/103	0.82(0.53-1.25)	0.97(0.56-1.68)	0.96(0.36-2.53)
T_3	136/105	1.49(1.01-2.20)	1.06(0.62-1.79)	1.29(0.74-2.25)
P		0.008	<0.001	-
Fatty fish				
T_1	119/110	1.00	1.00	1.00
T_2	105/102	1.05(0.71-1.55)	0.93(0.56-1.55)	0.89(0.43-1.69)
T_3	74/96	0.72(0.49-1.08)	0.50(0.29-0.87)	0.53(0.27-0.99)
P		0.145	<0.001	-
Fresh cheese				
T_1	150/153	1.00	1.00	1.00
T_2	224/33	0.64(0.32-1.28)	1.06(0.44-2.55)	1.11(0.66-1.87)
T_3	134/122	1.11(0.80-1.55)	1.10(0.70-1.72)	0.92(0.58-1.46)
P		0.272	<0.001	-
Other cheeses				
T_1	96/116	1.00	1.00	1.00
T_2	71/75	1.16(0.76-1.77)	1.51(0.86-2.63)	1.83(1.15-2.89)
T_3	141/117	1.46(1.01-2.12)	1.85(1.12-3.05)	1.87(1.11-3.16)

To be continued in the next page.

Continuation of Table 5.

Food subgroup intakes[1]	No. Case/Control	Model I[2]	Model II[3]	Model III[4]
		OR (95% CI)	OR (95% CI)	OR (95% CI)
P		0.112	<0.001	-
Fruits				
T_1	109/99	1.00	1.00	1.00
T_2	98/110	0.82(0.56-1.19)	1.08(0.63-1.85)	1.03(0.58-1.83)
T_3	101/99	0.92(0.62-1.37)	0.70(0.40-1.22)	0.68(0.37-1.26)
P		0.567	<0.001	-
Vegetables				
T_1	97/102	1.00	1.00	1.00
T_2	111/103	1.14(0.76-1.71)	0.98(0.55-1.73)	1.10(0.60-2.04)
T_3	100/103	1.03(0.68-1.57)	0.94(0.52-1.70)	1.10(0.58-2.11)
P		0.789	<0.001	-
Whole grains				
T_1	144/128	1.00	1.00	1.00
T_2	83/77	0.92(0.62-1.38)	0.86(0.52-1.42)	0.98(0.58-1.65)
T_3	81/103	0.68(0.46-1.01)	0.62(0.37-1.06)	0.62(0.39-0.98)
P		0.135	<0.001	

[1]Food groups consumption was categorized into tertiles according to the distribution in controls, and by sexes for food groups with significant differences according to sex; Tertiles of food groups: red meat, T_1<33.5 grams/day, T_2 33.5-54.9, T_3>54.9; processed meat, T_1<11.6, T_2 11.6-22.8, T_3>22.8; non-cheese dairy, T_1<225.0, T_2 225.0-325.0, T_3>325.0; cheese, T_1 <7.5, T_2 7.5-20.0, T_3>20.0; vegetables, T_1<152.9, T_2 152.9-237.2, T_3>237.2;Tertiles of food groups for men: fruits, T_1<207.5, T_2 207.5-392.9, T_3>392.9; whole grains, T_1<1.0, T_2 1.0-17.5, T_3>17.5; Tertiles of food groups for women: fruits T_1<242.9, T_2 242.9-425.0,: whole grains, T_1<2.0, T_2 2.0-30.0, T_3>30.0. [2]Model I, analyses were performed using crude conditional logistic regression, without taking into account confounding factors. [3]Model II, analyses were performed using conditional logistic regression analysis adjusted for age (50-59 years old, 60-69 years old), sex, Body Mass Index (underweight/normal weight, overweight/obesity), energy intake (kcal/day), physical exercise level (< 15 minutes/day of cycling/sports, ≥15 minutes/day), smoking status and intensity of smoking (never; past: quit smoking ≥ 11 years ago, quit < 11 years ago; smoker: ≤15 cigarettes/day,>15 cigarettes/day), Deprivation Index (quintile 1-3,quintile 4-5) and Predictive Risk Modelling (level 1-2, level 3-4), including food groups separately; participants with missing data for the confounding variables were included as a separate category for these variables. [4]Model III, model II including all the mean food groups. CI: confidence interval; No.: number; OR: odd ratio; T: tertile.

Table 6. Diet quality indices in the sample studied

	Cases (n=308)		Controls (n=308)		*P*
	Mean	SD	Mean	SD	
HEISD components[1]					
Meats	3.1	1.7	3.1	1.7	0.811
Processed meats	2.8	2.0	3.1	2.2	0.162
Legumes	8.6	2.1	8.5	2.3	0.716
Milk/Dairy	9.8	1.2	9.8	1.1	0.797
Fruits	9.1	1.8	9.1	1.9	0.464
Vegetables	8.9	1.7	8.9	1.6	0.816
Grains	9.9	0.9	9.9	1.9	0.862
Sweets	1.6	3.1	1.6	3.0	0.847
Soft-drink	8.8	2.5	8.7	2.6	0.583
Variety	8.0	1.7	8.0	1.7	0.646
Total HEISD	70.7	7.2	70.8	7.9	0.906
	Mean	SD	Mean	SD	
MDS components[2]					
Red meats and processed meats	0.7	1.0	0.8	1.2	0.134
Poultry	2.9	1.2	2.9	1.2	0.335
Fish	3.8	1.1	3.8	1.2	0.771
Legumes	2.4	1.2	2.3	1.1	0.482
Full fat dairy	2.0	1.8	2.0	1.9	0.618
Vegetables	4.9	0.6	4.9	0.5	0.599
Fruits	4.6	1.0	4.6	1.0	0.726
Potatoes	2.2	1.5	2.4	1.5	0.054
Whole grains	2.0	2.2	2.3	2.3	0.044
Alcoholic beverages	4.9	0.4	4.9	0.4	0.729
Olive oil	4.9	0.6	4.8	0.8	0.446
Total MDS	35.3	4.5	36.0	4.3	0.027

[1]Each component can contribute 10 points to the total score and the theoretical range is 0–100.

[2]Each component can contribute five points to the total score and the theoretical range is 0–55. HEISD: Healthy Eating Index for Spanish Diet; MDS: Mediterranean Diet Score; SD: standard deviation.

Supplementary Table S1. Food group intakes by tumor location

	Proximal cancer cases (n=74)		Paired controls (n=74)		Distal cancer cases (n=234)		Paired controls (n=74)		P^a	P^b	P^c
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Red and processed meat	67.3	38.5	55.6	32.7	72.1	36.0	69.3	41.2	0.206	0.109	0.230
Red meat	48.2	27.9	39.0	26.2	50.2	31.4	48.3	32.2	0.795	0.081	0.435
Processed meat	19.1	16.3	16.6	14.9	21.8	16.6	21.0	17.8	0.128	0.244	0.340
Fish	78.9	38.0	47.1	25.3	76.2	39.6	44.9	28.5	0.558	<0.001	<0.001
Eggs	21.2	13.5	17.6	10.9	20.7	12.5	19.0	11.6	0.948	0.099	0.125
Milk/dairy products	279.9	124.9	277.7	138.9	285.5	165.4	289.6	125.0	0.377	<0.001	<0.001
Non-cheese dairy products	270.2	128.2	260.0	123.7	274.3	123.1	267.1	164.0	0.465	0.755	0.132
Total cheeses	18.9	13.6	15.9	14.9	18.8	18.5	17.5	17.4	0.479	0.053	0.593

To be continued in the next page.

Continuation of Supplementary Table S1

	Proximal cancer cases (n=74)		Paired controls (n=74)		Distal cancer cases (n=234)		Paired controls (n=74)		P^a	P^b	P^c
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Fiber-containing foods	594.3	233.5	634.2	204.0	562.2	247.3	542.9	212.8	0.264	0.144	0.703
Fruits[d]	341.4	181.9	373.5	154.5	326.6	208.9	306.5	169.5	0.399	0.202	0.678
Vegetables	212.2	96.5	214.3	89.4	198.9	86.2	196.3	91.1	0.246	0.672	0.573
Whole grains	16.6	19.4	23.7	26.7	13.7	20.1	17.2	22.0	0.062	0.063	0.068
Fat	35.4	7.0	32.3	4.7	35.6	6.8	35.2	6.7	0.782	0.023	0.386
Nuts	10.7	11.2	12.8	12.0	8.6	9.7	10.3	9.9	0.122	0.287	0.113
Sweets and added sugar	101.8	91.8	83.0	90.1	110.4	96.6	119.4	124.7	0.420	0.134	0.399
Alcoholic beverage	117.3	128.9	81.7	89.2	99.1	89.8	101.6	110.4	0.418	0.057	0.765

[a]Differences by tumor location (comparison of independent samples).

[b]Differences between the proximal cancer cases and their paired controls (comparison of related samples).

[c]Differences between the distal cancer cases and their paired controls (comparison of related samples).

[d]Including natural fruit juices. SD: standard deviation.

Supplementary Table S2. Dietary quality indexes and their components by tumor location

	Proximal cancer cases (n=74)		Paired controls (n=74)		Distal cancer cases (n=234)		Paired controls (n=74)		P^a	P^b	P^c
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
HEISD components											
Meats	3.0	1.6	3.2	1.8	3.2	1.7	3.1	1.7	0.583	0.491	0.912
Processed meats	3.2	2.0	3.4	2.1	2.7	2.0	3.0	2.2	0.083	0.544	0.209
Legumes	8.6	1.9	8.5	2.5	8.6	2.1	8.5	2.2	0.515	0.851	0.604
Milk/dairy	9.7	1.7	9.7	1.4	9.8	1.1	9.8	1.0	0.791	0.794	0.872
Fruits	9.3	1.7	9.7	1.0	9.0	1.9	8.9	2.1	0.200	0.064	0.952
Vegetables	8.8	1.9	9.1	1.7	9.0	1.6	8.9	1.6	0.488	0.1135	0.543
Grains	10.0	0.3	9.9	0.4	9.9	0.6	9.9	0.6	0.159	0.564	1.000
Sweets	1.9	3.7	2.7	3.8	1.5	2.9	1.3	2.7	0.830	0.145	0.443
Soft-drink	9.0	2.4	9.0	2.3	8.8	2.6	8.7	2.7	0.407	0.929	0.517
Variety	8.2	1.7	8.6	1.5	8.0	1.6	7.8	1.7	0.294	0.037	0.600
Total HEISD	71.6	7.2	73.9	7.8	70.4	7.1	69.9	7.7	0.157	0.019	0.294
MDS components											
Red meats and processed meats	0.9	1.1	1.1	1.4	0.6	1.0	0.8	1.1	0.091	0.485	0.168

To be continued in the next page.

Continuation of Supplementary Table S2

	Proximal cancer cases (n=74)		Paired controls (n=74)		Distal cancer cases (n=234)		Paired controls (n=74)		P^a	P^b	P^c
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Poultry	2.9	1.1	2.9	1.3	2.9	1.1	2.9	1.1	0.583	0.470	0.485
Fish	4.0	1.1	3.8	1.2	3.8	1.1	3.8	1.2	0.233	0.294	0.816
Legumes	2.4	1.1	2.3	1.2	2.4	1.2	2.4	1.1	0.566	0.340	0.810
Full fat dairy	2.1	1.8	2.4	2.0	1.9	1.8	1.9	1.9	0.438	0.267	0.977
Vegetables	4.9	0.6	4.9	0.6	4.9	0.6	4.9	0.4	0.518	0.891	0.528
Fruits	4.7	0.9	4.9	0.5	4.6	1.0	4.6	1.1	0.529	0.107	0.819
Potatoes	2.2	1.3	2.2	1.6	2.2	1.5	2.5	1.4	0.742	0.812	0.040
Whole grains	2.3	2.2	2.6	2.4	1.8	2.2	2.3	2.2	0.051	0.587	0.045
Alcoholic beverages	4.9	0.7	4.9	0.2	5.0	0.2	4.9	0.5	0.307	0.167	0.168
Olive oil	5.0	0.3	5.0	0.2	4.9	0.6	4.8	0.9	0.265	0.785	0.394
Total MDS	36.2	5.0	37.1	4.7	35.0	4.3	35.7	4.1	0.037	0.257	0.055

[a]Differences by tumor location (comparison of independent samples).
[b]Differences between the proximal cancer cases and their paired controls (comparison of related samples).
[c]Differences between the distal cancer cases and their paired controls (comparison of related samples). HEISD: Healthy Eating Index for Spanish Diet; MDS: Mediterranean Diet Score; SD: standard deviation.

DISCUSSION

The results from this observational study indicate that high consumption of milk/dairy products, in particular high-fat cheeses, is associated with CRC risk, while a high intake of fiber-containing foods, specially whole grains, and fatty fish was associated with a lower risk for CRC. Moreover, a higher MD adherence in general and particularly a higher score for whole grains have been associated with a reduced CRC risk.

As other authors have previously reported[40] milk/dairy products were the food group with the highest adjusted OR for CRC risk, which is not in agreement with the probable evidence of protection of this food group against CRC[5]. Some cohort studies support the protective effect of total dairy products and milk[41-43]. This effect has been hypothetically associated with calcium, vitamin D, fats and other components such as lactoferrin or lactic bacteria in the case of fermented dairy products milk[41,42]. However, case-control studies published to date are heterogeneous and, on average, do not provide evidence of an association between total intake of total dairy products, milk, cheese or yogurt and CRC risk[41]. Regarding milk/dairy products consumption according to anatomical subsites of cases, the intake was higher in proximal tumor cases and lower in distal cases than in their matched controls. Although according to scientific literature, the effect of this food group seems to be similar across all locations of the bowel[43].

In general, epidemiological studies have not found evidence of either reduction or increase of CRC risk specifically associated with the consumption of cheese[41,42]. Although there are few pieces of research on cheese consumption that reported an inverse association with CRC[44] in the present research, high-fat cheeses are shown to be possible risk factors for CRC development. Some studies showed a positive relationship between fatty foods and CRC incidence[45]. Dairy products, e.g., mature, semi-mature and creamy cheeses, are rich in saturated fat, so this relationship might be due to the content of fat in these products. Several studies have suggested that high-fat consumption increases bile acid discharge. Moreover, an increase in the concentration of bile acids above physiological levels has been reported to promote CRC[46,47]. In any case, the association between milk/dairy products consumption and the risk of developing CRC is complex and some researchers indicated that the fat content contained within dairy products does not influence this association[43].

In line with previous studies[48-50], we also found that the consumption of fiber-containing foods was inversely associated with CRC risk. Specifically, consumption of more than 424.3 g/d in men and 537.9 g/d in women of fiber-containing foods decreased CRC risk

by about 50% (OR ~0.5) compared to lower consumption, in adjusted models. The preventive effect of dietary fiber can be explained by biological mechanisms that include increasing amounts of feces, decreasing gastrointestinal transit time, diluting intestinal cancer-causing factors, interfering absorption of those, and lowering intestinal acidity[51]. In addition, fermentation of fiber produced butyrate. This short-chain fatty acid showed anti-inflammatory, anti-proliferation and antineoplastic properties in colonocyte cells metabolism through microbiota homeostasis and genetic/epigenetic regulation[52].

Furthermore, our findings suggest that high consumption of whole grains (higher than 17.5 g/d in men and 30.0 g/d in women) may decrease the risk of CRC, after controlling confounding factors. There is convincing evidence that whole grains help to reduce CRC risk[5,53]. The observed reduction in CRC risk associated with high consumption of whole grains may partly be attributed to dietary fiber, resistant starch, and oligosaccharides that can influence the gut environment. Insoluble fiber increases the bulk of luminal contents, diluting potential carcinogens and promoters in the colon and decreasing transit time, and, consequently, reduces the exposure of the colonic epithelium to harmful compounds[54,55]. Additionally, other components such as vitamins (especially B-vitamins), minerals (e.g. magnesium and zinc), phenolic compounds, antinutrients (e.g. tannins), and phytoestrogens may also contribute to this protection[54].

On the other hand, the consumption of fatty fish (higher than 42.8 g/d) was associated with a decreased risk in CRC by about 50% (OR ~0.5) compared to lower consumption, after adjusting models for covariates. It should be noted that the Basque Country population has a higher consumption of total fish and fatty fish compared to other Spanish autonomous communities[55,56]. Recent cohort studies have observed that fatty fish was inversely associated with CRC incidence[57,58] and they have related this association with exposure to long-chain n-3 polyunsaturated fatty acids[57]. Evidence from animal and in vitro studies indicates that n-3 fatty acids present in fatty fish may inhibit carcinogenesis[59]. High intake of n-3 fatty acids suppresses the production of arachidonic acid-derived eicosanoids such as prostaglandin E2 and leukotriene B431. N-3 fatty acids could also suppress the expression of inducible nitric oxide synthase (NOS) and nuclear transcription factor κ B (NF-κ B)[60].

In relation to the diet quality, our findings on the MDS and CRC risk are supported by those of other researchers[13,61-63], who found significant associations between lower risk of CRC and adherence to Mediterranean dietary pattern. However, the HEISD was not associated with CRC risk, discrepancies in results obtained with the two dietary quality indices analysed are probably due to differences in their constructs and scoring criteria.

The overall MDS was inversely associated with CRC risk, being higher the total score in cases with the proximal location of cancer than for those with the distal location. These last results contrast with previous findings, which showed that the protective effects of adherence to the MD were mainly for distal colon and rectal cancer and not for proximal colon cancer[64]. In the total sample, investigation of the separate score components showed that whole grain score was lower for cases than for controls. This result is consistent with that obtained for the association between whole grains consumption and CRC risk.

Our study has several limitations. First, recall bias inherent in a case-control study design cannot be ruled out. The primary concern of this study is the low participation rate, which may have limited the representativeness of study samples. The decision to participate or not may be influenced by several factors, including social, educational and health conditions, which may again correlate with outcome risk factors. Second, self-reported data could be subject to measurement errors and the problem of food omissions due to memory failure and underreporting of unhealthy habits among disease subjects. However, previous validation studies indicate that the self-reported dietary information is reported with sufficient accuracy for use in epidemiology analyses[65]; and it should be noted that dietary changes are usually modest after participating in the CRCSP due to a lack of information and personalized advice[66,67]. Another limitation of this type of study could be the selection of controls (selection bias). To avoid this type of bias, we obtained controls from the same CRCSP and in the same period as cases, thus, it was confirmed that they did not suffer from CRC by colonoscopy.

Despite these limitations, the results allow us to conclude that high consumption of high-fat cheeses is associated with CRC risk, whereas, a high intake of fiber-containing foods, especially whole grains, and fatty fish, and adherence to the Mediterranean dietary pattern was associated with a lower risk for CRC. Future studies are needed to better understand the influence of the dietary habits on CRC prevention in this population that can provide leads for the design and tailoring of future interventions, and guide counselling strategies for promoting a healthy lifestyle.

ACKNOWLEDGMENTS

We thank all patients who agreed to participate in this study.

ARTICLE HIGHLIGHTS

Research background

Epidemiological evidence suggests that some foods may both protect against and promote the development of colorectal cancer (CRC). However, foods are not consumed in isolation but as part of a dietary pattern; therefore, the actual effect of diet on disease risk may be observed only when all components are considered jointly. For this purpose, several diet quality indexes have been developed using point systems to measure whole diet quality based on the alignment of food choices with dietary recommendations.

Research motivation

Some diet quality indexes have been used to begin assessing the relationships between overall diet quality and CRC risk, and the results show that high scores in these indices are associated with a lower CRC risk. However, the results vary considerably according to the index used and other factors such as sex and age. Therefore, there is a need to further examine these relationships in diverse population studies.

Research objectives

To study the relationships between food groups, diet quality and CRC risk, in an adult population of the Basque Country (North of Spain).

Research methods

This observational study included 308 patients diagnosed with CRC and 308 age- and sex-matched subjects as controls. During recruitment, dietary, anthropometric, lifestyle, socioeconomic, demographic and health status information was collected. Dietary intake was assessed using a short food frequency questionnaire that was adapted and validated for this population. Adherence to the dietary recommendations was evaluated utilizing the Healthy Eating Index for the Spanish Diet and the MedDietScore. Statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, USA) and STATA 13.0 (StataCorp LP, Texas, USA). Conditional logistic regressions were used to evaluate the associations of food group intakes, diet quality scores, categorized in tertiles, with CRC risk.

Research results

The adjusted models for potential confounding factors showed a direct association between milk/dairy products consumption, in particular high-fat cheeses (OR third tertile *vs* first tertile=1.87, 95% CI 1.11-3.16), and CRC risk. While the consumption of fiber-containing foods, especially whole grains (OR third tertile *vs* first tertile=0.62, 95% CI 0.39-0.98), and fatty fish (OR=0.53, 95% CI 0.27-0.99) was associated with a lower risk for CRC. Moreover, higher MD adherence was associated with a reduced CRC risk in adjusted models (OR=0.40, 95% CI 0.20-0.80).

Research conclusions

Direct associations were found for high-fat cheese, whereas an inverse relation was reported for fiber-containing foods and fatty fish, as well as adherence to a Mediterranean dietary pattern.

Research perspectives

Future studies are needed to better understand the influence of the dietary habits on CRC prevention in this population that can provide leads for the design and tailoring of future interventions, and guide counselling strategies for promoting a healthy lifestyle.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68:** 394-424 [PMID: 30207593 DOI:10.3322/caac.21492.]

2 **Altobelli E**, Lattanzi A, Paduano R, Varassi G, di Orio F. Colorectal cancer prevention in Europe: burden of disease and status of screening programs. *Prev Med* 2014; **62:** 132-41 [PMID: 24530610 DOI: 10.1016/j.ypmed.2014.02.010]

3 **Huxley RR**, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer* 2009; **125:** 171-80 [PMID: 19350627 DOI: 10.1002/ijc.24343]

4 **Vucenik I**, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. *Ann N Y Acad Sci* 2012; **1271:** 37-43 [PMID: 23050962 PMCID: PMC3476838 DOI:10.1111/j.1749-6632.2012.06750.x]

5 **World Cancer Research Fund/American Institute for Cancer Research. Continuous** Update Project Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. 2017. Available from: https://www.wcrf.org/sites/default/files/Colorectal-cancer-report.pdf

6 **Jacques PF,** Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? *Am J Clin Nutr* 2001; **73:** 1-2 [PMID:11124739]

7 **Reedy J,** Wirfält E, Flood A, Mitrou PN, Krebs-Smith SM, Kipnis V, Midthune D, Leitzmann M, Hollenbeck A, Schatzkin A, Subar AF. Comparing 3 dietary pattern methods--cluster analysis, factor analysis, and index analysis--With colorectal cancer risk: The NIH-AARP Diet and Health Study. *Am J Epidemiol* 2010; **171:** 479-87 [PMID:20026579 PMCID: PMC2842201 DOI: 10.1093/aje/kwp393]

8 **Steck SE,** Guinter M, Zheng J, Thomson CA. Index-based dietary patterns and colorectal cancer risk: a systematic review. *Adv Nutr* 2015; **6:** 763-73 [PMID: 26567200 PMCID: PMC4642428 DOI: 10.3945/an.115.009746]

9 **Reedy J,** Mitrou PN, Krebs-Smith SM, Wirfält E, Flood A, Kipnis V, Leitzmann M, Mouw T, Hollenbeck A, Schatzkin A, Subar AF. Index-based dietary patterns and risk of colorectal cancer: the NIH-AARP Diet and Health Study. *Am J Epidemiol* 2008; **168:** 38-48 [PMID:18525082 PMCID: PMC6287243 DOI: 10.1093/aje/kwn097]

10 **Miller PE,** Cross AJ, Subar AF, Krebs-Smith SM, Park Y, Powell-Wiley T, Hollenbeck A, Reedy J. Comparison of 4 established DASH diet indexes: examining associations of index scores and colorectal cancer. *Am J Clin Nutr* 2013; **98:** 794-803 [PMID:23864539 PMCID: PMC3743737 DOI: 10.3945/ajcn.113.063602]

11 **Idigoras I,** Arrospide A, Portillo I, Arana-Arri E, Martínez-Indart L, Mar J,de Koning HJ, Lastra R, Soto-Gordoa M, van der Meulen M, Lansdorp-Vogelaar I. Evaluation of the colorectal cancer screening Programme in the Basque Country (Spain) and its effectiveness based on the Miscan-colon model. *BMC Public Health* 2017; **18:** 78 (PMID: 28764731 PMCID: PMC5540568 DOI:10.1186/s12889-017-4639-3]

12 **Banqué M,** Raidó B, Masuet C, Ramon JM. Food groups and nutrient intake and risk of colorectal cancer: a hospital-based case-control study in Spain. *Nutr Cancer* 2012; **64:** 386-92. [PMID: 22369135 DOI: 10.1080/01635581.2012.657334]

13 **Castelló A,** Amiano P, Fernández de Larrea N, Martín V, Alonso MH, Castaño-Vinyals G, Pérez-Gómez B, Olmedo-Requena R, Guevara M, Fernandez-Tardon G, Dierssen-Sotos T, Llorens-Ivorra C, Huerta JM, Capelo R, Fernández-Villa T, Díez-Villanueva A, Urtiaga C, Castilla J, Jiménez-Moleón JJ, Moreno V, Dávila-Batista V, Kogevinas M, Aragonés N, Pollán M; MCC-Spain researchers. Low adherence to the western and high adherence to the mediterranean dietary patterns could prevent

colorectal cancer. *Eur J Nutr* 2019; **58:** 1495-1505 [PMID: 29582162 DOI:10.1007/s00394-018-1674-5]

14 **Rosato V,** Guercio V, Bosetti C, Negri E, Serraino D, Giacosa A, Montella M, La Vecchia C, Tavani A. Mediterranean diet and colorectal cancer risk: a pooled analysis of three Italian case-control studies. *Br J Cancer* 201627; **115:** 862-5 [PMID: 27537381 PMCID: PMC5046203 DOI: 10.1038/bjc.2016.245]

15 **Alegria-Lertxundi I,** Aguirre C, Bujanda L, Fernández FJ, Polo F, Ordovás JM, Etxezarraga MC, Zabalza I, Larzabal M, Portillo I, de Pancorbo MM, Palencia-Madrid L, Rocandio AM, Arroyo-Izaga M. Single nucleotide polymorphisms associated with susceptibility for development of colorectal cancer: Case-control study in a Basque population. *PLoS One* 2019; **14:** e0225779 [PMID: 31821333 PMCID: PMC6903717 DOI:10.1371/journal.pone.0225779]

16 **Edge SB,** Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17:** 1471-4 [PMID: 20180029 DOI:10.1245/s10434-010-0985-4].

17 **Rodríguez IT,** Ballart JF, Pastor GC, Jordà EB, Val VA. Validation of a short questionnaire on frequency of dietary intake: reproducibility and validity. *Nutr Hosp* 2008; **23:** 242-52 [PMID: 18560701]

18 **Telleria-Aramburu N,** Alegria-Lertxundi, Arroyo-Izaga M. Adaptation, validation and reproducibility of a short food frequency questionnaire to assess food group intake in the population resident in the Basque Country (Spain). *Public Health Nutr* 2020; In press

19 **Alegria-Lertxundi I**, Alvarez M, Rocandio AM, de Pancorbo MM, Arroyo-Izaga M. Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Postsurgery: A Pilot Study. *Nutr Cancer* 2016; **68:** 577-88 [PMID: 27144653. DOI:10.1080/01635581.2016.1158299]

20 **Carbajal A,** Sánchez-Muniz FJ. Guía de prácticas. In: García-Arias, MC García-Fernández *Nutrición y dietética*. León: Secretariado de Publicaciones y Medios Audiovisuales, Universidad de León, 2003: 1-30

21 **Elika.** Estudio cuantitativo del consumo de alimentos en la CAPV. Vitoria-Gasteiz: Dpto. Agricultura, Pesca y Alimentación, Gobierno Vasco, 2008

22 **Dapchich V,** Salador Castell G, Ribas Barba L, Pérez Rodrigo C, Aranceta Bartrina J, Serra Majem L. 1st ed. Madrid: Sociedad Española de Nutrición Comunitaria, 2004: 1-106

23 **Song M,** Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology* 2015; **148:** 1244-60.e16 [PMID: 25575572 PMCID: PMC4409470 DOI::10.1053/j.gastro.2014.12.035]

24 **Schwingshackl L,** Hoffmann G. Does a Mediterranean-Type Diet Reduce Cancer Risk? *Curr Nutr Rep* 2016; **5:** 9-17. [PMID:27014505 PMCID: PMC4778149]

25 **Ortega RM**, López-Sobaler AM, Andrés P, Requejo AM, Aparicio A, Molinero LM.DIAL software for assessing diets and food calculations (for Windows, version 8). Department of Nutrition (UCM) & Alce Ingeniería, S.L. Madrid, Spain, 2016 Available from: http://www.alceingenieria.net/nutricion/descarga.htm. Last access: [accesed: 20/10/2016].

26 **Norte Navarro AI**, Ortiz Moncada R. Spanish diet quality according to the healthy eating index. *Nutr Hosp* 2011; **26**: 330-6 [PMID: 21666971 DOI:10.1590/S0212-16112011000200014]

27 **Panagiotakos DB**, Milias GA, Pitsavos C, Stefanadis C. MedDietScore: a computer program that evaluates the adherence to the Mediterranean dietary pattern and its relation to cardiovascular disease risk. *Comput Methods Programs Biomed* 2006; **83:** 73-7. [PMID: 16806570]

28 **Ministerio de Sanidad, Servicios Sociales e Igualdad**. Encuesta Nacional de Salud 2011/12. Madrid: 2013. Available from: http://www.ine.es/jaxi/menu.do?typeDpcaxis&pathD/t15/p419&fileDinebase

29 **World Health Organization.** Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. 2000; **894:** i-xii, 1-253. [PMID: 11234459]

30 **Silva Rodrigues RA,** Martinez Espinosa M, Duarte Melo C, Rodrigues Perracini M, Rezende Fett WC, Fett CA. New values anthropometry for classification of nutritional status in the elderly. *J Nutr Health Aging* 2014; **18:** 655-61. [PMID: 25226103: DOI: 10.1007/s12603-014-0451-2]

31 **Domínguez-Berjón MF,** Borrell C, Cano-Serral G, Esnaola S, Nolasco A, Pasarín MI, Ramis R, Saurina C, Escolar-Pujolar A. Constructing a deprivation index based on census data in large Spanish cities (the MEDEA project). *Gac Sanit* 2008; **22:** 179-87 [PMID: 18579042]

32 **Johns Hopkins University, School of Public Health**. The Johns Hopkins University ACG Case-Mix System. Available from: http://www.acg.jhsph.org/index.php?option=com_content&view=article&id=46&Itemid=61

33 **Verisk Health's DxCGDCG_Methodology**. Available from: http://www.dxcg.com/resources/library

34 3**M™ Clinical Risk Grouping Software**. Available from: http://solutions.3m.com/wps/portal/3M/en_US/3M_Health_Information_Systems/HIS/Products/CRG/

35 **Orueta JF,** Nuño-Solinis R, Mateos M, Vergara I, Grandes G, Esnaola S. Predictive risk modelling in the Spanish population: a cross-sectional study. *BMC Health Serv Res* 2013; **13:** 269 [PMID: 23837560 PMCID: PMC3750562 DOI: 10.1186/1472-6963-13-269]

36 **Torres Stone RA,** Waring ME, Cutrona SL, Kiefe CI, Allison J, Doubeni CA. The association of dietary quality with colorectal cancer among normal weight, overweight and obese men and women: a prospective longitudinal study in the USA. *BMJ Open* 2017; **7:** e015619 [PMID:28679675 PMCID: PMC5734399 DOI: 10.1136/bmjopen-2016-015619]

37 **Doubeni CA,** Laiyemo AO, Major JM, Schootman M, Lian M, Park Y, Graubard BI,Hollenbeck AR, Sinha R. Socioeconomic status and the risk of colorectal cancer: an analysis of more than a half million adults in the National Institutes of Health-AARP Diet and Health Study. *Cancer* 2012; **118:** 3636-44 [PMID: 22898918 PMCID: PMC3422782 DOI: 10.1002/cncr.26677]

38 **Deshpande AD,** McQueen A, Coups EJ. Different effects of multiple health status indicators on breast and colorectal cancer screening in a nationally representative US sample. *Cancer Epidemiol* 2012; **36:** 270-5 [PMID: 22079763 PMCID: PMC3292673 DOI:10.1016/j.canep.2011.10.001]

39 **Romaguera D,** Vergnaud AC, Peeters PH, van Gils CH, Chan DS, Ferrari P, Romieu I, Jenab M, Slimani N, Clavel-Chapelon F, Fagherazzi G, Perquier F, Kaaks R, Teucher B, Boeing H, von Rüsten A, Tjønneland A, Olsen A, Dahm CC, Overvad K, Quirós JR, Gonzalez CA, Sánchez MJ, Navarro C, Barricarte A, Dorronsoro M, Khaw KT, Wareham NJ, Crowe FL, Key TJ, Trichopoulou A, Lagiou P, Bamia C, Masala G, Vineis P, Tumino R, Sieri S, Panico S, May AM, Bueno-de-Mesquita HB, Büchner FL, Wirfält E, Manjer J, Johansson I, Hallmans G, Skeie G, Benjaminsen Borch K, Parr CL, Riboli E, Norat T. Is concordance with World Cancer Research Fund/American Institute for Cancer Research guidelines for cancer prevention related to subsequent risk of cancer? Results from the EPIC study. *Am J Clin Nutr* 2012; **96:** 150-63 [PMID: 22592101 DOI: 10.3945/ajcn.111.031674]

40 **Abbastabar H,** Roustazadeh A, Alizadeh A, Hamidifard P, Valipour M, Valipour AA. Relationships of colorectal cancer with dietary factors and public health indicators: an ecological study. *Asian Pac J Cancer Prev* 2015; **16**(9): 3991-5 [PubMed PMID: 25987074]

41 **Norat T,** Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. *Eur J Clin Nutr* 2003; **57**(1): 1-17 [PMID: 12548291]

42 **Aune D,** Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, Norat T. Dairy products and colorectal cancer risk: a systematic review and meta-analysis of cohort studies. *Ann Oncol* 2012; **23:** 37-45 [PMID: 21617020 DOI: 10.1093/annonc/mdr269]

43 **Murphy N,** Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Olsen A, Tjønneland A, Dahm CC, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Nailler L, Kaaks R, Teucher B, Boeing H, Bergmann MM, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Pala V, Tumino R, Vineis P, Panico S, Peeters PH, Dik VK, Weiderpass E, Lund E, Garcia JR, Zamora-Ros R, Pérez MJ, Dorronsoro M, Navarro C, Ardanaz E, Manjer J, Almquist M, Johansson I, Palmqvist R, Khaw KT, Wareham N, Key TJ, Crowe FL, Fedirko V, Gunter MJ, Riboli E. Consumption of dairy products and colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS One* 2013; **8:** e72715. [PMID: 24023767 PMCID: PMC3759377 DOI: 10.1371/journal.pone.0072715]

44 **Larsson SC,** Bergkvist L, Wolk A. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. *Am J Clin Nutr* 2005; **82:** 894-900. [PMID: 16210722]

45 **Pais R,** Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. *World J Gastroenterol* 2009; **15:** 5141-8 [PMID: 19891012 PMCID: PMC2773892]

46 **Ou J,** DeLany JP, Zhang M, Sharma S, O'Keefe SJ. Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. *Nutr Cancer* 2012; **64:** 34-40. [PMID: 22136517 PMCID: PMC6844083 DOI:10.1080/01635581.2012.630164.

47 **Ajouz H,** Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. *World J Surg Oncol* 2012; **12:** 164 [PMID: 24884764 PMCID: PMC4041630 DOI: 10.1186/1477-7819-12-164]

48 **Dahm CC,** Keogh RH, Spencer EA, Greenwood DC, Key TJ, Fentiman IS, Shipley MJ, Brunner EJ, Cade JE, Burley VJ, Mishra G, Stephen AM, Kuh D, White IR, Luben R, Lentjes MA, Khaw KT, Rodwell Bingham SA. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. *J Natl Cancer Inst* 2010; **102:** 614-26 [PMID: 20407088 DOI: 10.1093/jnci/djq092]

49 **Tan J,** Chen YX. Dietary and Lifestyle Factors Associated with Colorectal Cancer Risk and Interactions with Microbiota: Fiber, Red or Processed Meat and Alcoholic Drinks. *Gastrointest Tumors* 2016; **3:** 17-24 [PMID:27722153 PMCID: PMC5040877]

50 **Global Burden of Disease Cancer Collaboration**, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandona R, Dandona L, Fleming T, Forouzanfar MH, Hancock J, Hay RJ, Hunter-Merrill R, Huynh

C, Hosgood HD, Johnson CO, Jonas JB, Khubchandani J, Kumar GA, Kutz M, Lan Q, Larson HJ, Liang X, Lim SS, Lopez AD, MacIntyre MF, Marczak L, Marquez N, Mokdad AH, Pinho C, Pourmalek F, Salomon JA, Sanabria JR, Sandar L, Sartorius B, Schwartz SM, Shackelford KA, Shibuya K, Stanaway J, Steiner C, Sun J, Takahashi K, Vollset SE, Vos T, Wagner JA, Wang H, Westerman R, Zeeb H, Zoeckler L, Abd-Allah F, Ahmed MB, Alabed S, Alam NK, Aldhahri SF, Alem G, Alemayohu MA, Ali R, Al-Raddadi R, Amare A, Amoako Y, Artaman A, Asayesh H, Atnafu N, Awasthi A, Saleem HB, Barac A, Bedi N, Bensenor I, Berhane A, Bernabé E, Betsu B, Binagwaho A, Boneya D, Campos-Nonato I, Castañeda-Orjuela C, Catalá-López F, Chiang P, Chibueze C, Chitheer A, Choi JY, Cowie B, Damtew S, das Neves J, Dey S,Dharmaratne S, Dhillon P, Ding E, Driscoll T, Ekwueme D, Endries AY, Farvid M, Farzadfar F, Fernandes J, Fischer F, G/Hiwot TT, Gebru A, Gopalani S, Hailu A,Horino M, Horita N, Husseini A, Huybrechts I, Inoue M, Islami F, Jakovljevic M,James S, Javanbakht M, Jee SH, Kasaeian A, Kedir MS, Khader YS, Khang YH, Kim D, Leigh J, Linn S, Lunevicius R, El Razek HMA, Malekzadeh R, Malta DC, Marcenes W, Markos D, Melaku YA, Meles KG, Mendoza W, Mengiste DT, Meretoja TJ, Miller TR,Mohammad KA, Mohammadi A, Mohammed S, Moradi-Lakeh M, Nagel G, Nand D, Le Nguyen Q, Nolte S, Ogbo FA, Oladimeji KE, Oren E, Pa M, Park EK, Pereira DM, Plass D, Qorbani M, Radfar A, Rafay A, Rahman M, Rana SM, Søreide K, Satpathy M, SawhneyM, Sepanlou SG, Shaikh MA, She J, Shiue I, Shore HR, Shrime MG, So S, Soneji S, Stathopoulou V, Stroumpoulis K, Sufiyan MB, Sykes BL, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tessema GA, Thakur JS, Tran BX, Ukwaja KN, Uzochukwu BSC, Vlassov VV, Weiderpass E, Wubshet Terefe M, Yebyo HG, Yimam HH, Yonemoto N, Younis MZ, Yu C, Zaidi Z, Zaki MES, Zenebe ZM, Murray CJL, Naghavi M. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* 2017; **3:** 524-548 [PMID: 27918777 PMCID: PMC6103527 DOI: 10.1001/jamaoncol.2016.5688]

51 **Aune D,** Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, Norat T. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ* 2011; **343:** d6617 [PMID: 22074852 PMCID: PMC3213242 DOI: 10.1136/bmj.d6617].

52 **Yang J,** Yu J. The association of diet, gut microbiota and colorectal cancer: what we eat may imply what we get. *Protein Cell* 2018; **9:** 474-487. [PMID: 29713943 PMCID: PMC5960467 DOI: 10.1007/s13238-018-0543-6]

53 **Vieira AR**, Abar L, Chan DSM, Vingeliene S, Polemiti E, Stevens C, Greenwood D, Norat T. Foods and beverages and colorectal cancer risk: a systematic review and meta-analysis of cohort studies, an update of the evidence of the WCRF-AICR Continuous Update Project. *Ann Oncol* 2017; **28:** 1788-1802 [PMID: 28407090 DOI:10.1093/annonc/mdx171. Review. PubMed]

54 **Slavin JL,** Martini MC, Jacobs DR Jr, Marquart L. Plausible mechanisms for the protectiveness of whole grains. *Am J Clin Nutr* 1999; **70** Suppl 3: 459S-463S [PMID: 10479218 DOI: 10.1093/ajcn/70.3.459s. Review. PubMed

55 **Ministerio de Agricultura, Alimentación y Medio Ambiente, Gobierno de España**. Informe del Consumo de Alimentación en España 2014. Available from: https://www.mapa.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2014_tcm30-104149.pdf;

56 **Federación Española del Corazón (FEC).** Encuesta de consumo de pescado azul en España 2015. Available from: https://fundaciondelcorazon.com/prensa/notas-de-prensa/2773-la-poblacion-adulta-espanola-suspende-en-el-consumo-de-pescado-azul-recomendado-.html

57 **Aglago EK,** Huybrechts I, Murphy N, Casagrande C, Nicolas G, Pischon T, Fedirko V, Severi G, Boutron-Ruault MC, Fournier A, Katzke V, Kühn T, Olsen A, Tjønneland A, Dahm CC, Overvad K, Lasheras C, Agudo A, Sánchez MJ, Amiano P, Huerta JM, Ardanaz E, Perez-Cornago A, Trichopoulou A, Karakatsani A, Martimianaki G, Palli D, Pala V, Tumino R, Naccarati A, Panico S, Bueno-de-Mesquita B, May A, Derksen JWG, Hellstrand S, Ohlsson B, Wennberg M, Van Guelpen B, Skeie G, Brustad M, Weiderpass E, Cross AJ, Ward H, Riboli E, Norat T, Chajes V, Gunter MJ. Consumption of Fish and Long-chain n-3 Polyunsaturated Fatty Acids Is Associated With Reduced Risk of Colorectal Cancer in a Large European Cohort. *Clin Gastroenterol Hepatol* 2020; **18:** 654-666.e6 [PMID: 31252190 DOI: 10.1016/j.cgh.2019.06.031]

58 **Spencer EA,** Key TJ, Appleby PN, Dahm CC, Keogh RH, Fentiman IS, Akbaraly T, Brunner EJ, Burley V, Cade JE, Greenwood DC, Stephen AM, Mishra G, Kuh D, Luben R, Mulligan AA, Khaw KT, Rodwell SA. Meat, poultry and fish and risk of colorectal cancer: pooled analysis of data from the UK dietary cohort consortium. *Cancer Causes Control* 2010; **21:** 1417-25 [PMID: 20437091 DOI:: 10.1007/s10552-010-9569-7]

59 **Rao CV,** Hirose Y, Indranie C, Reddy BS. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Res* 2001; **61:** 1927-33 [PMID: 11280748]

60 **Larsson SC**, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am J Clin Nutr* 2004; **79:** 935-45 [PMID: 15159222]

61 **Donovan MG**, Selmin OI, Doetschman TC, Romagnolo DF. Mediterranean Diet: Prevention of Colorectal Cancer. *Front Nutr* 2017; **4:** 59 [PMID: 29259973 PMCID: PMC5723389 DOI:10.3389/fnut.2017.00059]

62 **Jafari Nasab S,** Bahrami A, Rafiee P, Hekmatdoust A, Ghanavati M, Rashidkhani B, Sadeghi A, Asadzadeh Aghdaei H, Naja F, Hejazi E. Healthy Eating Index-2010 and Mediterranean-Style Dietary Pattern Score and the risk of colorectal cancer and adenoma: a case-control study. *Nutr Cancer* 2019; **5:** 1-10 [PMID: 31687849 DOI:10.1080/01635581.2019.1683212]

63 **Grosso G,** Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S, Drago F, Basile F. Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. *Nutr Cancer* 2014; **66:** 558-65 [PMID: 24754383 DOI: 10.1080/01635581.2014.902975]

64 **Bamia C,** Lagiou P, Buckland G, Grioni S, Agnoli C, Taylor AJ, Dahm CC, Overvad K, Olsen A, Tjønneland A, Cottet V, Boutron-Ruault MC, Morois S, Grote V, Teucher B, Boeing H, Buijsse B, Trichopoulos D, Adarakis G, Tumino R, Naccarati A, Panico S, Palli D, Bueno-de-Mesquita HB, van Duijnhoven FJ, Peeters PH, Engeset D, Skeie G, Lund E, Sánchez MJ, Barricarte A, Huerta JM, Quirós JR, Dorronsoro M, Ljuslinder I, Palmqvist R, Drake I, Key TJ, Khaw KT, Wareham N, Romieu I, Fedirko V, Jenab M, Romaguera D, Norat T, Trichopoulou A. Mediterranean diet and colorectal cancer risk: results from a European cohort. *Eur J Epidemio*l 2013; **28:** 317-28. [PMID: 23579425 DOI: 10.1007/s10654-013-9795-x]

65 **Kumanyika SK,** Mauger D, Mitchell DC, Phillips B, Smiciklas-Wright H, Palmer JR. Relative validity of food frequency questionnaire nutrient estimates in the Black Women's Health Study. *Ann Epidemiol* 2003; **13:** 111-8 {PMID: 12559670]

66 **Berstad P,** Løberg M, Larsen IK, Kalager M, Holme Ø, Botteri E, Bretthauer M, Hoff G. Long-term lifestyle changes after colorectal cancer screening: randomized controlled trial. *Gut* 2015; **64:** 1268-76 [PMID: 25183203 DOI: 10.1136/gutjnl-2014-307376]

67 **Anderson AS,** Steele R, Coyle J. Lifestyle issues for colorectal cancer survivors--perceived needs, beliefs and opportunities. Support *Care Cancer* 2013; **21:** 35-42 [PMID:2277329 DOI: 10.1007/s00520-012-1487-7]

Footnotes

Institutional review board statement: This study was approved by the Clinical Research Ethics Committee of the Basque Country (reference numbers PI2011006 and PI2014042).

Informed consent statement: Written informed consent was obtained from all the study participants.

Conflict-of-interest statement: None.

STROBE Statement:

Manuscript source: solicited manuscript
Specialty type: nutrition and dietetics
Country of origin: Spain

3.4. STUDY 4: "Single nucleotide polymorphisms associated with susceptibility for development of colorectal cancer: Case-control study in a Basque population"

Single nucleotide polymorphisms associated with susceptibility for development of colorectal cancer: Case-control study in a Basque population

Iker Alegria-Lertxundi[1,2], Carmelo Aguirre[2,3], Luis Bujanda[4], Francisco Javier Fernández[5], Francisco Polo[6], José Mª Ordovás[7,8], Mª Carmen Etxezarraga[9,10], Iñaki Zabalza[11], Mikel Larzabal[12], Isabel Portillo[13], Marian M. de Pancorbo[2], Leire Palencia-Madrid[2], Ana Mª Rocandio[1,2], Marta Arroyo-Izaga[1,2]

[1]Department of Pharmacy and Food Sciences.Faculty of Pharmacy, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain.
[2]BIOMICs Research Group, University of the Basque Country, UPV/EHU, Vitoria-Gasteiz, Spain
[3]Pharmacovigilance Unit, Galdakao-Usansolo Hospital, Osakidetza, Spain.
[4]Department of Gastroenterology, Donostia University Hospital / BioDonostia Institute. Biomedicine Research Networking Center - CIBER of Hepatic and Digestive Diseases (CIBERehd), University of the Basque Country, UPV/EHU, San Sebastian, Spain.
[5]Department of Gastroenterology, Galdakao-Usansolo Hospital, Osakidetza, Galdakao, Spain.
[6]Department of Gastroenterology, Basurto University Hospital, Osakidetza, Bilbao, Spain.
[7]Nutrition and Genomics Laboratory, Jean Mayer Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts, United States of America; IMDEA Food, Madrid, Spain.
[8]Nutritional Genomics and Epigenomics Group. Madrid Institute for Advanced Studies (IMDEA) Food Institute, Madrid, Spain.
[9]Department of Pathology, Basurto Hospital, Osakidetza, Bilbao, Spain.
[10]Department of Physician and Surgeon Specialities, University of the Basque Country, Leioa, UPV/EHU, Spain
[11]Department of Pathology, Galdakao-Usansolo Hospital, Osakidetza, Galdakao, Spain
[12]Department of Pathology, Donostia University Hospital / BioDonostia Institute. Biomedicine Research Networking Center - CIBER of Hepatic and Digestive Diseases (CIBERehd), San Sebastian, Spain.
[13]Colorectal cancer screening programme, The Basque Health Service, Bilbao, Spain.

Citation text: Alegria-Lertxundi I, Aguirre C, Bujanda L, Fernández FJ, Polo F, Ordovás JM, Etxezarraga MC, Zabalza I, Larzabal M, Portillo I, de Pancorbo MM, Palencia-Madrid L, Rocandio AM, Arroyo-Izaga M. Single nucleotide polymorphisms associated with

susceptibility for development of colorectal cancer: Case-control study in a Basque population. PLoS One. 2019 Dec 10;14(12):e0225779. doi: 10.1371/journal.pone.0225779. PMID: 31821333; PMCID: PMC6903717.

Abstract

Given the significant population diversity in genetic variation, we aimed to investigate whether single nucleotide polymorphisms (SNPs) previously identified in studies of colorectal cancer (CRC) susceptibility were also relevant to the population of the Basque Country (North of Spain). We genotyped 230 CRC cases and 230 healthy controls for 48 previously reported CRC-susceptibility SNPs. Only the rs6687758 in *DUPS10* exhibited a statistically significant association with CRC risk based on the crude analysis. The rs6687758 *AG* genotype conferred about 2.13-fold increased risk for CRC compared to the *AA* genotype. Moreover, we found significant associations in cases between smoking status, physical activity, and the rs6687758 SNP. The results of a Genetic Risk Score (GRS) showed that the risk alleles were more frequent in cases than controls and the score was associated with CRC in crude analysis. In conclusion, we have confirmed a CRC susceptibility locus and the existence of associations between modifiable factors and the rs6687758 SNP; moreover, the GRS was associated with CRC. However, further experimental validations are needed to establish the role of this SNP, the function of the gene identified, as well as the contribution of the interaction between environmental factors and this locus to the risk of CRC.

Introduction

Colorectal cancer (CRC)is the fourth most common type of tumour, being 6.1% of the total new cases of cancer diagnosed in 2018and one of the major causes of cancer-related morbidity and mortality globally(9.2% of cancer deaths) [1].There is wide geographical variation in incidence with rates varying 8-fold (colon cancer) and 6-fold (rectal cancer) in both sexes worldwide [1].In this sense, Spain is one of the countries with the highest incidence of CRC, and taking into account both sexes, it was the most frequent cancer diagnosed in 2018 with 13.7% of new cancer cases [2] and is the main cause of cancer related deaths [3]. Considering the magnitude of the problem, the use of screening tests for early detection and effective treatment of CRC during the initial stages would have a significant impact on public health. In this sense, US Preventive Services Task Force and the American Cancer Society recommend the screening for CRC by annual faecal occult blood testing (FOBT), flexible sigmoidoscopy or (every 5 years) or colonoscopy (every 10 years), in subjects aged 50 years or older [4].

The mechanisms underlying CRC occurrence and progression are complicated and mainly involve genetic and environmental factors, such as sex [5,6], diet and physical activity [5,7].Various oncogenes and tumour suppressors, such as *KRAS, APC, BRAF, TP53*, and *SMAD4*, have been identified by CRC-related studies and may be useful for diagnosing and treating CRC in the future [5,8,9]. There is a direct association between sporadic tumour occurrence and susceptibility variants carried by an individual [10]. Many candidate gene [11] and genome-wide association studies (GWAS) [12] have evaluated common genetic risk factors for CRC; however, only a few of these have been replicated in subsequent studies [10].Thus, in this study, we aimed to test the hypothesis that some of the previously reported CRC-related SNPs are associated with CRC susceptibility in the Basque population, in which there are no previous studies of this kind. Therefore, we investigated possible associations between 48 susceptibility SNPs and development of sporadic CRC in the adult population of the Basque Country.

Methods

Design

This is an observational, matched case-control study in a population group residing in the Basque Country (Spain).

Study Population

Participants in this study were recruited among patients attending, between January 2012 and December 2014, any of the three hospitals of the Osakidetza/Basque Health Service (Basurto, Galdakao and Donostia)belong of the Basque Country Colorectal Cancer Screening Programme (CRCSP) [13].To be eligible for this CRCSP, average risk people from 50 to 69 years, asymptomatic for colorectal symptoms and registered with the Osakidetza/Basque Health Service [13]. Subjects with symptoms suggesting CRC or with high CRC risk, such as individuals with familial adenomatous polyposis or hereditary nonpolyposis are managed outside this programme and are not included in this analysis. Subjects were invited to participate in this study by the gastroenterologists who performed the colonoscopies as a confirmatory test.

The recruitment and data collection for the present study were conducted between 2014 and 2016. All the patients who were newly diagnosed with CRC (n=601) were invited to participate in this study, that is, the individuals with a positive result, (abnormal) to an immunochemical faecal occult blood test (iFOBT), being the faecal-Haemoglobin cut-off point of 20 μg Hb/g faeces for both sexes [13] and a colonoscopy [13]. Of those, 283 refused to participate in the study, and 10 were excluded due to missing information. Ultimately, 308 subjects (66.2% men) consented to participate in the survey and completed all the questionnaires.

In addition, for each case, three age- (±9.0 years) and sex-matched control patients were randomly sought from the list of CRC-free subjects (n=1,836) who participated in the CRCSP during the same period as the cases. The matched controls were patients with positive results (abnormal) for iFOBT and negative colonoscopy results (normal). The participation rate of the controls was 37.6%, and 17 subjects were excluded due to missing information. Finally, the matched case-to-control ratio was 1:1, and the final dataset included 308 cases who were diagnosed with CRC and 308 age- and sex-matched controls. The flowchart displaying the selection process for the CRC cases and controls is shown in Fig 1. Thirty-three cases, 39 controls and 6 cases-controls initially included in this study were excluded from the genetic analysis because incomplete genotyping by insufficient DNA available for the assay, and the respective partners of cases and controls were also excluded of the study. Finally, genotyping data were obtained from 230 cases and 230 controls.

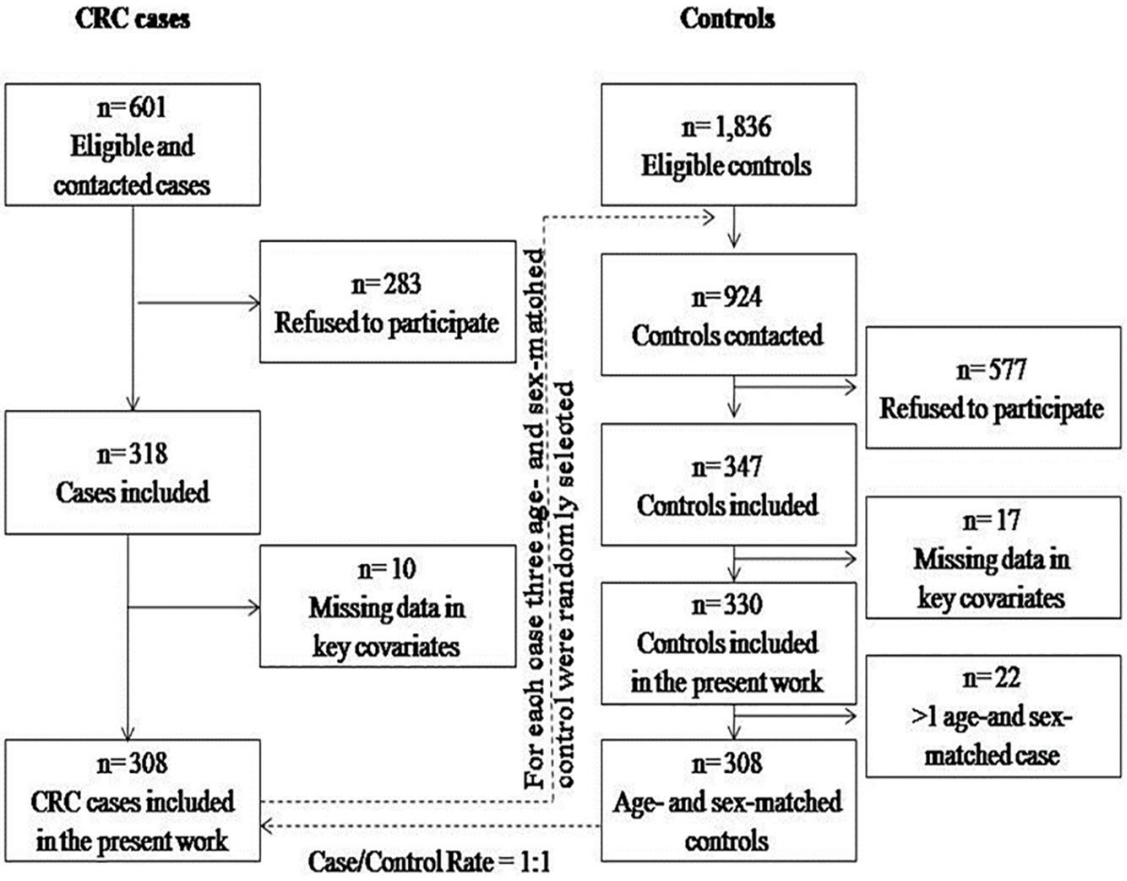


Fig 1. Flow chart of the process of obtaining the sample. CCR, Colorectal cancer.

The time spent between the participation in the CRCSP and in the present study was 1.8(1.0) years (range: 0.4-4.6) in cases and 1.6(1.5) years (range: 0.2-3.7) in controls, without significant differences (P=0.119). Consenting participants self-completed and returned a detailed Food Frequency Questionnaire (FFQ) and one general questionnaire (GQ). The questions referred to the behaviours before participating in the CRCSP. Assistance from the study staff was available to help the patients to understand the items on the questionnaires.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the Clinical Research Ethics Committee of the Basque Country (reference numbers PI2011006 and PI2014042). Written informed consent was obtained from all the study participants.

Biological Samples and Genotyping

In this study, healthy tissues or saliva samples of 230 CRC patients and 230 controls were collected and genotyped. Samples were provided by the Basque Biobank for Research-OEHUN www.biobancovasco.org and were processed following standard

operating procedures with appropriate ethical approval. DNA was extracted using AllPrep DNA / RNA kit (Qiagen) for paraffin-embedded tissue samples and AutoGenFlex Tissue DNA Extraction kit (Autogen) for mouthwash saliva samples and then was quantified with NanoDrop™ Spectrophotometer (ThermoFisher).

Double-stranded DNA was quantified by fluorometry using theQuant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, CA) on a DTX 880 Multimode Detector (Beckman Coulter) to normalize DNA concentration.After an updated summary of the published SNPs associated with susceptibility for development of CRC [14,15], those shown in Table 1 were selected. These SNPs were organized in the context of the gene(s) at or near locus and chromosome locus. The allelic discrimination was assessed using the MassARRAY® System (Agena Bioscience) on CeGen-PRB2-ISCII (Nodo USC) following the procedure provided by the manufacturer. Quality control samples were included in the genotyping assays.

Table 1. 48 SNPs associated with susceptibility for the development of CRC and analyzed in this study.

SNP	Gene(s) at or near locus, variant type	Chr. locus	OR[a]	Risk allele[b]	SNP	Gene(s) at or near locus, variant type	Chr. locus	OR[a]	Risk allele[b]
rs12080929	*TRABD2B,* intron variant	1p33	0.87	*C* [61]	rs1535	*FADS2,* intron variant, FADS1, upstream gene variant	11q12.2	1.15	*A* [69]
rs6687758	*DUSP10,* regulatory region variant	1q41	1.04	*G* [62]	rs3802842	*COLCA1,* upstream gene variant*, COLCA2,* intron variant	11q23.1	1.14	*C* [69]
rs6691170	*LOC105372950, DUSP10,* intergenic variant	1q41	1.01	*T* [62]	rs10849432	*LOC105369625,* intron variant, non coding transcript variant	12p13.31	1.14	*T* [70]
rs10911251	*LAMC1*, intron variant	1q25.3	1.11	*A* [62]	rs3217810[e]	*CCND2,* intron variant, *CCND2-AS1*, upstream gene variant	12p13.32	1.19	*T* [62]
rs11903757	*NABP1/SDPR,* Intergenic variant	2q.32.3	1.14	*C* [62]	rs3217901	*CCND2,* intron variant	12p13.32	1.10	*G*[71]
rs10936599	*MYNN,* upstream gene variant	3q26.2	1.02	*C* [62]	rs10774214	*CCND2,* intron variant, non coding transcript variant	12p13.32	1.17	*T* [72]
rs647161	*C5orf66,* Intron variant, non coding transcript variant	5q31.1	1.07	*A* [62]	rs7136702	*LARP4/DIP2B, ATF1,* intergenic variant	12q13.12	1.10	*T* [62]
rs2736100	*TERT,* 3 prime UTR variant	5p15.33	1.07	*A* [63]	rs11169552	*LARP4/DIP2B, ATF1,* upstream gene variant	12q13.12	1.05	*C* [62]
rs1321311	*SRSF3/CDKN1A,* regulatory region variant	6p21.2	1.07	*A* [62]	rs59336	*TBX3,* intron variant	12q24.21	1.15	*T* [62]
rs11987193	*DUSP4,* intergenic variant	8p12	0.79	*T* [61]	rs4444235	*BMP4/ATP5C1P1/CDKN3/MIR5580,* downstream gene variant	14q22.2	1.11	*C* [73]

To be continued in the next page.

Continuation of Table 1.

SNP	Gene(s) at or near locus, variant type	Chr. locus	OR[a]	Risk allele[b]	SNP	Gene(s) at or near locus, variant type	Chr. locus	OR[a]	Risk allele[b]
rs16892766	*TRPS1/EIF3H/UTP23,* downstream gene variant	8q23.3	1.25	*C* [63]	rs1957636	*LOC105370507,* regulatory region variant	14q22.2	1.03	*T* [74]
rs6983267	*CCAT2,* intron variant, non coding transcript variant, *CCAT2*, non coding transcript exon variant	8q24.21	1.15	*G* [63]	rs4779584	*SCG5, GREM1, FMN1,* intergenic variant	15q13.3	1.18	*T* [70]
rs10505477	*CASC8,* intron variant, non coding transcript variant	8q24.21	1.11	*A* [64]	rs16969681	*GREM 1,* downstream gene variant	15q13.3	1.18	*T* [75]
rs7014346	*CASC8,* intron variant, non coding transcript variant, *POU5F1B*, intron variant	8q24.21	1.20	*A* [65]	rs11632715	*SCG5, GREM1, FMN1,* intergenic variant	15q13.3	1.12	*A* [76]
rs719725	*TPD52L3/UHRF2/GLDC,* intergenic variant	9p24.1	1.08	*A* [61]	rs9929218	*CDH1,* intron variant	16q22.1	1.10	*A* [75]
rs10795668	*LOC105376400,* upstream gene variant	10p14	1.32	*A* [66]	rs12603526	*NXN,* intron variant	17p13.3	1.10	*C* [69]
rs704017	*ZMIZ1-AS1,* intron variant, non coding transcript variant	10q22.3	1.13	*G* [67]	rs4939827	*SMAD7,* intron variant	18q21.1	1.16	*T* [77]
rs1035209	*ABCC2/MRP2,* intergenic variant	10q24.2	1.13	*T* [68]	rs10411210	*RHPN2,* intron variant	19q13.11	1.15	*C* [73]
rs12241008	*VTI1A,* intron variant	10q25.2	1.19	*C* [38]	rs1800469	*TGFB1,* upstream gene variant *B9D2,* downstream gene variant, *TMEM91*, intron variant	19q13.2	1.09	*G*[69]

To be continued in the next page.

Continuation of Table 1.

SNP	**Gene(s) at or near locus, variant type**	**Chr. locus**	**OR[a]**	**Risk allele[b]**	**SNP**	**Gene(s) at or near locus, variant type**	**Chr. locus**	**OR[a]**	**Risk allele[b]**
rs11196172	*TCF7L2,* intron variant	10q25.2	1.14	*A* [69]	rs2241714	*TGFB1, TMEM91,* upstream gene variant, *B9D2,* missense variant	19q13.2	1.09	*C* [70]
rs1665650	*HSPA12A,* intron variant	10.q25.3	0.95	*T* [64]	rs961253	*BMP2/HAO1/FERMT1,* upstream gene variant	20p12.3	1.12	*A* [73]
rs174537	*TNEM258,* downstream gene variant, *MYRF,* intron variant	11q12.2	1.16	*G* [64]	rs4813802	*BMP2/HAO1/FERMT1,* regulatory region variant	20p12.3	1.10	*C* [70]
rs4246215	*TNEM258,* upstream gene variant *FEN1,* 3 prime UTR variant, FADS1, downstream gene variant, MIR611, upstream gene variant, FADS2, intron variant	11q12.2	1.15	*G* [69]	rs2423279	*HAO1/PLCB1,* downstream gene variant	20p12.3	1.10	*C* [72]
rs174550	*FADS1,* intron variant	11q12.2	1.15	*T* [69]	rs5934683	*SHROOM,* upstream gene variant, GPR143, intron variant	Xp22.2	1.04	*C* [31]

Chr, Chromosome; OR, odds ratio; SNP, single nucleotide polymorphism

[a]Odds ratios of previous studies are reported to calculate weighted Genetic Score

[b] Superscript numbers correspond with the studies in References

Associated data

The questionnaire mentioned above, the GQ was used to gather information on weight status (self-reported weight and height) and environmental factors (demographic factors: age and sex; and lifestyle information: physical activity (PA) and smoking consumption). These questions were taken from the Spanish Health Questionnaire [16]. Body mass index (BMI), estimated from self-reported height and weight was classified according to the WHO criteria for those under 65 years of age [17] and according to the criteria proposed by Silva Rodríguez *et al.* for those 65 years and older [18].

Diet was assessed using a short FFQ that is a modified version of the Rodríguez *et al.* questionnaire [19]. This adaptation was validated with multiple 24- recalls in a subsample of the participants [20]. It consists of 67 items and requires the subjects to recall the number of times each food item was consumed either per week or per month. The respondents might also record the consumption of other foods that were not included on the food list.

Average portion sizes were employed to convert FFQ consumptions [21]. For items that included several foods, each food's contribution was estimated with weighting coefficients that were obtained from the usual consumption data [22]. All the food items that were consumed were entered into DIAL 2.12 (2011ALCE INGENIERIA), a type of dietary assessment software, to estimate energy intake (kcal/d).Moreover, the FFQ included specific questions about their frequency of intake of five major types of alcohol beverages: beer, wine, cider, aperitif with alcohol and liquor. In terms of the amount consumed, 10 g of alcohol was considered a standard drink [23]. Participants were categorized into non-drinker/moderate consumption and risk consumption, according to the SENC criteria that consider moderate drinking is up to 1 standard drink per day for women and up to 2 standard drinks per day for men [23]. Alcohol consumption was also expressed in tertiles of ml per day according to sex (men: T1, ≤ 70.6; T2, 70.7-138.8; T3, ≥ 138.9; and women: T1 ≤ 5.8; T2, 5.9-69.8; T3, ≥ 69.9).

Additionally, socioeconomic data was assessed with an index that was obtained from the clinical databases developed by the Health Department of the Basque Government, namely the socioeconomic deprivation index (DI). This index was estimated using the MEDEA project criteria [24] from simple indicators in the 2001 Census, namely unemployment, manual workers, casual workers, low education level and low education level among young people. The DI was divided into quintiles (Q), with the first being the least disadvantaged and the fifth being the most disadvantaged. The DI was successfully

assigned to 82.4% of participants, while the address information quality did not permit the linking of the remaining 17.6%.

Quality management

In the present research, we apply a similar quality management that those used in the IDEFICS study [25]. A unique subject identification number was attached to each recording sheet, questionnaire, and sample, as in other researches. The identification number had to be entered twice before the document could be entered into its respective database. All data were entered twice independently, and deviating entries were corrected. Inconsistencies that were identified by additional plausibility checks were rectified.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, USA), STATA 13.0 (StataCorp LP, Texas, USA). Categorical variables are shown as a percentage, and continuous variables are shown as the means and standard deviations (s.d.). Normality was checked using Kolmogorov-Smirnov-Lilliefors test. Paired *t*-test or Wilcoxon rank-sum test was used to two related means comparison, and a $\chi 2$ test was used to evaluate differences. Tests for association and deviation from Hardy-Weinberg equilibrium were performed separately in CRC patients and healthy controls. When expected frequencies were lesser than 5, Fisher's exact test was used.

In the case-control study, we estimated the odds ratio (OR) and 95% confidence interval (95% CI) for the polymorphism selected using conditional logistic regression adjusted for age (50-59 years old *vs.* 60-69 years old),sex(women *vs.* men), BMI (underweight/normal weight *vs*. overweight/obesity), physical activity (≥15 min/d *vs.*<15 min/d), smoking status (never smoker *vs.* current and former smoker and quit smoking: ≥ 11 years ago *vs.*< 11 years ago), alcohol consumption (T1, T2 and T3) and Deprivation Index (DI) (quintile 1-3 *vs.* quintile 4-5) as categorical variables and energy intake as quantitative (kcal/d).ORs were calculated for the codominant model, dominant model, recessive model, and allelic comparison. The most frequent genotype (homozygous) was considered the reference group to calculate ORs in a codominant and dominant model, and the most frequent genotype (homozygous) and the heterozygous genotype containing the risk allele were considered the reference group in the recessive model. The significance level was corrected using a Bonferroni correction by dividing the standard *P* value (two-tailed) (0.05) by the total number of SNPs analyzed (*n*=48), assuming alpha was equal to 0.001 (α=0.05/48).

Additionally, correspondence analysis (CA) was performed using PAST 3.21 to identify potential associations between SNPs associated with CRC and associated data.CA is a multivariate statistical technique which provides Cartesian diagrams based on the association of the variables examined. All variables were represented in graphs and the more closed are the points the more higher is the level of association between variables [26].

To assess genetic susceptibility, two methods were used as a simple, unweighted count method (count Genetic Risk Scores, c-GRS) and a weighted method (w-GRS) [27,28]. Both methods assumed each SNP to be independently associated with risk [29]. An additive genetic model was assumed: weightings of 0, 1, and 2 were given according to the number of risk alleles present [29,30].

The count method assumed that each SNP contributed equally to CRC risk and was calculated by summing the number of risk alleles across the panel of SNPs tested. This produced a score between 0 and twice the number of SNPs, i.e., representing the total number of risk alleles. The weighted GRS was calculated by multiplying each β-coefficient for the CRC phenotype from the discovery set by the number of corresponding risk alleles (0, 1, or 2 copies of the risk allele except for the SNP rs5934683 in chromosome X that was coded 0, 0.5, and 1) and then summing the products [31].

Finally, we defined the GRS as the count of risk alleles across all 48 SNPs, ranging from 0 to 95 for c-GRS and 0 to 105 for w-GRS. Since the published effects of each SNP were similar, an unweighted GRS was preferred. However, we also explored the models using weights derived from the GWAS publications and models fitted to our data [32].

Gene expression association analyses

Gene expression changes in tumour and normal colon tissue associated to SNPs with significant association with CRC risk were analyzed using publicly available data and bioinformatic tools. In the first place Genomic Data Commons Data Portal (GDC) (https://portal.gdc.cancer.gov) was used to examine data generated by the TCGA (The Cancer Genome Atlas) research network (https://www.cancer.gov/tcga), but for SNPs with unavailable data in GDC portal alternative bioinformatic tools were applied. On the one hand, gene expression data from between case and control samples of colon and rectum adenocarcinomas were compared using GEPIA (Gene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/index.html) [33] . On the other hand, GTEx (The Genotype-Tissue expression project) (https://gtexportal.org/home/) was used

to check the relationship between SNPs and the expression level of genes related to these SNPs in colon tissue of healthy donors.

Results

Table 2 shows the comparisons of associated data between cases and controls. Cases had a higher consumption of cigarettes/day and were more engaged in regular physical activity at a medium-high level as compared with controls. In addition, in the total sample, there were more smokers in men than in women (70.6% vs. 54.5%; *P*<0.001); and had a higher consumption of cigarettes/day (11.6(11.1) vs. 9.0(11.4); *P*=0.030). Among controls 51.9% of women and 65.4% of men were smokers (*P*=0.049); and among cases, 57.1% of women and 75.8% of men were smokers (*P*=0.004).

The distribution of genotypes and alleles at SNPs selected in the CRC group and in the control group that deviated from the Hardy-Weinberg equilibrium are shown in Supplementary Material(S1 Table).The SNPs that were not following the Hardy-Weinberg equilibrium in cases were rs12080929 and rs5934683.None of the genotype or allele frequencies for the SNPs analysed reached statistically significant differences between cases and controls, after Bonferroni correction application.

Table 3 presents some results of the association of susceptibility genotypes and alleles with the risk of CRC in the codominant model. Other SNPs analyzed in this study are shown in Supplementary Material (S2 Table). Adjusting for potential confounders did not appreciably alter the observed ORs. Only the rs6687758 exhibited a statistically significant association with CRC risk based on the crude analysis. The AG genotype of rs6687758 conferred about 2.13-fold increased risk for CRC compared to the AA genotype.

Table 2. Comparison of associated data between cases and controls with genotyping data.

	Cases (n=230)	**Controls (n=230)**	***P*[a]-*value***
Age, years, mean(s.d.)	61.5(5.4)	60.9(5.5)	0.333
BMI classification, %			
NonOv/Ob	42.2	33.0	
Ov/Ob	57.8	67.0	**0.043**
Physical activity level, %			
Low	65.7	77.4	
Medium and high	34.3	22.6	**0.005**

To be continued in the next page.

Continuation of Supplementary Table 2.

	Cases (n=230)	**Controls (n=230)**	***P*[a]-*value***
Smoking status, %[b]			
Non-smoker	30.4	39.1	
Smoker	69.6	60.9	0.050
Cigarettes, cigarettes/day, mean(s.d.)	10.7(11.2)	8.3(10.9)	**0.007**
Number of cigarettes, %[b]			
< 15	49.3	66.9	
≥ 15	50.7	33.1	**0.003**
Alcoholic beverage intake, ml/day, mean(s.d.)	98.0(91.5)	97.2(107.5)	0.637
Tertiles of alcohol intake, ml/day[c], %			
T1	32.6	33.9	
T2	31.3	35.7	
T3	36.1	30.4	0.404
Standard drink units, classification, %			
Abstemious /low risk	72.1	79.1	
High risk	27.9	20.9	0.078
DI, %[b]			
Q1-Q3	73.5	69.6	
Q4-Q5	26.5	30.4	0.409

BMI, body mass index; DI, deprivation index; Ob, obesity; Ov, overweight, Q, quintile; s.d. standard deviation

[a]P<0.05 wassignificant

[b]Valid percentages

[c]Men: T1, ≤ 70.6; T2, 70.7-138.8; T3, ≥ 138.9; and women: T1 ≤ 5.8; T2, 5.9-69.8; T3, ≥ 69.9

Table 3. Association between genetic variants associated with susceptibility and the risk of CRC in the codominant model.

Gene, SNP ID[a]	**N (cases/controls)**	**Model I[b]**		**Model II[c]**	
		OR (95% CI)	***P*[d]-value**	**OR (95% CI)**	***P*[d]-value**
rs6687758					
AA	136/169	1.00	-	1.00	-
AG	87/51	2.13(1.39-3.25)	<0.001	1.95(1.05-3.60)	0.034
GG	7/9	1.02(0.37-2.82)	0.967	1.06(0.21-5.28)	0.945
A	359/389	1.00	-	1.00	-
G	101/69	1.60(1.13-2.28)	0.009	1.54(0.97-2.46)	0.067
rs6691170					
GG	72/87	1.00	-	1.00	-

To be continued in the next page.

Continuation of Table 3.

Gene, SNP ID[a]	N (cases/controls)	Model I[b]		Model II[c]	
		OR (95% CI)	*P*[d]-value	OR (95% CI)	*P*[d]-value
GT	112/108	1.22(0.82-1.82)	0.331	1.20(0.64-2.26)	0.570
TT	45/31	1.79(1.01-3.16)	0.045	1.70(0.74-3.89)	0.207
G	256/282	1.00	-	1.00	-
T	202/170	1.23(0.94-1.62)	0.124	1.27(0.89-1.79)	0.185
rs719725					
AA	63/91	1.00	-	1.00	-
AC	116/106	1.46(0.97-2.18)	0.068	1.99(1.07-3.71)	0.030
CC	51/31	2.14(1.27-3.64)	0.005	1.80(0.78-4.17)	0.168
A	242/288	1.00	-	1.00	-
C	218/168	1.60(1.22-2.11)	<0.001	1.49(1.05-2.10)	0.025
rs12241008					
TT	196/204	1.00	-	1.00	-
CT	33/24	1.47(0.82-2.64)	0.192	1.49(0.75-2.95)	0.253
CC	½	0.50(0.05-5.51)	0.571	0.78(0.05-12.84)	0.862
T	425/435	1.00	-	1.00	-
C	35/28	1.22(0.72-2.09)	0.455	1.34(0.66-2.72)	0.412
rs7136702					
CC	80/91	1.00	-	1.00	-
CT	108/114	1.11(0.75-1.65)	0.593	1.03(0.56-1.89)	0.826
TT	42/25	1.98(1.09-3.64)	0.026	2.83(1.12-7.17)	0.028
C	268/296	1.00	-	1.00	-
T	192/164	1.34(1.03-1.74)	0.030	1.28(0.91-1.80)	0.154
rs2241714					
CC	116/101	1.00	-	1.00	-
CT	94/105	0.79(0.55-1.15)	0.217	0.54(0.31-0.95)	0.034
TT	20/23	0.72(0.37-1.38)	0.321	0.28(0.09-0.89)	0.031
C	326/307	1.00	-	1.00	-
T	134/151	0.80(0.61-1.06)	0.125	0.74(0.51-1.07)	0.114
rs961253					
CC	101/124	1.00	-	1.00	-
AC	103/76	1.65(1.11-2.46)	0.013	1.79(0.67-4.78)	0.247
AA	26/30	1.03(0.57-1.85)	0.925	1.04(0.41-2.63)	0.941
C	305/324	1.00	-	1.00	-
A	155/136	1.20(0.90-1.59)	0.208	1.11(0.76-1.62)	0.584

A, adenine; C, cytosine; CI, confidence interval; G, guanine; OR, odds ratio; rs, reference single nucleotide polymorphism; SNP, single nucleotide polymorphism; T, thymine

[a]The most frequent genotype (homozygous) was considered the reference group

[b]Model I, crude conditional logistic regression model

[c]Model II, conditional logistic regression adjusted forage, sex, BMI, physical activity, smoking status, alcohol consumption, Deprivation Index and energy intake. Participants

with missing data for the confounding variables were included as a separate category for these variables

[d]*P*<0.001 was significant

Moreover, there was an association between smoking status, physical activity and the rs6687758 SNP for CRC risk in cases (Fig 2).We did not find an association between the risk genotype for rs6687758 and other associated variables (BMI, sex, alcohol consumption, DI and age).The results of CA for all cases are shown in a Cartesian diagram. The first three axes accounted for more than 50.0% of the total variance in all cases (axis 1: 23.0%; axis 2: 19.6% and axis 3: 13.4%).An inverse association can be observed between the variable DI (which plotted at the negative end of axis 1) and age, positioned in the positive segment of axis 1. Overall, axis 1 represents a gradient that runs from low values for DI (0: Q1-Q3; 1: Q4-Q5) to high values for age (0:50-59 y; 1:60-69 y).From the genetic viewpoint, the SNP that showed the closest association with associated variables wasrs6687758, which also plotted in the quadrant delimited by the positive segments of axis 1 and 2.

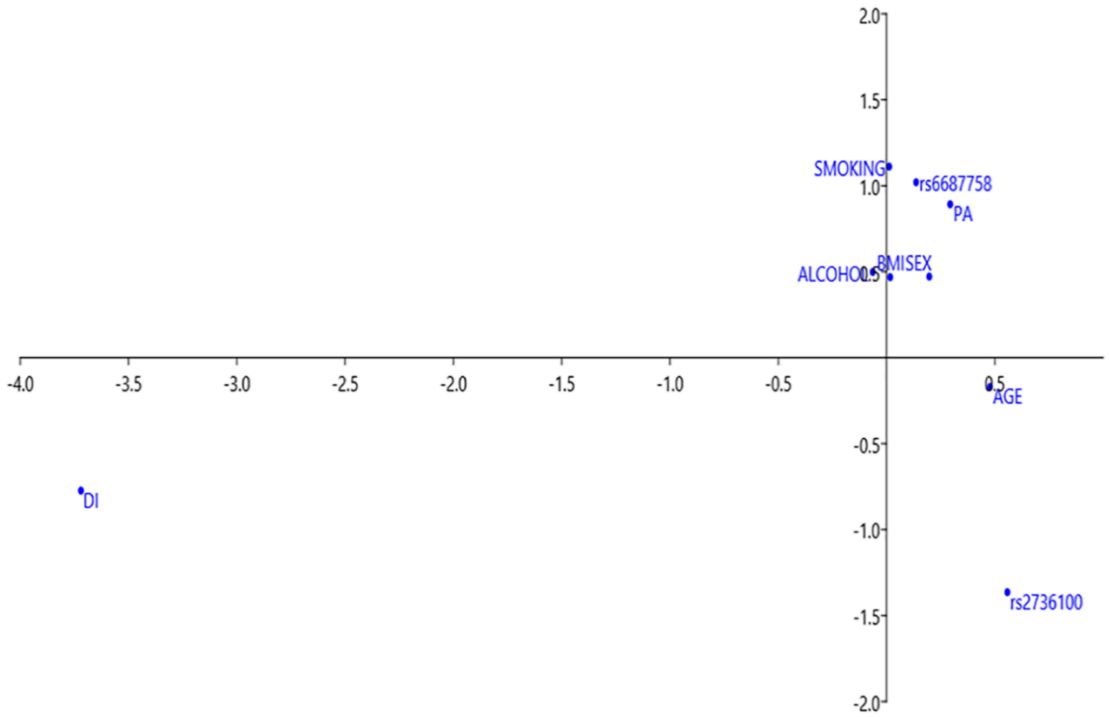


Fig 2. Cartesian diagram of correspondence analysis for studied associations between genetic and environmental factors in cases. BMI, body mass index; DI, deprivation index; PA, physical activity.

Analyses performed to study possible changes in gene expression associated with rs6687758 in tumour versus normal colon tissue showed that gene *DUSP10* is more expressed in colon sigmoid tissue when rs6687758 has GG genotype (in healthy

individuals) (S1 Fig), but also, that it has higher expression in cases of colon and rectum adenocarcinomas than in healthy persons (S2 Fig).

For SNP rs719725,an increased CRC risk was found to be associated with the CC genotype in dominant and recessive models for crude analysis, compared with the AA and AC genotype (OR_{CC}: 1.77; 95% CI=1.09-2.86; *P*=0.020 in recessive model and OR_{AC+CC}: 1.64; 95% CI = 1.12-2.38; *P*=0.010 in dominant model). Moreover, significantly elevated CRC risk was found to be associated with rs2736100, rs11987193and rs961253by using dominant model (for rs2736100 OR_{AA+AC}:1.72; 95%CI = 1.00-2.94; *P*=0.048 in adjusted model; for rs11987193 OR_{CC+CT}: 1.45; 95%CI = 1.01-2.49; *P*=0.046 in crude analysis; and for rs961253 OR_{AA+AC}: 1.47; 95%CI = 1.02-2.11; *P*=0.038 in crude analysis).

Finally, the unweighted GRS of the sample studied was 38.6(4.6) (range: 25-52), with statistically significant differences between cases and controls (39.2(4.4) (range: 28-50.5) vs. 37.95(4.6) (range: 25-52); *P*=0.002). The GRS built as the unweighted count of risk alleles was significantly associated with CRC risk, with an average per-allele OR of 1.07 (95%CI=1.02-1.11; *P*=0.002) in crude analysis. However, this association was not statistically significant in the adjusted model (OR: 1.04; 95% CI=1.00-1.10; *P*=0.066).On the other hand, w-GRS was 44.7(5.5) for the total sample, with statistically significant differences between cases and controls (45.3(5.4) (range: 32.2-58.6)*vs.*44.1(5.6) (range: 27.7-57.6); *P*=0.036).The w-GRS was associated with CRC risk (OR: 1.04; 95% CI=1.00-1.09; *P*=0.037) in crude analysis but not in the adjusted one (OR: 1.01; 95% CI=0.97-1.05; *P*=0.588).

Supporting information

S1 Table. Deviation from Hardy-Weinberg equilibrium and differences in allele frequencies and genotype distribution between cases and controls**.**

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs12080929	*TT*	123(53.5)		132(57.4)		
	CT	8938.7)		83(36.1)		
	CC	18(7.8)	<0.001	15(6.5)	0.690	0.670
	T	335(72.8)		347(75.4)		
	C	125(27.2)	-	113(24.6)	-	0.653
rs6687758	*AA*	136(59.1)		169(73.8)		
	AG	887(37.8)		51(23.3)		
	GG	7(3.0)	0.116	9(3.9)	0.050	0.001
	A	359(78.0)		389(84.6)		
	G	101(22.0)	-	69(15.4)	-	0.016

To be continued in the next page.

Continuation of Supplementary Table S1.

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs6691170	*GG*	72(31.4)		87(38.5)		
	GT	112(48.9)		108(47.8)		
	TT	45(19.7)	0.903	31(13.7)	0.784	0.132
	G	256(55.7)		282(61.3)		
	T	202(44.3)	-	170(38.7)	-	0.150
rs10911251	*AA*	87(37.8)		74(32.3)		
	AC	110(47.8)		107(46.7)		
	CC	33(14.3)	0.852	48(21.0)	0.420	0.145
	A	284(61.7)		255(55.4)		
	C	176(38.3)	-	203(44.6)	-	0.066
rs11903757	*TT*	150(65.2)		168(73.4)		
	CT	74(32.2)		57(24.9)		
	CC	6(2.6)	0.376	4(1.7)	0.934	0.170
	T	374(81.3)		393(85.4)		
	C	86(18.7)	-	65(14.6)	-	0.044
rs10936599	*CC*	150(65.2)		135(58.7)		
	CT	74(32.2)		81(35.2)		
	TT	6(2.6)	0.376	14(6.1)	0.692	0.116
	C	374(81.3)		351(76.3)		
	T	86(18.7)	-	109(23.7)	-	0.053
rs647161	*AA*	101(44.9)		104(45.6)		
	AC	95(42.2)		105(46.1)		
	CC	29(12.9)	0.374	19(8.3)	0.292	0.273
	A	297(64.6)		313(68.0)		
	C	153(35.4)	-	143(32.0)	-	0.359
rs2736100	*CC*	61(26.6)		69(30.0)		
	AC	121(52.8)		119(51.7)		
	AA	47(20.5)	0.358	42(18.3)	0.455	0.674
	C	243(52.8)		257(55.9)		
	A	215(47.2)	-	203 (44.1)	-	0.235
rs1321311	*CC*	116(50.9)		129 (56.3)		
	AC	102(44.7)		88 (38.4)		
	AA	10(4.4)	0.033	12 (5.2)	0.544	0.384
	C	334(72.6)		346 (75.2)		
	A	122(27.4)	-	112 (24.8)	-	0.086
rs11987193	*CC*	105(45.7)		127 (55.2)		
	CT	110(47.8)		90 (39.1)		
	TT	15(6.5)	0.050	13 (5.7)	0.570	0.121
	C	320(69.6)		344 (74.8)		
	T	140(30.4)	-	116 (25.2)	-	0.067
rs16892766	*AA*	202(87.8)		209(90.9)		
	AC	27(11.7)		21(9.1)		
	CC	1(0.4)	1.000	0(0.0)	0.907	0.393
	A	431(93.7)		439(95.4)		
	C	29(6.3)	-	21(4.6)	-	0.130
rs6983267	*GG*	75(32.6)		64(27.8)		
	GT	115(50.0)		117(50.9)		
	TT	40(17.4)	0.719	49(21.3)	0.742	0.407
	G	265(57.6)		245(53.3)		
	T	195(42.4)	-	215(46.7)	-	0.144

To be continued in the next page.

Continuation of Supplementary Table S1.

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs10505477	*AA*	71(32.3)		64(30.9)		
	AG	110(50.0)		110(47.8)		
	GG	39(17.7)	0.667	53(23.0)	0.667	0.304
	A	252(54.8)		238(51.7)		
	G	188(45.2)	-	216(48.3)	-	0.110
rs7014346	*GG*	95(4.3)		108(47.2)		
	AG	107(46.5)		90(39.3)		
	AA	28(12.2)	0.800	31(13.5)	0.085	0.294
	G	297(64.6)		306(66.5)		
	A	163(35.4)	-	152(33.5)	-	0.357
rs719725	*AA*	63(27.4)		91(39.9)		
	AC	116(50.4)		106(46.5)		
	CC	51(22.2)	0.862	31(13.6)	0.988	0.005
	A	242(52.6)		288(62.6)		
	C	218(47.4)	-	168(37.4)	-	0.002
rs10795668	*GG*	110(47.8)		104(45.4)		
	AG	100(43.5)		104(45.4)		
	AA	20(8.7)	0.685	21(9.2)	0.490	0.874
	G	320(69.6)		312(67.8)		
	A	140(30.4	-	146(32.2)	-	0.222
rs704017	*AA*	67(29.5)		63(27.4)		
	AG	116(51.1)		121(52.6)		
	GG	44(19.4)	0.623	46(20.0	0.772	0.881
	A	250(54.3)		247(53.7)		
	G	204(45.7)	-	213(46.3)	-	0.632
rs1035209	*CC*	146(63.8)		154(67.2)		
	CT	75(32.8)		61(26.5)		
	TT	8(3.5)	0.666	14(6.1)	0.024	0.193
	C	367(79.8)		369(80.2)		
	T	91(20.2)	-	89(19.8)	-	0.870
rs12241008	*TT*	196(85.2)		204(88.7)		
	CT	33(14.3)		24(10.4)		
	CC	1(0.4)	0.973	2(0.9)	0.419	0.442
	T	425(92.4)		432(93.9)		
	C	35(7.6)	-	28(6.1)	-	0.430
rs11196172	*GG*	174(76.0)		172(74.8)		
	AG	53(23.1)		49(21.3)		
	AA	2(0.9)	0.640	9(3.9)	0.092	0.099
	G	401(87.2)		393(85.4)		
	A	57(12.8)	-	67(14.6)	-	0.257
rs1665650	*CC*	139(60.4)		149(64.8)		
	CT	75(32.6)		73(31.7)		
	TT	16(7.0)	0.189	8(3.5)	0.797	0.219
	C	353(76.7)		371(80.6)		
	T	107(23.3)	-	89(19.4)	-	0.230
rs174537	*GG*	113(49.3)		102(44.5)		
	GT	98(42.8)		103(45.0)		
	TT	18(7.9)	0.609	24(10.5)	0.790	0.462
	G	324(70.4)		307(66.7)		
	T	134(29.6)	-	151(33.3)	-	0.255

To be continued in the next page.

Continuation of Supplementary Table S1.

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs4246215	*GG*	113(49.1)		101(43.9)		
	GT	98(42.6)		105(45.7)		
	TT	19(8.3)	0.727	24(10.4)	0.668	0.473
	G	324(70.5)		307(66.7)		
	T	136(29.5)	-	153(33.3)	-	0.238
rs174550	*TT*	114(49.6)		101(43.9)		
	CT	97(42.2)		104(45.2)		
	CC	19(8.3)	0.818	25(10.9)	0.797	0.397
	T	325(70.7)		306(66.5		
	C	135(29.3)	-	154(33.5)	-	0.121
rs1535	*AA*	116(50.4)		96(41.7)		
	AG	93(40.4)		107(46.5)		
	GG	21(9.1)	0.705	27(11.7)	0.733	0.164
	A	325(70.7)		299(65.0)		
	G	135(29.3)	-	161(35.0)	-	0.067
rs3802842	*AA*	109(47.6)		107(46.5)		
	AC	99(43.2)		104(45.2)		
	CC	21(9.2)	0.827	19(8.3)	0.367	0.887
	A	317(68.9)		318(69.1)		
	C	141(31.1)	-	142(30.9)	-	0.965
rs10849432	*TT*	171(74.3)		174(75.7)		
	CT	57(24.8)		53(23.0)		
	CC	2(0.9)	0.495	3(1.3)	0.902	0.839
	T	399(86.7)		401(87.2)		
	C	61(13.3)	-	59(2.8)	-	0.923
rs3217810	*CC*	182(79.5)		191(83.4)		
	CT	45(19.7)		38(16.6)		
	TT	2(0.9)	0.909	0(0.0)	0.646	0.271
	C	409(88.9)		420(91.3)		
	T	49(11.1)	-	38(8.7)	-	0.923
rs3217901	*AA*	85(37.1)		90(39.5)		
	AG	111(48.5)		111(48.7)		
	GG	33(37.1)	0.738	27(11.8)	0.413	0.691
	A	281(61.1)		291(63.3)		
	G	177(38.9)	-	165(36.7)	-	0.236
rs10774214	*CC*	106(46.7)		109(47.4)		
	CT	101(44.5)		95(41.3)		
	TT	20(8.7)	0.557	26(11.3)	0.446	0.610
	C	313(68.0)		313(68.0)		
	T	141(32.0)	-	147(32.0)	-	0.675
rs7136702	*CC*	80(34.8)		91(39.6)		
	CT	108(46.9)		114(49.6)		
	TT	42(18.3)	0.601	25(10.8)	0.224	0.075
	C	268(58.3)		296(64.3)		
	T	192(41.7)	-	164(35.7)	-	0.043
rs11169552	*CC*	151(65.9)		128(56.9)		
	CT	71(31.0)		89(39.6)		
	TT	7(3.1)	0.698	8(3.6)	0.113	0.139
	C	373(81.1)		345(75.0)		
	T	85(18.9)	-	105(25.0)	-	0.229

To be continued in the next page.

Continuation of the Supplementary Table S1.

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs59336	*AA*	63(27.4)		58(25.2)		
	AT	110(47.8)		109(47.4)		
	TT	57(24.8)	0.516	63(27.4)	0.433	0.774
	A	236(51.3)		225(48.9)		
	T	224(48.7)	-	235(51.1)	-	0.323
rs4444235	*CC*	61(26.5)		69(30.0)		
	CT	118(51.3)		113(49.1)		
	TT	51(22.2)	0.671	48(20.9)	0.890	0.708
	C	240(52.2)		251(54.6)		
	T	220(47.8)	-	209(45.4)	-	0.644
rs1957636	*CC*	80(34.9)		97(42.2)		
	CT	109(47.6)		95(41.3)		
	TT	40(17.5)	0.784	38(16.5)	0.079	0.267
	C	269(58.5)		289(62.8)		
	T	189(41.5)	-	171(37.2)	-	0.411
rs4779584	*CC*	164(71.6)		166(72.2)		
	CT	60 26.2)		57(24.8)		
	TT	5(2.2)	0.858	7(3.0)	0.442	0.811
	C	388(84.3)		389(84.6)		
	T	67(15.7)	-	71(15.4)	-	0.627
rs16969681	*CC*	191(83.0)		180(78.3)		
	CT	37(16.1)		48(20.9)		
	TT	2(0.9)	1.000	2(0.9)	0.856	0.378
	C	419(91.1)		408(88.7)		
	T	41(8.9)	-	61(11.3)	-	0.660
rs11632715	*GG*	84(36.7)		84(36.8)		
	AG	108(47.2)		105(46.1)		
	AA	37(16.2)	0.817	39(17.1)	0.530	0.955
	G	276(60.3)		273(59.9)		
	A	182(39.7)		183(40.1)	-	0.808
rs9929218	*GG*	111(48.5)		111(48.3)		
	AG	101(44.1)		100(43.5)		
	AA	17(7.4)	0.357	19(8.3)	0.593	0.945
	G	323(70.2)		322 (70.0)		
	A	135(29.8)	-	135 (30.0)	-	1.000
rs12603526	*TT*	228(99.1)		228(99.1)		
	CT	2(0.9)		2(0.9)		
	CC	0(0.0)	1.000	0(0.0)	1.000	1.000
	T	458(99.6)		458(99.6)		
	C	2(0.4)	-	2(0.4)	-	1.000
rs4939827	*TT*	66(28.8)		70(30.6)		
	CT	125(54.6)		112(48.9)		
	CC	38(16.6)	0.101	47(20.5)	0.857	0.410
	T	257(55.9)		252(54.8)		
	C	201(44.1)	-	206(45.2)	-	0.232
rs10411210	*CC*	180(78.6)		178(77.4)		
	CT	45(19.7)		51(22.2)		
	TT	4(1.7)	0.699	1(0.4)	0.362	0.336
	C	405(88.0)		407(88.5)		
	T	53(12.0)	-	53(12.5)	-	0.743

To be continued in the next page.

Continuation of the Supplementary Table S1.

SNP ID	Genotype Alleles	Cases n(%)[a]	HWE *P*[b]-value	Controls n(%)[a]	HWE *P*[b]-value	Diff.[c] *P*[b]-value
rs1800469	*GG*	118(51.8)		104(45.4)		
	AG	91(39.9)		102(44.5)		
	AA	19(8.3)	0.806	23(10.0)	0.783	0.389
	G	327(71.1)		310(67.4)		
	A	129(28.9)	-	148(32.6)	-	0.127
rs2241714	*CC*	116(50.4)		101(44.1)		
	CT	94(40.9)		105(45.9)		
	TT	20(8.7)	0.877	23(10.0)	0.572	0.396
	C	326(70.9)		307(66.7)		
	T	134(29.1)	-	151(33.3)	-	0.220
rs961253	*CC*	101(43.9)		124(53.9)		
	AC	103(44.8)		76(33.0)		
	AA	26(11.3)	0.973	30(13.0)	0.002	0.035
	C	305(66.3)		324(70.4)		
	A	155(33.7)	-	136(29.6)	-	0.157
rs4813802	*TT*	113(49.3)		112(48.9)		
	GT	88(38.4)		94(41.0)		
	GG	28(12.2)	0.100	23(10.0)	0.618	0.707
	T	314(68.3)		318(69.1)		
	G	144(31.7)	-	140(30.9)	-	0.775
rs2423279	*TT*	94(40.9)		109(47.4)		
	CT	113(49.1)		101(43.9)		
	CC	23(10.0)	0.192	20(8.7)	0.618	0.370
	T	301(65.4)		319(69.3)		
	C	159(34.6)	-	141(30.7)	-	0.325
rs5934683	*C*	102(37.6)		116(32.2)		
	CT	41(17.9)		40(17.4)		
	T	86(44.5)	<0.001	74(50.4)	0.001	0.405
	C	245(53.3)		272(59.1)		
	T	213(46.7)	-	188(41.9)	-	0.127

A, adenine; C, cytosine; G, guanine; HWE, Hardy-Weinberg equilibrium; rs, reference single nucleotide polymorphism; SNP, single nucleotide polymorphism; T, thymine.

[a]Valid percentages.

[b]P<0.001 was significant.

[c]Differences in allele frequencies and genotype distribution between cases and controls.

S2 Table. Association between genetic variants associated with susceptibility and the risk of CRC in the codominant model.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	*P*[d]-value	OR (95%CI)	*P*[d]-value
TRABD2B (Chr 1)				
rs12080929				
TT	1.00	-	1.00	-
CT	1.15(0.78-1.68)	0.480	0.81(0.25-2.61)	0.721
CC	1.30(0.62-2.76)	0.488	0.52(0.17-1.58)	0.249
T	1.00	-	1.00	-
C	1.14(0.84-1.55)	0.390	1.30(0086-1.97)	0.218
***LAMC1* (Chr 1)**				
rs10911251				
AA	1.00	-	1.00	-
AC	0.88(0.59-1.30)	0.525	0.77(0.41-1.45)	0.422
CC	0.56(0.32-0.99)	0.046	0.40(0.16-0.98)	0.045
A	1.00	-	1.00	-
C	0.76(0.58-0.99)	0.043	0.71(0.51-0.99)	0.046
***NABP1/SDPR* (Chr 2)**				
rs11903757				
TT	1.00	-	1.00	-
CT	1.42(0.95-2.14)	0.090	3.81(0.55-26.36)	0.175
CC	1.67(0.47-5.99)	0.430	1.67(0.93-3.00)	0.087
T	1.00	-	1.00	-
C	1.47(1.03-2.10)	0.035	1.54(0.96-2.45)	0.073
***MYNN* (Chr 3)**				
rs10936599				
CC	1.00	-	1.00	-
CT	0.84(0.57-1.22)	0.350	0.89(0.49-1.61)	0.692
TT	0.40(0.15-1.06)	0.065	0.39(0.10-1.52)	0.175
C	1.00	-	1.00	-
T	0.75(0.55-1.04)	0.083	0.81(0.54-1.21)	0.302
PITX1/H2AFY				
rs647161				
AA	1.00	-	1.00	-
AC	0.97(0.66-1.44)	0.897	1.09(0.60-1.96)	0.784
CC	1.70(0.87-3.37)	0.123	2.84(0.93-8.65)	0.066
A	1.00	-	1.00	-
C	1.13(0.86-1.50)	0.359	1.07(0.75-1.53)	0.705
***TERT* (Chr 5)**				
rs2736100				
CC	1.00	-	1.00	-
AC	1.14(0.73-1.77)	0.562	2.83(1.13-4.83)	0.023
AA	1.25(0.73-2.16)	0.420	1.87(0.81-4.34)	0.143
C	1.00	-	1.00	-
A	1.13(0.87-1.48)	0.348	1.13(0.81-1.59)	0.468
***SRSF3/CDKN1A* (Chr 6)**				
rs1321311				
CC	1.00	-	1.00	-
AC	1.45(0.56-3.78)	0.439	1.19(0.25-3.79)	0.962
AA	1.08(0.41-2.79)	0.881	0.97(0.65-2.20)	0.572
C	1.00	-	1.00	-
A	1.12(0.84-1.51)	0.439	1.23(0.84-1.80)	0..278

To be continued in the next page.

Continuation of Supplementary Table S2.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	*P*[d]-value	OR (95%CI)	*P*[d]-value
***DUSP4* (Chr 8)**				
rs11987193				
CC	1.00		1.00	-
CT	1.45(1.00-2.10)	0.050	1.15(0.67-1.99)	0.614
TT	1.47(0.65-3.29)	0.353	1.35(0.39-4.63)	0.636
C	1.00	-	1.00	-
T	1.32(0.99-1.77)	0.057	1.13(0.78-1.64)	0.527
***TRPS1/EIF3H/UTP23* (Chr 8)**				
rs16892766				
AA	1.00	-	1.00	-
AC	1.33(0.72-2.46)	0.356	2.06(0.80-5.35)	0.136
CC	NA	-	NA	-
A	1.00	-	1.00	-
C	1.65(0.90-3.05)	0.102	2.24(1.02-4.88)	0.043
***CCAT2* (Chr 8)**				
rs6983267				
GG	1.00	-	1.00	-
GT	0.83(0.53-1.29)	0.399	0.81(0.41-1.60)	0.546
TT	0.69(0.41-1.19)	0.183	0.75(0.33-1.68)	0.483
G	1.00	-	1.00	-
T	0.82(0.63-1.06)	0.131	0.78(0.56-1.09)	0.145
***CASC8* (Chr 8)**				
rs10505477				
AA	1.00	-	1.00	-
AG	0.94(0.60-1.47)	0.794	0.98(0.50-1.95)	0.962
GG	0.70(0.41-1.19)	0.184	0.71(0.32-1.57)	0.402
A	1.00	-	1.00	-
G	0.80(0.61-1.04)	0.089	0.78(0.56-1.09)	0.149
rs7014346		-		
GG	1.00	0.986	1.00	-
AG	1.30(0.90-1.89)	0.167	1.46(0.83-2.57)	0.188
AA	0.99(0.55-1.79)	-	1.56(0.62-3.96)	0.345
G	1.00	0.374	1.00	-
A	1.13(0.86-1.48)		1.23(0.87-1.73)	0.240
***KRT8P16/TCEB1P3* (Chr 10).**				
rs10795668				
GG	1.00	-	1.00	-
AG	1.11(0.76-1.61)	0.591	1.16(0.66-2.02)	0.611
AA	1.12(0.56-2.26)	0.744	0.34(0.10-1.11)	0.074
G	1.00	-	1.00	-
A	1.09(0.82-1.44)	0.563	1.09(0.75-1.58)	0.655
***ZMIZ1-AS1* (Chr 10)**				
rs704017				
AA	1.00	-	1.00	-
AG	0.91(0.58-1.42)	0.731	1.03(0.50-2.11)	0.938
GG	0.91(0.52-1.58)	0.670	0.73(0.30-1.79)	0.497
A	1.00	-	1.00	-
G	1.08 (0.83-1.42)	0.542	0.87(0.61-1.23)	0.417
***ABCC2/MRP2* (Chr 10)**				
rs1035209				
CC	1.00	-	1.00	-

To be continued in the next page.

Continuation of Supplementary Table S2.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	***P*[d]-value**	**OR (95%CI)**	***P*[d]-value**
CT	1.29(0.85-1.94)	0.235	1.74(0.93-3.24)	0.081
TT	0.61(0.25-1.47)	0.270	0.72(0.19-2.70)	0.627
C	1.00	-	1.00	-
T	1.01 (0.71-1.34)	0.973	0.88(0.56-1.38)	0.576
***TCF7L2* (Chr 10)**				
rs11196172				
GG	1.00	-	1.00	-
AG	1.11(0.71-1.76)	0.642	0.95(0.49-1.86)	0.889
AA	0.22(0.05-1.03)	0.054	0.13(0.01-1.63)	0.114
G	1.00	-	1.00	-
A	0.80(0.54-1.18)	0.260	0.96(0.57-1.61)	0.871
***HSPA12A* (Chr 10)**				
rs1665650				
CC	1.00	-	1.00	-
CT	1.13(0.76-1.69)	0.566	1.05(0.57-1.96)	0.874
TT	2.46(0.93-6.52)	0.070	2.19(0.58-8.31)	0.248
C	1.00	-	1.00	-
T	1.25(0.90-1.73)	0.184	1.18(0.77-1.80)	0.437
***MYRF, FEN1, FADS1, FADS2* (Chr 11)**				
rs174537				
GG	1.00	-	1.00	-
GT	0.85(0.57-1.26)	0.407	0.85(0.48-1.49)	0.566
TT	0.71(0.37-1.32)	0.274	0.70(0.27-1.79)	0.472
G	1.00	-	1.00	-
T	0.85(0.64-1.12)	0.253	0.76(54-1.09)	0.135
rs4246215				
GG	1.00	-	1.00	
GT	0.84(0.57-1.24)	0.382	0.80(0.46-1.40)	0.437
TT	0.74(0.40-1.38)	0.349	0.83(0.33-2.14)	0.707
G	1.00	-	1.00	-
T	0.86(0.65-1.13)	0.276	0.77(0.54-1.09)	0.145
rs174550				
TT	1.00	-	1.00	-
CT	0.82(0.56-1.23)	0.270	1.22(0.45-3.34)	0.694
CC	0.71(0.38-1.31)	0.353	1.44(0.56-3.68)	0.452
T	1.00	-	1.00	-
C	0.81(0.61-1.08)	0.159	0.69(0.47-0.99)	0.047
rs1535				
AA	1.00	-	1.00	-
AG	0.73(0.49-1.07)	0.106	0.80(0.45-1.40)	0.427
GG	0.68(0.37-1.23)	0.203	0.68(0.27-1.71)	0.414
A	1.00	-	1.00	-
G	0.78(0.59-1.03)	0.079	0.69(0.48-0.98)	0.037
***LOC120376, FL45803, c11orf53, POU2AF1* (Chr 11)**				
rs3802842				
AA	1.00	-	1.00	-
AC	0.95(0.65-1.38)	0.780	0.96(0.53-1.74)	0.899
CC	1.08(0.56-2.06)	0.833	1.03(0.36-2.94)	0.958
A	1.00	-	1.00	-
C	0.99(0.74-1.32)	0.941	1.14(0.78-1.67)	0.485
***CD9* (Chr 9)**				
rs10849432				

To be continued in the next page.

Continuation of Supplementary Table S2.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	***P*[d]-value**	**OR (95%CI)**	***P*[d]-value**
TT	1.00	-	1.00	-
CT	1.10(0.71-1.70)	0.659	1.47(0.11-19.03)	0.768
CC	0.67(0.11-3.99)	0.657	0.88(0.45-1.71)	0.709
T	1.00	-	1.00	-
C	0.92(0.62-1.35)	0.658	1.24(0.74-2.07	0.420
***CCND2* (Chr 12)**				
rs3217810				
CC	1.00	-	1.00	-
CT	1.32(0.79-2.21)	0.295	1.21(0.57-2.56)	0.620
TT	NA	-	NA	-
C	1.00	-	1.00	-
T	1.56(0.98-2.47)	0.059	1.26(0.73-2.20)	0.408
rs3217901				
AA	1.00	-	1.00	-
AG	1.05(0.71-1.55)	0.799	1.10(0.59-2.04)	0.761
GG	1.30(0.72-2.35)	0.388	0.94(0.38-2.36)	0.897
A	1.00	-	1.00	-
G	1.15(0.88-1.53)	0.293	1.22(0.85-1.74)	0.285
rs10774214				
CC	1.00	-	1.00	-
CT	1.08(0.73-1.60)	0.688	0.80(0.44-1.44)	0.452
TT	0.81(0.43-1.52)	0.509	0.72(0.28-1.81)	0.480
C	1.00	-	1.00	-
T	1.08(0.81-1.44)	0.616	1.19(0.82-1.71)	0.353
***ATF1* (Chr 12)**				
rs11169552				
CC	1.00	-	1.00	-
CT	0.69(0.47-1.02)	0.061	0.53(0.29-0.98)	0.044
TT	0.77(0.28-2.16)	0.622	0.90(0.18-4.51)	0.897
C	1.00	-	1.00	-
T	0.80(0.58-1.10)	0.165	0.78(0.52-1.19)	0.247
***TBX3* (Chr 12)**				
rs59336				
AA	1.00	-	1.00	-
AT	0.92(0.58-1.44)	0.706	0.83(0.42-1.65)	0.604
TT	0.83(0.49-1.39)	0.472	0.46(0.19-1.10)	0.081
A	1.00	-	1.00	-
T	0.84(0.65-1.10)	0.210	1.25(0.89-1.76)	0.206
***BMP4/ATP5C1P1/CDKN3/MIR5580* (Chr 14)**				
rs4444235				
CC	1.00	-	1.00	-
CT	1.21(0.76-1.92)	0.416	1.50(0.73-3.07)	0.272
TT	1.23(0.71-2.13)	0.460	0.56(0.24-1.34)	0.194
C	1.00	-	1.00	-
T	1.02(0.79-1.33)	0.867	1.12(0.79-1.60)	0.515
rs1957636				
CC	1.00	-	1.00	-
CT	1.46(0.95-2.26)	0.085	1.45(0.75-2.80)	0.271
TT	1.28(0.73-2.23)	0.384	0.68(0.30-1.54)	0.360
C	1.00	-	1.00	-
T	1.09(0.83-1.43)	0.534	0.99(0.69-1.41)	0.947

To be continued in the next page.

Continuation of Supplementary Table S2.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	***P*[d]-value**	**OR (95%CI)**	***P*[d]-value**
***SCG5, GREM1, FMN1* (Chr 15)**				
rs4779584				
CC	1.00	-	1.00	-
CT	1.09(0.71-1.69)	0.698	0.79(0.39-1.60)	0.519
TT	0.61(0.15-2.58)	0.504	0.31(0.06-1.70)	0.177
C	1.00	-	1.00	-
T	1.11(0.77-1.58)	0.584	0.06(0.56-1.45)	0.679
rs16969681		0.193		
CC	1.00	-	1.00	-
CT	0.73(0.46-1.17)	0.193	0.69(0.33-1.46)	0.332
TT	0.93(0.13-6.62)	0.938	0.67(0.05-8.47)	0.756
C	1.00	-	1.00	-
T	0.88(0.57-1.37)	0.577	0.77(0.42-1.42)	0.402
rs11632715				
GG	1.00	-	1.00	-
AG	1.06(0.70-1.61)	0.796	1.13(0.57-2.25)	0.727
AA	1.00(0.59-1.71)	0.991	0.58(0.27-1.27)	0.173
G	1.00	-	1.00	-
A	0.97(0.74-1.28)	0.854	0.96(0.67-1.37))	0.815
***CDH1* (Chr 16)**				
rs9929218				
GG	1.00	-	1.00	-
AG	1.02(0.70-1.50)	0.900	1.24(0.71-2.18)	0.930
AA	0.90(0.45-1.78)	0.776	0.95(0.32-2.83)	0.447
G	1.00	-	1.00	-
A	0.94(0.71-1.25)	0.663	1.10(0.76-1.61)	0.605
***NXN* (Chr 17)**				
rs12603526				
TT	1.00	-	1.00	-
CT	1.00(0.14-7.10)	1.000	1.49(0.08-28.04)	0.790
CC	NA	-	NA	-
T	1.00	-	1.00	-
C	1.00(0.14-7.10)	1.000	0.74(0.10-5.65)	0.774
***SMAD7* (Chr 18)**				
rs4939827				
TT	1.00	-	1.00	-
CT	1.19(0.79-1.81)	0.408	1.14(0.60-2.17)	0.691
CC	0.90(0.52-1.54)	0.694	0.80(0.35-1.82)	0.592
T	1.00	-	1.00	-
C	0.87(0.66-1.13)	0.288	0.90(0.64-1.27)	0.554
***RHPN2* (Chr 19)**				
rs10411210				
CC	1.00	-	1.00	-
CT	0.90(0.59-1.39)	0.646	0.52(0.26-1.03)	0.060
TT	3.77(0.42-34.22)	0.238	4.44(0.33-58.89)	0.262
C	1.00	-	1.00	-
T	1.01(0.67-1.52)	0.959	0.98(0.57-1.68)	0.942
***TGFB1* (Chr 19)**				
rs1800469				
GG	1.00	-	1.00	-
AG	0.80(0.55-1.15)	0.257	0.56(0.32-0.99)	0.047

To be continued in the next page.

Continuation of Supplementary Table S2.

Gene, SNP ID[a]	Model I[b]		Model II[c]	
	OR (95%CI)	***P*[d]-value**	**OR (95%CI)**	***P*[d]-value**
AA	0.68(0.35-1.33)	0.230	0.32(0.10-0.99)	0.049
G	1.00	-	1.00	-
A	0.80(0.60-1.06)	0.115	0.77(0.53-1.12)	0.171
***BMP2/HAO1/FERMT1* (Chr 20)**				
rs4813802				
TT	1.00	-	1.00	-
GT	0.91(0.62-1.33)	0.550	0.90(1.28-9.70)	0.754
GG	1.20(0.66-2.21)	0.627	3.52(1.28-9.70)	0.015
T	1.00	-	1.00	-
G	1.03(0.77-1.37)	0.855	0.82(0.56-1.18)	0.283
HAO1/PLCB1				
rs2423279				
TT	1.00	-	1.00	-
CT	1.26(0.87-1.83)	0.213	1.04(0.61-1.79)	0.880
CC	1.28(0.68-2.43)	0.441	0.63(0.23-1.68)	0.352
T	1.00	-	1.00	-
C	1.19(0.89-1.57)	0.235	1.12(0.78-1.60)	0.534
SHROOM (Chr X)				
rs5934683				
C	1.00	-	1.00	-
CT	1.16(0.63-2.18)	0.626	1.20(0.47-3.09)	0.702
T	1.30(0.87-1.94)	0.207	1.47(0.79-2.75)	0.225
C	1.00	-	1.00	-
T	1.20(0.91-1.59)	0.197	1.21(0.83-1.76)	0.322

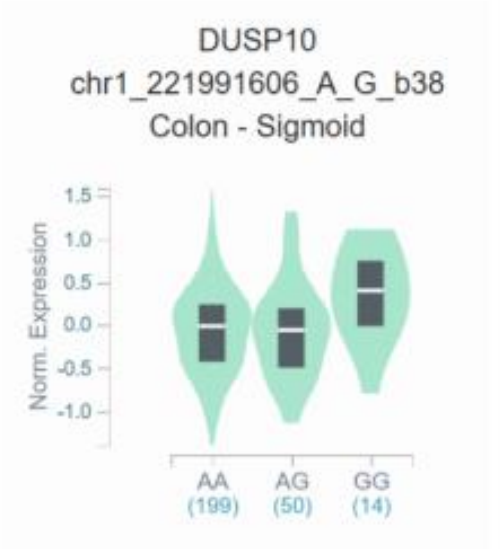
A, adenine; C, cytosine; CI, confidence interval; G, guanine; NA, no available data; OR, odds ratio; rs, reference single nucleotide polymorphism; SNP, single nucleotide polymorphism; T, thymine.

[a]The most frequent genotype was considered the reference group.

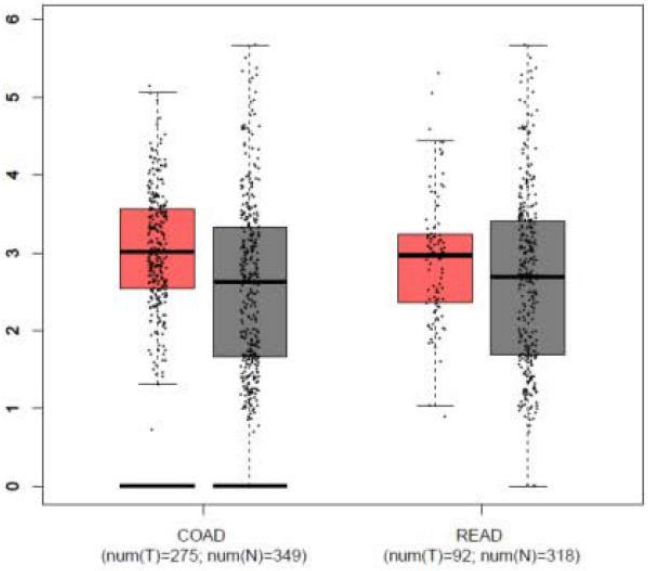
[b]Model I, crude conditional logistic regression model.

[c]Model II, conditional logistic regression adjusted for: age, sex, BMI, physical activity, smoking status, alcohol consumption, Deprivation Index and energy intake. Participants with missing data for the confounding variables were included as a separate category for these variables.

[d]P<0.001 was significant.



S1 Fig. eQTL violin plot showing gene association results for *DUSP10* gene, rs6687758 and colon sigmoid healthy tissue. A, adenine. G, guanine. Data Source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2).



S2 Fig. Box plot for comparing the difference of expression for DUSP10 gene between cases (in red) and controls (in grey) in colon adenocarcinoma and rectum adenocarcinoma. COAD, colon adenocarcinoma. N, normal. READ, rectum adenocarcinoma. T, tumour. The method for differential analysis is one-way ANOVA, using disease state (Tumor or Normal) as variable for calculating differential expression. Data Source: TCGA and GTEx data, using GEPIA.

Discussion

In this study, we investigated SNPs associated with susceptibility for the development of CRC in a Basque population who took part in the population screening programme. We found that out of 48 analysed SNPs, only the rs6687758 was associated with the risk of CRC in this population. This is in agreement with previous GWAS that reported a positive association between this SNP and CRC also in European population [15,34]. Some authors have also observed relationships between this SNP and colorectal polyp risk [35]; although this SNPs is not associated significantly with adenoma risk and has their effects on the malignant stage of colorectal tumorigenesis [36]. The frequency of the risk allele of rs6687758 (G) in the European population (22.2%) [37] is similar to that registered in the cases of the present study and higher than that of the controls.

The other 47 risk SNPs did not replicate in our population. This may be due to differences in the underlying linkage patterns given the ethnic differences in populations studied. Twenty-one of the SNPs analyzed have been replicated in Asian, American-Caucasian or African, but not in European (rs11903757, rs1321311, rs10505477, rs719725, rs704017, rs12241008, rs11196172, rs174537, rs4246215, rs174550, rs1535, rs10849432, rs3217901, rs4444235, rs11632715, rs4939827, rs10411210, rs1800469, rs2241714, rs961253 and rs4813802); and 4 were not replicated in population studies; however, they were associated with susceptibility for development of CRC in GWAS (rs1665650, rs59336, rs1957636 and rs12603526). The effect sizes of some of these associations were small (OR <1.20, P<0.05, for rs1321311, rs12241008, and rs704017) [38-40]. Additionally, it may be that the distribution of environmental factors in our population differs from that of the populations in which these genetic variants were discovered.

The SNP rs6687758 is in a regulatory region, flanking the promoter of *DUSP10*, at ~250 kb from the start of the gene. Hence, it is likely to affect the expression of this gene. Polymorphisms in *DUSP10 gene (dual* specificity protein phosphatase 10) have been previously demonstrated to be associated with CRC risk [41,42]. In this study, we confirmed this CRC susceptibility locus in the Basque population sample. Earlier analyses have found frequent dysregulation of dual specificity protein phosphatase 10 (*DUSP10*/*MKP-5*) in CRC [41]. *DUSP10* belongs to the dual kinase phosphatase family. These proteins are associated with cellular proliferation and differentiation, and they act as tumour suppressors [41,43].

Target kinases of DUSPs are inactivated by dephosphorylation of both phosphoserine/threonine and phosphotyrosine residues [41,42]. They act at several levels, taking part in fine-tuning signalling cascades. DUSPs negatively regulate members of the mitogen-activated protein kinase (*MAPK*) superfamily [41,44], which are implicated in some activities that are often dysregulated in cancer, such as cell proliferation, survival, and migration [41]. *MAPK* signalling also plays a key role in determining the response of tumour cells to cancer therapies, since its abnormal signalling has important consequences for the development and progression of human cancer [44]. Several studies have already shown the involvement of *DUSP*s as major modulators of critical signalling pathways dysregulated in different cancers [43], such as in the case of the overexpression of *DUSP1*/*MKP-1* in the early phases of cancer and its decreasing during tumour progression [42].

There is abundant evidence that *DUSP10*, in particular, may play an important role in tumorigenesis and could alter CRC risk [45,46]. It inactivates *p38* and *JNK in vitro* [41,47], and its upregulation are very common in CRC[48]. The activation of JNK protein is due to the protein kinase *G (PKG)/MEKK1/SEK1/JNK* cascade, and it is related with cell proliferation and inducing apoptosis [41,49]. Moreover, *p38* is involved in the promotion of cellular senescence as a means of eluding oncogene-induced transformation; it participates in cell cycle regulation suppressing cell proliferation and tumorigenesis [41,49].

On the other hand, the results extracted from gene expression association analyses **show** a higher expression of *DUSP10*gene in CRC cases, but also that there is a higher expression of this gene in colon tissue of healthy controls when they have the GG genotype for rs6687758. Thus, it would be likely to find a relationship between higher expression of the gene and the presence of allele G in rs6687758 in tumour tissue. Nonetheless, it would be interesting to further explore this aspect through future analyses to compare gene expression between individuals carrying the risk variant and control individuals. Previous studies have pointed in the same direction that there is overall increase in patients' relapse-free survival when *DUSP10* expression is upregulated, and that *DUSP10* mRNA was increased in the tumour compared with normal tissue adjacent to the tumours [46,49,50].

We found an association between smoking status and the rs6687758 SNP for CRC risk in cases .Other authors have also observed this association [51].Benzo[a]pyrene, one of the carcinogenic compounds included in cigarette smoke, up-regulated *COX-2* in mouse cells [52], which in turn could either activate or be dependent on the *MAPK* pathway,

suggesting a possible gene-smoking interaction [53,54].Concerning the association between physical activity, the rs6687758 SNP and CRC risk, as far as we know, there are no precedents in the literature. However, other studies have found interactions between polymorphisms associated with growth hormone (*GH1*) and insulin-like growth factor I (*IGF-I*) (rs647161, rs2665802), physical activity and CRC [53,54]. According our results, rs6687758, medium-high physical activity level and CRC would be associated. However, this outcome, contrary o what it could be expected, could be related to changes in the lifestyles, including physical activity level, in cases after diagnosis [55].

We also analyzed unweighted and weighted GRS models. We observed that cases had more risk alleles than controls, this result was according to expectations considering the previous studies [56]. In the crude analysis, we observed that patients that had a higher number of risk alleles had a higher risk of CRC. Other authors observed similar results using an adjusted unweighted model [32]. However, some other authors did not find this association [57]. It should be noted that common allele variants generally have modest effect sizes [58], but the combination of multiple loci with modest effects into a global GRS might improve the identification of patients with genetic risk for common complex diseases, such cancer [59]. In this sense, Ortlepp *et al.* [60] concluded that more than 200 polymorphisms might be necessary for “reasonable” genetic discrimination.

Our study has several limitations and strengths. The principal limitations of this study were the small sample size that makes difficult to detect possible associations between polymorphisms and disease risk since some genotypes showed very low frequencies in our population. Another disadvantage of the small sample size is that they can produce false-positive results; in order to avoid it, the Bonferroni correction was used. The strengths of the study were that although controls tested positive in iFOBT, in CRCSP were confirmed that they were free of the disease through colonoscopy. Colonoscopy was used as diagnosis criteria to identify the cases in order to avoid false positives and negatives.

In **conclusion**, most SNPs analyzed were not associated with risk of CRC. Only one of the 48 SNPs analyzed, rs6687758, was associated with risk of CRC, in this population (on crude analysis). Moreover, there were significant associations between smoking status, physical activity, the rs6687758SNP and CRC risk. On the other hand, the results of the GRS showed that the risk alleles were more frequent in cases than controls and this score was associated with this type of cancer in crude analysis. Therefore, in this study, we have confirmed a CRC susceptibility locus and the existence of associations between modifiable factors such as smoking and physical activity and the presence of

the risk genotype for rs6687758. However, further experimental validations are needed to establish the role of this SNP, the function of the gene identified, as well as the contribution of the interaction between environmental factors and this polymorphism to the risk of CRC.

Acknowledgments

The genotyping service was carried out at CEGEN-PRB2-ISCIII; it is supported by grant PT13/0001, ISCIII-SGEFI / FEDER. We want to particularly acknowledge the patients enrolled in this study for their participation and the Basque Biobank for Research-OEHUN for its collaboration.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality world wide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394-424. doi:10.3322/caac.21492.
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, *et al.* Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer;2018. [cited 2019 Apr 4].Available from: https://gco.iarc.fr/today
3. Castillejo A, Mata-Balaguer T, Montenegro P, Ochoa E, Lázaro R, Martínez-Cantó A, *et al.* The TGFBR1*6A allele is not associated with susceptibility to colorectal cancer in a Spanish population: a case-control study. BMC Cancer. 2009;9:193. doi: 10.1186/1471-2407-9-193.
4. Wolf AMD, Fontham ETH, Church TR, Flowers CR, Guerra CE, La Monte SJ, *et al.* Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin. 2018;68(4):250-281. doi:10.3322/caac.21457.
5. Chen Y, Du M, Chen W, Zhu L, Wu C, Zhang Z, Wang M, Chu H, Gu D, Chen J. Polymorphism rs2682818 in miR-618 is associated with colorectal cancer susceptibility in a Han Chinese population. Cancer Med. 2018;7(4):1194-1200. doi: 10.1002/cam4.1409
6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7-34. doi: 10.3322/caac.21551.
7. Marley AR, Nan H. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet. 2016;7(3):105-114.

8. Sameer AS. Colorectal cancer: molecular mutations and polymorphisms. Front Oncol. 2013;3:114. doi: 10.3389/fonc.2013.00114.
9. Zhong R, Liu L, Zou L, Sheng W, Zhu B, Xiang H,*et al.* Genetic variations in the TGFβ signaling pathway, smoking and risk of colorectal cancer in a Chinese population. Carcinogenesis. 2013;34(4):936-42. Doi:10.1093/carcin/bgs395.
10. Yang CY, Lu RH, Lin CH, Jen CH, Tung CY, Yang SH, Lin JK, Jiang JK, Lin CH. Single nucleotide polymorphisms associated with colorectal cancer susceptibility and loss of heterozygosity in a Taiwanese population. PLoS One. 2014;9(6):e100060. doi: 10.1371/journal.pone.0100060.
11. Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. JAMA. 2008;299(20):2423-36. doi:10.1001/jama.299.20.2423.
12. Castellví-Bel S, Abulí A, Castells A. Meta-analysis of several GWAS sets yields additional genetic susceptibility variants for colorectal cancer: first X-linked component identified. Gastroenterology. 2012;143(6):1684-5. doi:10.1053/j.gastro.2012.10.008.
13. Idigoras I, Arrospide A, Portillo I, Arana-Arri E, Martínez-Indart L, Mar J, *et al.* Evaluation of the colorectal cancer screening Programme in the Basque Country (Spain) and its effectiveness based on the Miscan-colon model. BMC Public Health. 2017 Aug 1;18(1):78. doi: 10.1186/s12889-017-4639-3. Erratum in: BMC Public Health. 2017;17 (1):736.
14. Carethers JM, Jung BH. Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer. Gastroenterology. 2015;149(5):1177-1190.e3. doi:10.1053/j.gastro.2015.06.047.
15. Real LM, Ruiz A, Gayán J, González-Pérez A, Sáez ME, Ramírez-Lorca R, *et al.* A colorectal cancer susceptibility new variant at 4q26 in the Spanish population identified by genome-wide association analysis. PLoS One. 2014;9(6):e101178. doi: 10.1371/journal.pone.0101178.
16. Ministerio de Sanidad, Servicios Sociales e Igualdad / Instituto Nacional de Estadística. Encuesta Nacional de Salud. España 2011/12.Madrid: Ministerio de Sanidad, Servicios Sociales e Igualdad;2012. Available from: http://www.msssi.gob.es/estadEstudios/estadisticas/sisInfSanSNS/nivelSalud.htm
17. World Health Organization (WHO). Report of WHO consultation: Obesity: preventing and managing the global epidemic. Geneva; 2000.
18. Silva Rodrigues RA, Martinez Espinosa M, Duarte Melo C, Rodrigues Perracini M, Rezende Fett WC, *et al.* New values anthropometry for classification of

nutritional status in the elderly. J Nutr Health Aging. 2014;18(7):655-61. doi: 10.1007/s12603-014-0451-2.

19. Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. Validation of a short questionnaire on frequency of dietary intake: reproducibility and validity. NutrHosp. 2008;23(3):242-52. Spanish.
20. Alegria-Lertxundi I, Alvarez M, Rocandio AM, de Pancorbo MM, Arroyo-Izaga M. Nutritional Adequacy and Diet Quality in Colorectal Cancer Patients Postsurgery: A Pilot Study. Nutr Cancer. 2016;68(4):577-88. doi: 10.1080/01635581.2016.1158299.
21. Carbajal A, Sánchez-Muniz FJ. Guía de prácticas. In: García-Arias MT, García-Fernández MC, editors. Nutrición y dietética. Secretariado de Publicaciones y Medios Audiovisuales, Universidad de León; 2003. p. 1a-130a.
22. Gobierno Vasco, Departamento de Agricultura Pesca y Alimentación. Estudio cuantitativo del consumo de alimentos en la CAPV, año 2007. Vitoria-Gasteiz: Servicio Central de Publicaciones del Gobierno Vasco; 2008.
23. Aranceta J, Serra Majem L, Arija V, Gil A, Martínez de Vitoria E, Ortega R, *et al.* Objetivos nutricionales para la población Española Consenso de la Sociedad Española de Nutrición Comunitaria 2011. Rev Esp Nutr Comunitaria. 2011;17(4):178-199.
24. Domínguez-Berjón MF, Borrell C, Cano-Serral G, Esnaola S, Nolasco A, Pasarín MI, *et al.* Constructing a deprivation index based on census data in large Spanish cities(the MEDEA project). Gac Sanit. 2008;22(3):179-87.
25. Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, Hebestreit A, Krogh V, Lissner L, Mårild S, Molnár D, Moreno LA, Pitsiladis YP, Reisch L, Tornaritis M, Veidebaum T, Pigeot I; IDEFICS Consortium. The IDEFICS cohort: design, characteristics and participation in the baseline survey. Int J Obes (Lond). 2011;35 Suppl1:S3-15. doi: 10.1038/ijo.2011.30.
26. Celorrio D, Muñoz X, Amiano P, Dorronsoro M, Bujanda L, Sánchez MJ *et al.* Influence of Dopaminergic System Genetic Variation and Lifestyle Factorson Excessive Alcohol Consumption. Alcohol Alcohol. 2016;51(3):258-67. doi: 10.1093/alcalc/agv114
27. Piccolo SR, Abo RP, Allen-Brady K, Camp NJ, Knight S, Anderson JL, *et al.* Evaluation of genetic risk scores for lipid levels using genome-wide markers in the Framingham Heart Study. BMC Proc. 2009;3 Suppl7:S46..
28. Jung KJ, Won D, Jeon C, Kim S, Kim TI, Jee SH, *et al.* A colorectal cancer prediction model using traditional and genetic risk scores in Koreans. BMC Genet. 2015;16:49. doi: 10.1186/s12863-015-0207-y.

29. Cornelis MC, Qi L, Zhang C, Kraft P, Manson J, Cai T, *et al.* Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. Ann Intern Med. 2009;150(8):541-50.
30. Balding DJ. A tutorial on statistical methods for population association studies. Nat Rev Genet. 2006;7(10):781-91.
31. Ibáñez-Sanz G, Díez-Villanueva A, Alonso MH, Rodríguez-Moranta F, Pérez-Gómez B, Bustamante M, *et al.* Risk Model for Colorectal Cancer in Spanish Population Using Environmental and Genetic Factors: Results from the MCC-Spain study. Sci Rep. 2017;7:43263. doi:10.1038/srep43263.
32. MacArthur J, Bowler E, Cerezo M, Gil L, Hall P, Hastings E, *et al.* The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res. 2017;45(D1):D896-D901. doi: 10.1093/nar/gkw1133.
33. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017 Jul 3;45(W1):W98-W102. doi: 10.1093/nar/gkx247.
34. Houlston RS, Cheadle J, Dobbins SE, Tenesa A, Jones AM, Howarth K, *et al.* Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. Nat Genet. 2010;42(11):973-7. doi: 10.1038/ng.670.
35. Zhang B, Shrubsole MJ, Li G, Cai Q, Edwards T, Smalley WE, *et al.* Association of genetic variants for colorectal cancer differs by subtypes of polyps in the colorectum. Carcinogenesis. 2012;33(12):2417-23. doi:10.1093/carcin/bgs308.
36. Carvajal-Carmona LG, Zauber AG, Jones AM, Howarth K, Wang J, Cheng T *et al.* Much of the genetic risk of colorectal cancer is likely to be mediated through susceptibility to adenomas. Gastroenterology. 2013;144(1):53-5. doi: 10.1053/j.gastro.2012.09.016.
37. Ensembl genome database project [Internet]; 2018Available from: https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=1:221991106-221992106;v=rs6687758;vdb=variation;vf=3766274
38. Wang H, Burnett T, Kono S, Haiman CA, Iwasaki M, Wilkens LR, *et al.* Trans-ethnic genome-wide association study of colorectal cancer identifies a new susceptibility locus in VTI1A. Nat Commun. 2014;5:4613. doi:10.1038/ncomms5613.
39. Dunlop MG, Dobbins SE, Farrington SM, Jones AM, Palles C, Whiffin N, *et al.* Common variation near CDKN1A, POLD3 and SHROOM2 influences colorectal cancer risk. Nat Genet. 2012;44(7):770-6. doi: 10.1038/ng.2293.

40. Abe M, Ito H, Oze I, Nomura M, Ogawa Y, Matsuo K. The more from East-Asian, the better: risk prediction of colorectal cancer risk by GWAS-identified SNPs among Japanese. J Cancer Res Clin Oncol. 2017;143(12):2481-2492. doi:10.1007/s00432-017-2505-4.
41. Duan X, Gao Y, Yang H, Feng T, Jin T, Long Y, *et al.* Poly morphisms in the DUSP10 gene are associated with sex-specific colorectal cancer risk in a Han population. Int J Clin Exp Pathol. 2015;8(2):2018-25.
42. Bermudez O, Pagès G, Gimond C. The dual-specificity MAP kinas phosphatases: critical roles in development and cancer. Am J Physiol Cell Physiol. 2010;299(2):C189-202. doi:10.1152/ajpcell.00347.2009.
43. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J. 2009;418(3):475-89.
44. Keyse SM. Dual-specificity MAP kinase phosphatases (MKPs) and cancer. Cancer Metastasis Rev. 2008;27(2):253-61.
45. Tanoue T, Moriguchi T, Nishida E. Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5. J Biol Chem. 1999;274(28):19949-56.
46. Nomura M, Shiiba K, Katagiri C, Kasugai I, Masuda K, Sato I, *et al.* Novel function of MKP-5/DUSP10, a phosphatase of stress-activated kinases, on ERK-dependent gene expression, and up regulation of its gene expression in colon carcinomas. Oncol Rep. 2012;28(3):931-6. doi:10.3892/or.2012.1862.
47. Fang JY, Richardson BC. The MAPK signaling pathways and colorectal cancer. Lancet Oncol. 2005;6(5):322-7.
48. Han J, Sun P. The pathways to tumour suppression via route p38. Trends Biochem Sci. 2007;32(8):364-71.
49. Png CW, Weerasooriya M, Guo J, James SJ, Poh HM, Osato M, *et al.* DUSP10 regulates intestinal epithelial cell growth and colorectal tumorigenesis. Oncogene. 2016;35(2):206-17. doi:10.1038/onc.2015.74.
50. Jiménez-Martínez M, Stamatakis K, Fresno M. The Dual-Specificity Phosphatase 10 (DUSP10): Its Role in Cancer, Inflammation, and Immunity. Int J Mol Sci. 2019;20(7). pii: E1626. doi: 10.3390/ijms20071626.
51. Song N, Shin A, Jung HS, Oh JH, Kim J. Effects of interactions between common genetic variants and smoking on colorectal cancer. BMC Cancer. 2017;17(1):869. doi: 10.1186/s12885-017-3886-0.
52. Ouyang W, Ma Q, Li J, Zhang D, Ding J, Huang Y, Xing MM, *et al.* Benzo[a]pyrene diol-epoxide (B[a]PDE) upregulates COX-2 expression through

MAPKs/AP-1 and IKK beta/NF-kappa B in mouse epidermal Cl41 cells. Mol Carcinog. 2007;46(1):32-41.

53. Gunathilake MN, Lee J, Cho YA, Oh JH, Chang HJ, Sohn DK, *et al.* Interaction between physical activity, PITX1 rs647161 genetic polymorphism and colorectal cancer risk in a Korean population: a case-control study. Oncotarget. 2018;9(7):7590-7603. doi: 10.18632/oncotarget.24136.
54. Khoury-Shakour S, Gruber SB, Lejbkowicz F, Rennert HS, Raskin L, Pinchev M, *et al.* Recreational physical activity modifies the association between a common GH1 polymorphism and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2008;17(12):3314-8.
55. Dennis DL, Waring JL, Payeur N, Cosby C, Daudt HM. Making lifestyle changes after colorectal cancer: insights for program development. Curr Oncol. 2013;20(6):e493-511. doi: 10.3747/co.20.1514.
56. Dunlop MG, Tenesa A, Farrington SM, Ballereau S, Brewster DH, Koessler T, *et al.* Cumulative impact of common genetic variants and other risk factors on colorectal cancer risk in 42,103 individuals. Gut. 2013;62(6):871-81. doi:10.1136/gutjnl-2011-300537.
57. Phipps AI, Newcomb PA, Garcia-Albeniz X, Hutter CM, White E, Fuchs CS, *et al.* Association between colorectal cancer susceptibility loci and survival time after diagnosis with colorectal cancer. Gastroenterology. 2012;143(1):51-4.e4. doi:10.1053/j.gastro.2012.04.052.
58. Hindorff LA, Gillanders EM, Manolio TA. Genetic architecture of cancer and other complex diseases: lessons learned and future directions. Carcinogenesis. 2011;32(7):945-54. doi: 10.1093/carcin/bgr056.
59. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk to disease from genome-wide association studies. Genome Res. 2007;17(10):1520-8.
60. Ortlepp JR, Lauscher J, Janssens U, Minkenberg R, Hanrath P, Hoffmann R. Analysis of several hundred genetic polymorphisms may improve assessment of the individual genetic burden for coronary artery disease. Eur J Intern Med. 2002;13(8):485-492.
61. Fernandez-Rozadilla C, Cazier JB, Tomlinson IP, Carvajal-Carmona LG, Palles C, Lamas MJ, *et al.* A colorectal cancer genome-wide association study in a Spanish cohort identifies two variants associated with colorectal cancer risk at 1p33 and 8p12. BMC Genomics. 2013;14:55. doi: 10.1186/1471-2164-14-55.
62. Kantor ED, Hutter CM, Minnier J, Berndt SI, Brenner H, Caan BJ, *et al.* Gene-environment interaction involving recently identified colorectal cancer

susceptibility Loci. Cancer Epidemiol Biomarkers Prev. 2014;23(9):1824-33. doi:10.1158/1055-9965.EPI-14-0062.

63. Abulí A, Castells A, Bujanda L, Lozano JJ, Bessa X, Hernández C, *et al.* Genetic Variants associated with Colorectal Adenoma Susceptibility. PLoS One. 2016;11(4):e0153084. doi:10.1371/journal.pone.0153084.
64. Yao K, Hua L, Wei L, Meng J, Hu J. Correlation Between CASC8, SMAD7 Polymorphisms and the Susceptibility to Colorectal Cancer: An Updated Meta-Analysis Based on GWAS Results. Medicine (Baltimore). 2015;94(46):e1884. doi: 10.1097/MD.0000000000001884.
65. Li L, Lv L, Liang Y, Shen X, Zhou S, Zhu J, *et al.* Association of 8q23-24 region (8q23.3 loci and 8q24.21 loci) with susceptibility to colorectal cancer: a systematic and updated meta-analysis. Int J Clin Exp Med. 2015;8(11):21001-13.
66. Hong Y, Wu G, Li W, Liu D, He K. A comprehensive meta-analysis of genetic associations between five key SNPs and colorectal cancer risk. Oncotarget. 2016 Nov 8;7(45):73945-73959. doi: 10.18632/oncotarget.12154.
67. Song N, Shin A, Park JW, Kim J, Oh JH. Common risk variants for colorectal cancer: an evaluation of associations with age at cancer onset. Sci Rep. 2017;7:40644. doi: 10.1038/srep40644.
68. Whiffin N, Hosking FJ, Farrington SM, Palles C, Dobbins SE, Zgaga L, *et al.* Identification of susceptibility loci for colorectal cancer in a genome-wide meta-analysis. Hum Mol Genet. 2014;23(17):4729-37. doi: 10.1093/hmg/ddu177.
69. Zhang B, Jia WH, Matsuda K, Kweon SS, Matsuo K, Xiang YB, *et al.* Large-scale genetic study in East Asians identifies six new loci associated with colorectal cancer risk. Nat Genet. 2014;46(6):533-42. doi: 10.1038/ng.2985.
70. Peters U, Hutter CM, Hsu L, Schumacher FR, Conti DV, Carlson CS, *et al.* Meta-analysis of new genome-wide association studies of colorectal cancer risk. Hum Genet. 2012;131(2):217-34. doi: 10.1007/s00439-011-1055-0.
71. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, *et al.* Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. Gastroenterology. 2013;144(4):799-807.e24. doi: 10.1053/j.gastro.2012.12.020.
72. Jia WH, Zhang B, Matsuo K, Shin A, Xiang YB, Jee SH, *et al.* Genome-wide association analyses in East Asians identify new susceptibility loci for colorectal cancer. Nat Genet. 2013;45(2):191-6. Doi:10.1038/ng.2505.

73. Lubbe SJ, Whiffin N, Chandler I, Broderick P, Houlston RS. Relationship between 16 susceptibility loci and colorectal cancer phenotype in 3146 patients. Carcinogenesis. 2012;33(1):108-12. doi: 10.1093/carcin/bgr243.
74. Houlston RS, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, *et al.* Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. Nat Genet. 2008;40(12):1426-35. doi: 10.1038/ng.262.
75. Tomlinson IP, Carvajal-Carmona LG, Dobbins SE, Tenesa A, Jones AM, Howarth K, *et al.* Multiple common susceptibility variants near BMP pathway loci GREM1, BMP4, and BMP2 explain part of the missing heritability of colorectal cancer. PLoS Genet. 2011;7(6):e1002105. doi:10.1371/journal.pgen.1002105.
76. Kupfer SS, Skol AD, Hong E, Ludvik A, Kittles RA, Keku TO, *et al.* Shared and independent colorectal cancer risk alleles in TGFβ-related genes in African and European Americans. Carcinogenesis. 2014;35(9):2025-30.
77. Broderick P, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, Rowan A, *et al.* A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. Nat Genet. 2007 ;39(11):1315-7.

4. DISCUSSION

The main objective of this thesis was to study the association between dietary and genetic factors and the risk of development of CRC in a sample of cases and controls from the population-based CRCSP of the Osakidetza/Basque Health Service. To achieve this aim, a case-control study based on a standardised protocol was carried out, which included not only dietary and genetic factors but also certain health determinants (such as age, lifestyle and socioeconomic conditions), weight status, perceived quality of life and stress and use of drugs related to decreasing the CRC risk. In addition, the other aim of this thesis was to assess the adequacy of the nutrients consumed and the diet quality in a group of CRC patients postsurgery.

Pilot study of CRC patients postsurgery

Before starting the case-control study, we conducted a pilot study in a group of CRC patients postsurgery who participated in the CRCSP of the Osakidetza/Basque Health Service. The objectives of this pilot study were, on one hand, to assess the adequacy of the nutrients consumed and the diet quality and, on the other hand, to identify possible associations between nutritional adequacy and diet quality and certain health determinants (such as age, weight status, lifestyles and socioeconomic conditions) in the aforementioned group. Regarding diet of CRC patients in this pilot study, this tended to follow the western pattern. This dietary pattern is characterized by a high protein and fat intake and a low consumption of carbohydrates. Scientific evidence demonstrates that this type of diet has a causative link to colon cancer; however, the mechanisms of action have not been fully elucidated [1].

Regarding micronutrient intakes, a significant proportion of subjects did not meet daily requirements for folic acid, vitamins A, D and E, Ca, Mg and I. Inadequate intakes of these nutrients were also noted by other authors in cancer patients [2,3] and in the general population [4]. In addition, the inadequacy for nutrient intakes was greater in men than women for the following micronutrients: thiamine, vitamin A, Mg and Zn ($P<0.05$). Consistent with our results, Lim *et al.* [5] reported more dietary habit problems and poor nutritional balance in males with gastric cancer than in females. Moreover, in the present study, patients who were overweight/obese presented greater inadequate intake of folic acid, vitamin A and Zn than those whose weight was normal ($P<0.05$).

In relation to the diet quality, the mean percentage for adherence to the MD was 66.6%, and a significant proportion of subjects did not meet food group recommendations; 85.1% had "no healthy diet or need changes" according to the HEISD. The percentage of subjects classified as "no healthy diet or need changes" was similar to the general

population of the Basque Country [6]. Although CRC patients claimed to have changed to a healthy diet after being diagnosed with CRC [7,8], these patients often either receive no dietary information or dietary advice is scarce [9,10]. This fact could influence dietary adequacy and quality. Furthermore, participants who were 61 years of age or older obtained higher HEISD scores than subjects younger than 61 (P<0.05); this result agrees with the findings of other studies [6].

Other factors related to diet quality scores (HEISD and/or MDS) were educational attainment, most recent occupation, smoking status and physical activity participation. Although no significant association was identified, there was a medium size effect. In addition, regression analyses demonstrated that those without studies or primary education had a low adherence to the MD and greater thiamine inadequacy (P<0.05). Results concerning educational level and MDS agreed with the findings of other authors [11]. However, care must be taken in the interpretation of our analysis of logistic regression due to sample size. In relation to lifestyle factors, the greatest size effect occurred in the smoking status variable for both diet quality indices. In our study, tobacco consumption was associated with a greater inadequacy in the intake of folic acid (P<0.05) and could be associated with a low diet quality, even though the P-value was not significant. Other authors have observed relationships between smoking and dietary intake [12]. Furthermore, significant interactions were observed between smoking and CRC, suggesting a potential mediating effect of the MD [13,14].

In summary, our results demonstrated that the diet of the studied group was inadequate in many respects, including nutrients and food intakes. The inadequacy of some nutrients was associated with male gender, excess weight, smoking and low educational level, and the low adherence to the MD was pronounced in those with a low educational level. These results confirm the hypothesis that the diet of CRC patients postsurgery is inadequate in many respects, including nutrients and food intakes, and this dietetic and nutritional inadequacy is associated with certain health determinants.

Participants in the case-control study of the CRCSP *of the Osakidetza/Basque Health Service*

To contextualize this section of the doctoral thesis, the rate of participation, as well as certain characteristics of cases and controls should be mentioned. The participation rate of the cases was 52.9% and that of the controls was 37.6%. More men than women participated (1.96:1.0), most being elderly people (average age in cases= 61.5; and in controls=61.1 years), which was consistent with previous literature on CRCSP of the

Osakidetza/Basque Health Service [15]. Although the average participation rate in this CRCSP was higher in women than men (70.9% vs. 65.6%), the proportion of CRC diagnosed was higher in men than in women (4.8% vs. 2.1%) [15]. Regarding pathological staging of the cases, 72% were diagnosed with in early-stage (I/II) CRC, 76% had distal location of the cancer, 80.5% of tumours were well/moderately differentiated and 73.7% had undergone surgical resection. The cases were invited to take part in this survey at least one month after finishing their last treatment (surgery, chemotherapy or radiotherapy) (median, 1.3 years; range, 0.1 to 4.2 years). No statistically significant differences were found either in the time elapsed between participation in the CRCSP and collaboration in this survey or between cases and controls.

Significant differences between cases and controls were found for educational level, smoking, and weight status; with a higher percentage of cases with low-medium educational level, past or current smoking status and with overweight/obesity compared to controls (P<0.01). This last result is in agreement with previous studies [16-18] that have confirmed that obesity is associated with an increased risk of CRC. Even though the biological mechanisms underlying the association between excess body-fat and CRC remain unclear [19], evidence seems to support the important role of metabolic syndrome, insulin resistance [20], systemic inflammation and immunity [21], microbial dysbiosis [22] , as well as certain genetic factors especially in early-onset CRC [23,24]. Elucidating the mediating role of these factors in obesity-induced CRC should be very useful in the prevention and treatment of this type of cancer. In addition to the direct contribution of obesity to CRC risk, excess body-fat could, in turn, be associated with other risk factors for CRC, such as unhealthy diet and sedentary lifestyle [25,26]. It is worth noting that we also observed a slightly higher proportion of controls whose main daily activity was sedentary compared to cases, but this result could be influenced by a greater awareness of the associations between diseases and lifestyle factors in cases.

On the other hand, in this study, we have not found a more frequent use of certain drugs related to a decreasing CRC risk [27]. In addition, alcohol consumption, perceived quality of life and stress levels did not differ between cases and controls. Lastly, significant differences between cases and controls were found for DI and PRM, with a higher percentage of controls than cases in Q_{1-3} (the least disadvantaged) for DI, and a higher percentage of cases than controls in L_{1-2} (these levels included those with a risk of high health resource consumption) for PRM (P<0.001). Briefly, results indicated significant differences in favour of controls for smoking habit, weight status, socioeconomic level and health status. So, the hypothesis that there are significant differences between cases

and controls with regard to lifestyle, weight status, use of drugs related to decreasing CRC risk, socioeconomic level, health status, quality of life and stress level was supported in part.

Diet of the case-control sample of the CRCSP *of the Osakidetza/Basque Health Service*

The diet of the case-control sample was assessed from several point of views: (1) adequacy of nutrient intake; (2) food groups; and (3) diet quality. First, the diet of participants, both cases and controls, was characterized by high intakes of protein, fat, SFA, and low intakes of carbohydrates and dietary fibre; thus, following a western diet pattern. This dietary pattern has been associated before with an elevated CRC incidence [28]. Moreover, the percentage of cases whose consumption of SFA and cholesterol did not comply with NOSP was higher than that for controls. This result is in agreement with those reported by other authors who observed a higher CRC risk among subjects with high intake of both SFA and cholesterol (highest vs. lowest) [29,30]. The mechanisms involved in the influence of fat on the colorectal carcinogenesis are complex and appear to be related with its effect on the insulin-signal pathway and the c-Jun N-terminal kinase (JNK) pathway that promote the colonic cell proliferation [31].

On the other hand, in the present study, we have not found a higher intake in controls than in cases of protective factors associated with a decrease in CRC risk according to the scientific literature, such as, for example, Ca, Mg, fibre diet, vitamin D or B_6 [27]. However, the average intake of vitamin B_2 and the Ca/P ratio was higher in controls than in cases. Some studies have indicated before that vitamin B_2 intake is inversely associated with CRC risk [32]. With respect to the Ca/P ratio, Botron *et al.* [33] reported a case-control study in which they analysed the possible association between this ratio and colorectal carcinogenesis, and found positive associations, but the intake of phosphorous did not appear to have any modulating effect on the relation between dietary Ca intake and CRC.

In addition, the frequency of use of salt added to cooking was significantly higher in cases than controls. In other case-control studies, a positive association between sodium intake and CRC was also observed [34]. In any case, our average intake of Na from SFFQ was similar for cases and for controls, probably due to the difficulty to estimate this intake from self-reported data on added salt [35]. In summary, the results were in agreement with the hypothesis that diet, from the nutritional perspective, is significantly different between cases and controls, being in some aspects more favourable in controls,

particularly with regard to intake of SFA, cholesterol, vitamin B_2, the Ca/P ratio and the use of salt added to cooking.

Second, in respect to consumption by food groups, a high consumption of high-fat cheeses was associated with CRC risk, whereas, a high intake of fibre-containing foods, especially whole grains, and fatty fish, was associated with a lower risk for CRC. As other authors have previously reported [36] milk/dairy products was the food group with the highest adjusted OR for CRC risk, which is not in agreement with the likelihood of evidence that this food group may have a protector effect against CRC [37]. However, case-control studies published to date are heterogeneous and, on average, do not provide evidence of an association between total intake of total dairy products, milk, cheese or yogurt and CRC risk [37]. Regarding milk/dairy product consumption according to anatomical subsites of cases, the intake was higher in proximal tumour cases and lower in distal cases than in their matched controls. Although according to scientific literature, the effect of this food group seems to be similar across all locations of the bowel [38].

In general, epidemiological studies have not found evidence of either a reduction or an increase in CRC risk specifically associated with the consumption of cheese [39,40]. Although there are few research papers on cheese consumption that have reported an inverse association with CRC [41], in this research, high-fat cheeses are shown to be possible risk factors for CRC development. Some studies showed a positive relationship between fatty foods and CRC incidence [42]. Dairy products, e.g., mature, semi-mature and creamy cheeses, are rich in saturated fat, so this relationship might be due to the content of fat in these products. In any case, the association between milk/dairy product consumption and the risk of developing CRC is complex and some researchers indicated that the fat content contained within dairy products does not influence this association [38].

In line with previous studies [43-45], we also found that the consumption of fibre-containing foods was inversely associated with CRC risk. The preventive effect of dietary fibre can be explained by biological mechanisms that include increasing amounts of faeces, decreasing gastrointestinal transit time, diluting intestinal cancer-causing factors, interfering absorption of those, and the lowering of intestinal acidity [46]. Furthermore, our findings suggest that high consumption of whole grains (higher than 17.5 g/d in men and 30.0 g/d in women) may decrease the risk of CRC, after controlling confounding factors. There is convincing evidence that whole grains help to reduce CRC risk [37,47,48]. The observed reduction in CRC risk associated with high consumption of whole grains may partly be attributed to dietary fibre, resistant starch, and

oligosaccharides that can influence the gut environment. In addition, recent research about the influence of dietary intake on gene expression in colon tissue has observed that the genes that are differentially expressed with a high intake of whole grain and vegetable consumption are associated with NF-κB signalling, regulation of apoptosis, cytoskeleton dynamics, and carbohydrate metabolism [49].

On the other hand, the consumption of fatty fish was associated with a decreased risk in CRC, after adjusting models for covariates. It should be noted that the consumption of total fish and fatty fish is higher in the Basque Country compared to other Spanish autonomous communities [50,51]. Recent cohort studies have observed that fatty fish was inversely associated with CRC incidence [52,53] and they have related this association with exposure to long-chain n-3 polyunsaturated fatty acids [52]. Evidence from animal and in vitro studies indicates that n-3 fatty acids present in fatty fish may inhibit carcinogenesis [54]. According to the hypothesis raised by Larsson *et al.* (2004) [55], n-3 fatty acids can suppress arachidonic acid-derived eicosanoid biosynthesis; influence transcription factor activity, gene expression, and signal transduction pathways; modulate estrogen metabolism; increase or decrease the production of free radicals and reactive oxygen species; and influence insulin sensitivity and membrane fluidity.

In summary, these results confirm part of Hypothesis 3 of the thesis, about the association between certain foods with increasing or decreasing risk for CRC. Although association between CRC risk and certain foods such as red meat and processed meat or alcoholic drinks, that are well documented in the literature [37], were not confirmed in this case-control sample. Furthermore, food group intakes were not substantially different between proximal and distal cancer cases, except for fish, milk/dairy products and fat.

Finally, adherence to the MD pattern was associated with a lower risk of CRC. These findings on the MDS and CRC risk are supported by those of other researchers [56-61], who found significant associations between lower risk of CRC and adherence to the Mediterranean dietary pattern. However, the HEISD was not associated with CRC risk, discrepancies in results obtained with the two dietary quality indices analysed are probably due to differences in their constructs and scoring criteria. The overall MDS was inversely associated with CRC risk, the total score in cases with the proximal location of cancer being higher than for those with the distal location. These last results contrast with previous findings, which showed that the protective effects of adherence to the MD were mainly for distal colon and rectal cancer and not for proximal colon cancer [13]. In the total sample, investigation of the separate score components showed that whole

grain score was lower for cases than for controls. This result is consistent with that obtained for the association between whole grain consumption and CRC risk. Therefore, we are able to confirm part of Hypothesis 3 put forward in this doctoral thesis with reference to the association between the MD pattern and decreasing risk for CRC, and to differences depending on tumour location.

SNPs associated with susceptibility for the development of CRC in the case-control sample from the CRCSP *of the Osakidetza/Basque Health Service*

The study of SNPs associated with susceptibility for the development of CRC showed that out of 48 analysed SNPs, only the rs6687758 was associated with the risk of CRC in this population. This is in agreement with previous GWAS that also reported a positive association between this SNP and CRC in the European population [62,63]. Some authors have also observed relationships between this SNP and colorectal polyp risk [64]; although this SNP is not associated significantly with adenoma risk and effects the malignant stage of colorectal tumorigenesis [65]. The frequency of the risk allele of rs6687758 (G) in the European population (22.2%) [66] is similar to that registered in the cases of the present study and higher than that of the controls.

The SNP rs6687758 is in a regulatory region, flanking the promoter of *dual* specificity protein phosphatase 10 (*DUSP10*), also known as MAP kinase phosphatase 5 (*MKP5*), at ~250 kb from the start of the gene. Hence, it is likely to affect the expression of this gene. Polymorphisms in *DUSP10 gene* have previously been demonstrated to be associated with CRC risk [67,68]. *DUSP10* is considered to be an inhibitor of inflammation [69] and was shown to negatively regulate the proliferation of intestinal epithelial cells and act as a suppressor for CRC [70]. It seems likely that *DUSP10* inhibits the intestinal epithelial cell barrier function via inhibition of ERK1/2 activation and *KLF5* expression, which in turn reduces intestinal epithelial cell proliferation, necessary for wound healing [70]. In any case, the harmful effect will occur when, for example, the intestine is exposed to carcinogenic compounds leading to the development of cancer-associated mutation.

Target kinases of DUSPs are inactivated by dephosphorylation of both phosphoserine/threonine and phosphotyrosine residues [67,68]. They act at several levels, taking part in fine-tuning signalling cascades. DUSPs negatively regulate members of the mitogen-activated protein kinase (*MAPK*) superfamily [67,71], which are implicated in some activities that are often dysregulated in cancer, such as cell proliferation, survival, and migration [67]. The most important groups of MAPKs are the

p38, JNK1/2 and extracellular signal-regulated kinase (ERK1/2). *MAPK* signalling also plays a key role in determining the response of tumour cells to cancer therapies, since its abnormal signalling has important consequences for the development and progression of human cancer [71].

Several studies have already shown the involvement of *DUSP*s as major modulators of critical signalling pathways dysregulated in different cancers [72], such as in the case of the overexpression of *DUSP1*/*MKP-1* in the early phases of cancer and its decrease during tumour progression [68]. There is abundant evidence that *DUSP10*, in particular, may play an important role in tumorigenesis and could alter CRC risk [71,73]. It inactivates *p38* and *JNK in vitro* [67,74], and its upregulation is very common in CRC [75]. The activation of the JNK protein is due to the protein kinase *G (PKG)/MEKK1/SEK1/JNK* cascade, and it is related to cell proliferation and apoptosis induction [67,70]. Moreover, *p38* is involved in the promotion of cellular senescence as a means of eluding oncogene-induced transformation; it participates in cell cycle regulation, suppressing cell proliferation and tumorigenesis [67,70].

On the other hand, the results extracted from gene expression association analyses show a higher expression of the *DUSP10* gene in CRC cases, but also that there is a higher expression of this gene in colon tissue of healthy controls when they have the GG genotype for rs6687758. Thus, a relationship is likely to be found between higher expression of the gene and the presence of allele G in rs6687758 in tumour tissue. Nonetheless, it would be interesting to further explore this aspect through future analyses to compare gene expression between individuals carrying the risk variant and control individuals. Previous studies have concurred to some extent that there is an overall increase in patients' relapse-free survival when *DUSP10* expression is upregulated, and that *DUSP10* mRNA was increased in the tumour compared with normal tissue adjacent to the tumours [70,73,76].

In addition, we found an association between smoking status and the rs6687758 SNP for CRC risk in cases. Other authors have also observed this association [77]. Benzo[a]pyrene, one of the carcinogenic compounds included in cigarette smoke, up-regulated *COX-2* in mouse cells [78], which in turn could either activate or be dependent on the *MAPK* pathway, suggesting a possible gene-smoking interaction [79,80]. Regarding the association between physical activity, the rs6687758 SNP and CRC risk, as far as we know, there are no precedents in the literature. However, other studies have found interactions between polymorphisms associated with growth hormone (*GH1*) and insulin-like growth factor I (*IGF-I*) (rs647161, rs2665802), physical activity and CRC [79,80]. According to our results, rs6687758, medium-high physical activity level and

CRC would be associated. However, this outcome, contrary to what might be expected, could be related to lifestyle changes, including increased physical activity level, in cases following diagnosis [81].

The remaining 47 SNPs analysed in this doctoral thesis were not replicated in our population. This may be due to differences in the underlying linkage patterns given the ethnic differences in the populations studied. Twenty-one of the SNPs analysed have been replicated in Asian, American-Caucasian or African but not in European populations; and four were not replicated in any population studies; however, they were associated with susceptibility for development of CRC in GWAS. The effect sizes of some of these associations were small (OR <1.20, P<0.05, for rs1321311, rs12241008, and rs704017) [82-84]. Additionally, it may be that the distribution of environmental factors in our population differs from that of the populations in which these genetic variants were discovered.

Finally, the unweighted and weighted GRS models showed that cases had more risk alleles than controls; this result was according to expectations considering the previous studies [85]. In the crude analysis, we observed that patients that had a higher number of risk alleles had a higher risk of CRC. Other authors observed similar results using an adjusted unweighted model [87]. However, some other authors did not find this association [88]. It should be noted that common allele variants generally have modest effect sizes [89], but the combination of multiple loci with modest effects in a global GRS might improve the identification of patients with genetic risk for common complex diseases, such cancer [90]. In this sense, Ortlepp *et al.* [91] concluded that more than 200 polymorphisms might be necessary for “reasonable” genetic discrimination.

Briefly, the results of this section of the thesis showed that most SNPs analysed were not associated with risk of CRC. Only one of the 48 SNPs analysed, rs6687758, was associated with a risk of CRC, in this population (on crude analysis). Moreover, there were significant associations between smoking status, physical activity, the rs6687758SNP and CRC risk. On the other hand, the results of the GRS showed that the risk alleles were more frequent in cases than in controls and this score was associated with this type of cancer in crude analysis.

Strengths and limitations

One of the strengths of this thesis comes from the originality of the pilot study, to the best of our knowledge, this nutritional study is the first one dedicated to nutritional adequacy and diet quality in CRC patients postsurgery. But the main strength is the fact that it

provides information based on a standardised protocol including not only dietary and genetic factors but also other possible determinants of CRC such as health determinants and weight status, among others. A further strength of the case-control study compared to others [56,92,93] is that colonoscopy was used as a diagnosis criterion to identify the cases in order to avoid false positives and negatives.

However, there are a number of limitations that should be mentioned. First, the small sample size of the pilot study carried out in CRC patients postsurgery did not allow a more precise analysis of the dietary adequacy of this group. Second, recall bias inherent in a case-control study design cannot be ruled out. The primary concern of this study is the low participation rate, which may have limited the representativeness of study samples. The decision to participate or not may have been influenced by several factors, including social, educational and health conditions, which may again correlate with outcome risk factors. Third, self-reported data could be subject to measurement errors and the problem of food omissions due to memory failure and underreporting of unhealthy habits among disease subjects. Despite that, previous validation studies indicate that the self-reported dietary information is reported with sufficient accuracy for use in epidemiology analysis [94,95]; and it should be noted that dietary changes are usually modest after participating in the CRCSP due to a lack of information or personalized advice [96-98].

Fourth, to avoid selection bias of controls we obtained controls from the same CRCSP and in the same period as cases, thus, it had been confirmed by colonoscopy that they did not suffer from CRC. Fifth, the lack of control of some possible confounders, such as comorbidities and other conditions that could affect food consumption and the capacity to absorb and to use nutrients, should be noted. Finally, sixth, the sample size of the case-control study makes it difficult to detect possible associations between polymorphisms and disease risk since some genotypes showed very low frequencies in our population. Another disadvantage of the small sample size is that it can produce false-positive results; in order to avoid this the Bonferroni correction was used.

Despite these limitations and considering all the results obtained in this doctoral thesis, we can conclude that the diet of the studied CRC patients postsurgery is inadequate in many respects, including nutrients and food intakes, and that this inadequacy is associated with certain health determinants. Further studies are needed to confirm these determinants with the purpose of applying educational programs in target groups and individualized nutritional counselling sessions to improve life quality and reduce the risk of CRC mortality. On the other hand, we also conclude that there are direct associations between CRC risk and high-fat cheese, and inverse associations with fibre-containing

foods and fatty fish, as well as adherence to an MD pattern, in the case-control sample analysed. With respect to genetic factors, we have confirmed a CRC susceptibility locus and the existence of associations between modifiable factors and the rs6687758 SNP; moreover, the GRS was associated with CRC. However, further studies are needed to better understand the influence of the dietary habits on CRC prevention and to establish the role of the genetic factors, as well as the contribution of the gene-diet interactions to the risk of CRC in this population.

Future perspectives

The results obtained in this thesis will be confirmed in later studies with larger sample sizes. The population of the Basque Autonomous Community offers us a great opportunity due to the relatively high incidence of this type of cancer and the existence of a public health system that facilitates the obtaining of clinical histories, pathology reports and tissue samples needed for the study. Therefore, in the future, it is expected to be able to extend the project to other Osakidetza Hospitals in order to increase the sample size. It should be remembered that this doctoral thesis is part of a line of research on the impact of gene-diet interactions on the risk of CRC in the Basque Country.

References

1. Kasdagly M, Radhakrishnan S, Reddivari L, Veeramachaneni DN, Vanamala J. Colon carcinogenesis: influence of Western diet-induced obesity and targeting stem cells using dietary bioactive compounds. *Nutrition*. 2014 Nov-Dec;30(11-12):1242-56. doi: 10.1016/j.nut.2014.02.016. Epub 2014 Mar 12. PMID: 25280404.
2. Zhu Y, Wu H, Wang PP, Savas S, Woodrow J, Wish T, Jin R, Green R, Woods M, Roebothan B, Buehler S, Dicks E, McLaughlin JR, Campbell PT, Parfrey PS. Dietary patterns and colorectal cancer recurrence and survival: a cohort study. *BMJ Open*. 2013 Feb 7;3(2):e002270. doi: 10.1136/bmjopen-2012-002270. PMID: 23396503; PMCID: PMC3586110.
3. Gómez Valiente da Silva H, Fonseca de Andrade C, Bello Moreira AS. Dietary intake and nutritional status in cancer patients; comparing adults and older adults. *Nutr Hosp.* 2014 Apr 1;29(4):907-12. doi: 10.3305/nh.2014.29.4.7131. PMID: 24679035.
4. Sharkey JR, Branch LG, Zohoori N, Giuliani C, Busby-Whitehead J, Haines PS. Inadequate nutrient intakes among homebound elderly and their correlation with

individual characteristics and health-related factors. *Am J Clin Nutr*. 2002 Dec;76(6):1435-45. doi: 10.1093/ajcn/76.6.1435. PMID: 12450914.

5. Lim H, Cho G, Kim S. Evaluation of nutrient intake and diet quality of gastric cancer patients in Korea. *Nutr Res Pract*. 2012 Jun;6(3):213-20. doi: 10.4162/nrp.2012.6.3.213. Epub 2012 Jun 30. PMID: 22808345; PMCID: PMC3395786.
6. Norte Navarro AI, Ortiz Moncada R. [Spanish diet quality according to the healthy eating index]. *Nutr Hosp*. 2011;26(2):330-336. doi:10.1590/S0212-16112011000200014 PMID**:** 21666971
7. Dixon LB, Subar AF, Peters U, Weissfeld JL, Bresalier RS, Risch A, Schatzkin A, Hayes RB. Adherence to the USDA Food Guide, DASH Eating Plan, and Mediterranean dietary pattern reduces risk of colorectal adenoma. *J Nutr*. 2007 Nov;137(11):2443-50. doi: 10.1093/jn/137.11.2443. PMID: 17951483.
8. Yusof AS, Isa ZM, Shah SA. Perceptions of Malaysian colorectal cancer patients regarding dietary intake: a qualitative exploration. *Asian Pac J Cancer Prev*. 2013;14(2):1151-4. doi: 10.7314/apjcp.2013.14.2.1151. PMID: 23621204.
9. Cha R, Murray MJ, Thompson J, Wall CR, Hill A, Hulme-Moir M, Merrie A, Findlay MP. Dietary patterns and information needs of colorectal cancer patients post-surgery in Auckland. *N Z Med J*. 2012 Jun 8;125(1356):38-46. PMID: 22729057.
10. Gudny Geirsdottir O, Thorsdottir I. Nutritional status of cancer patients in chemotherapy; dietary intake, nitrogen balance and screening. *Food Nutr Res*. 2008;52. doi: 10.3402/fnr.v52i0.1856. Epub 2008 Dec 12. PMID: 19158940; PMCID: PMC2615643.
11. Hu EA, Toledo E, Diez-Espino J, Estruch R, Corella D, Salas-Salvado J, Vinyoles E, Gomez-Gracia E, Aros F, Fiol M, Lapetra J, Serra-Majem L, Pintó X, Portillo MP, Lamuela-Raventos RM, Ros E, Sorli JV, Martinez-Gonzalez MA. Lifestyles and risk factors associated with adherence to the Mediterranean diet: a baseline assessment of the PREDIMED trial. *PLoS One*. 2013 Apr 29;8(4):e60166. doi: 10.1371/journal.pone.0060166. PMID: 23637743; PMCID: PMC3639284.
12. Ma J, Betts NM, Hampl JS. Clustering of lifestyle behaviors: the relationship between cigarette smoking, alcohol consumption, and dietary intake. *Am J Health Promot*. 2000;15(2):107-117. doi:10.4278/0890-1171-15.2.107
13. Grosso G, Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S, Drago F, Basile F. Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. *Nutr Cancer*. 2014;66(4):558-65. doi: 10.1080/01635581.2014.902975. Epub 2014 Apr 22. PMID: 24754383.

14. Bamia C, Lagiou P, Buckland G, et al. Mediterranean diet and colorectal cancer risk: results from a European cohort. *Eur J Epidemiol*. 2013;28(4):317-328. doi:10.1007/s10654-013-9795-x
15. Idigoras Rubio I, Arana-Arri E, Portillo Villares I, Bilbao Iturribarrria I, Martínez-Indart L, Imaz-Ayo N, de la Cruz M, de Castro V, López de Munain A, Torrejón Perez I, Gutiérrez-Ibarluzea I. Participation in a population-based screening for colorectal cancer using the faecal immunochemical test decreases mortality in 5 years. *Eur J Gastroenterol Hepatol*. 2019 Feb;31(2):197-204. doi: 10.1097/MEG.0000000000001338. PMID: 30543575.
16. Alsheridah N, Akhtar S. Diet, obesity and colorectal carcinoma risk: results from a national cancer registry-based middle-eastern study. *BMC Cancer*. 2018 Dec 7;18(1):1227. doi: 10.1186/s12885-018-5132-9. PMID: 30526552; PMCID: PMC6286580.
17. Ulaganathan V, Kandiah M, Shariff ZM. A case-control study on the association of abdominal obesity and hypercholesterolemia with the risk of colorectal cancer. *J Carcinog*. 2018 Sep 25;17:4. doi: 10.4103/jcar.JCar_2_18. PMID: 30294246; PMCID: PMC6166417.
18. Liu PH, Wu K, Ng K, Zauber AG, Nguyen LH, Song M, He X, Fuchs CS, Ogino S, Willett WC, Chan AT, Giovannucci EL, Cao Y. Association of Obesity With Risk of Early-Onset Colorectal Cancer Among Women. Version 2. *JAMA Oncol.* 2019 Jan 1;5(1):37-44. doi: 10.1001/jamaoncol.2018.4280. Erratum in: *JAMA Oncol*. 2019 Apr 1;5(4):579. PMID: 30326010; PMCID: PMC6382547.
19. Bardou M, Barkun AN, Martel M. Obesity and colorectal cancer. *Gut*. 2013 Jun;62(6):933-47. doi: 10.1136/gutjnl-2013-304701. Epub 2013 Mar 12. PMID: 23481261.
20. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006 Dec 14;444(7121):840-6. doi: 10.1038/nature05482. PMID: 17167471.
21. Hanyuda A, Cao Y, Hamada T, Nowak JA, Qian ZR, Masugi Y, da Silva A, Liu L, Kosumi K, Soong TR, Jhun I, Wu K, Zhang X, Song M, Meyerhardt JA, Chan AT, Fuchs CS, Giovannucci EL, Ogino S, Nishihara R. Body mass index and risk of colorectal carcinoma subtypes classified by tumor differentiation status. *Eur J Epidemiol*. 2017 May;32(5):393-407. doi: 10.1007/s10654-017-0254-y. Epub 2017 May 16. PMID: 28510098; PMCID: PMC5507723.
22. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z, Su L, Li X, Li X, Li J, Xiao L, Huber-Schönauer U, Niederseer D, Xu X, Al-Aama JY, Yang H, Wang J, Kristiansen K, Arumugam M, Tilg H, Datz C, Wang

J. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun*. 2015 Mar 11;6:6528. doi: 10.1038/ncomms7528. PMID: 25758642.

23. Kirzin S, Marisa L, Guimbaud R, De Reynies A, Legrain M, Laurent-Puig P, Cordelier P, Pradère B, Bonnet D, Meggetto F, Portier G, Brousset P, Selves J. Sporadic early-onset colorectal cancer is a specific sub-type of cancer: a morphological, molecular and genetics study. *PLoS One*. 2014 Aug 1;9(8):e103159. doi: 10.1371/journal.pone.0103159. PMID: 25083765; PMCID: PMC4118858.
24. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, Goldberg RM, Paskett E, Shields PG, Freudenheim JL, Stanich PP, Lattimer I, Arnold M, Liyanarachchi S, Kalady M, Heald B, Greenwood C, Paquette I, Prues M, Draper DJ, Lindeman C, Kuebler JP, Reynolds K, Brell JM, Shaper AA, Mahesh S, Buie N, Weeman K, Shine K, Haut M, Edwards J, Bastola S, Wickham K, Khanduja KS, Zacks R, Pritchard CC, Shirts BH, Jacobson A, Allen B, de la Chapelle A, Hampel H; Ohio Colorectal Cancer Prevention Initiative Study Group. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. *JAMA Oncol*. 2017 Apr 1;3(4):464-471. doi: 10.1001/jamaoncol.2016.5194. PMID: 27978560; PMCID: PMC5564179.
25. Kerr J, Anderson C, Lippman SM. Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. *Lancet Oncol*. 2017 Aug;18(8):e457-e471. doi: 10.1016/S1470-2045(17)30411-4. Epub 2017 Jul 26. PMID: 28759385.
26. Keum N, Cao Y, Oh H, *et al.* Sedentary behaviors and light-intensity activities in relation to colorectal cancer risk. *Int J Cancer*. 2016;138(9):2109-2117. doi:10.1002/ijc.29953
27. Thanikachalam K, Khan G. Colorectal Cancer and Nutrition. *Nutrients*. 2019 Jan 14;11(1):164. doi: 10.3390/nu11010164. PMID: 30646512; PMCID: PMC6357054.
28. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC. Major dietary patterns and the risk of colorectal cancer in women. *Arch Intern Med*. 2003 Feb 10;163(3):309-14. doi: 10.1001/archinte.163.3.309. PMID: 12578511.
29. Tayyem RF, Bawadi HA, Shehadah IN, Abu-Mweis SS, Agraib LM, Bani-Hani KE, Al-Jaberi T, Al-Nusairr M, Heath DD. Macro- and micronutrients consumption and the risk for colorectal cancer among Jordanians. *Nutrients*. 2015 Mar

10;7(3):1769-86. doi: 10.3390/nu7031769. PMID: 25763533; PMCID: PMC4377880.

30. Arafa MA, Waly MI, Jriesat S, Al Khafajei A, Sallam S. Dietary and lifestyle characteristics of colorectal cancer in Jordan: a case-control study. *Asian Pac J Cancer Prev.* 2011;12(8):1931-6. PMID: 22292627.
31. Endo H, Hosono K, Fujisawa T, Takahashi H, Sugiyama M, Yoneda K, Nozaki Y, Fujita K, Yoneda M, Inamori M, Wada K, Nakagama H, Nakajima A. Involvement of JNK pathway in the promotion of the early stage of colorectal carcinogenesis under high-fat dietary conditions. *Gut*. 2009 Dec;58(12):1637-43. doi: 10.1136/gut.2009.183624. Epub 2009 Jun 30. PMID: 19570763.
32. Ben S, Du M, Ma G, Qu J, Zhu L, Chu H, Zhang Z, Wu Y, Gu D, Wang M. Vitamin B_2 intake reduces the risk for colorectal cancer: a dose-response analysis. *Eur J Nutr*. 2019 Jun;58(4):1591-1602. doi: 10.1007/s00394-018-1702-5. Epub 2018 May 9. PMID: 29744609.
33. Boutron MC, Faivre J, Marteau P, Couillault C, Senesse P, Quipourt V. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case--control study. *Br J Cancer*. 1996 Jul;74(1):145-51. doi: 10.1038/bjc.1996.330. PMID: 8679449; PMCID: PMC2074613.
34. Yakoob MY, Baig-ansari N. Dietary Sodium (salt) Intake and Risk of Colorectal Cancer: A Systematic Review (P05-039-19). *Curr Dev Nutr*. 2019 Jun 13;3(Suppl 1):nzz030.P05-039-19. doi: 10.1093/cdn/nzz030.P05-039-19. PMCID: PMC6574693.
35. Quader ZS, Zhao L, Harnack LJ, Gardner CD, Shikany JM, Steffen LM, Gillespie C, Moshfegh A, Cogswell ME. Self-Reported Measures of Discretionary Salt Use Accurately Estimated Sodium Intake Overall but not in Certain Subgroups of US Adults from 3 Geographic Regions in the Salt Sources Study. *J Nutr*. 2019 Sep 1;149(9):1623-1632. doi: 10.1093/jn/nxz110. PMID: 31179499; PMCID: PMC6735801.
36. Abbastabar H, Roustazadeh A, Alizadeh A, Hamidifard P, Valipour M, Valipour AA. Relationships of colorectal cancer with dietary factors and public health indicators: an ecological study. *Asian Pac J Cancer Prev*. 2015;16(9):3991-5. doi: 10.7314/apjcp.2015.16.9.3991. PMID: 25987074.
37. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. 2017..
38. Murphy N, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Olsen A, Tjønneland A, Dahm CC, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F,

Nailler L, Kaaks R, Teucher B, Boeing H, Bergmann MM, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Pala V, Tumino R, Vineis P, Panico S, Peeters PH, Dik VK, Weiderpass E, Lund E, Garcia JR, Zamora-Ros R, Pérez MJ, Dorronsoro M, Navarro C, Ardanaz E, Manjer J, Almquist M, Johansson I, Palmqvist R, Khaw KT, Wareham N, Key TJ, Crowe FL, Fedirko V, Gunter MJ, Riboli E. Consumption of dairy products and colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS One*. 2013 Sep 2;8(9):e72715. doi: 10.1371/journal.pone.0072715. PMID: 24023767; PMCID: PMC3759377.

39. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. *Eur J Clin Nutr*. 2003 Jan;57(1):1-17. doi: 10.1038/sj.ejcn.1601522. PMID: 12548291.
40. Aune D, Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, Norat T. Dairy products and colorectal cancer risk: a systematic review and meta-analysis of cohort studies. *Ann Oncol*. 2012 Jan;23(1):37-45. doi: 10.1093/annonc/mdr269. Epub 2011 May 26. PMID: 21617020.
41. Larsson SC, Bergkvist L, Wolk A. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. *Am J Clin Nutr*. 2005 Oct;82(4):894-900. doi: 10.1093/ajcn/82.4.894. PMID: 16210722.
42. Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. *World J Gastroenterol*. 2009 Nov 7;15(41):5141-8. doi: 10.3748/wjg.15.5141. PMID: 19891012; PMCID: PMC2773892.
43. Dahm CC, Keogh RH, Spencer EA, Greenwood DC, Key TJ, Fentiman IS, Shipley MJ, Brunner EJ, Cade JE, Burley VJ, Mishra G, Stephen AM, Kuh D, White IR, Luben R, Lentjes MA, Khaw KT, Rodwell Bingham SA. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. *J Natl Cancer Inst*. 2010 May 5;102(9):614-26. doi: 10.1093/jnci/djq092. Epub 2010 Apr 20. Erratum in: *J Natl Cancer Inst.* 2011 Oct 5;103(19):1484. PMID: 20407088.
44. Tan J, Chen YX. Dietary and Lifestyle Factors Associated with Colorectal Cancer Risk and Interactions with Microbiota: Fiber, Red or Processed Meat and Alcoholic Drinks. Gastrointest Tumors. 2016 Sep;3(1):17-24. doi: 10.1159/000442831. Epub 2015 Dec 18. Erratum in: *Gastrointest Tumors*. 2018 Feb;4(3-4):104. PMID: 27722153; PMCID: PMC5040877.
45. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandona R, Dandona L, Fleming T, Forouzanfar MH, Hancock J, Hay RJ, Hunter-Merrill

R, Huynh C, Hosgood HD, Johnson CO, Jonas JB, Khubchandani J, Kumar GA, Kutz M, Lan Q, Larson HJ, Liang X, Lim SS, Lopez AD, MacIntyre MF, Marczak L, Marquez N, Mokdad AH, Pinho C, Pourmalek F, Salomon JA, Sanabria JR, Sandar L, Sartorius B, Schwartz SM, Shackelford KA, Shibuya K, Stanaway J, Steiner C, Sun J, Takahashi K, Vollset SE, Vos T, Wagner JA, Wang H, Westerman R, Zeeb H, Zoeckler L, Abd-Allah F, Ahmed MB, Alabed S, Alam NK, Aldhahri SF, Alem G, Alemayohu MA, Ali R, Al-Raddadi R, Amare A, Amoako Y, Artaman A, Asayesh H, Atnafu N, Awasthi A, Saleem HB, Barac A, Bedi N, Bensenor I, Berhane A, Bernabé E, Betsu B, Binagwaho A, Boneya D, Campos-Nonato I, Castañeda-Orjuela C, Catalá-López F, Chiang P, Chibueze C, Chitheer A, Choi JY, Cowie B, Damtew S, das Neves J, Dey S, Dharmaratne S, Dhillon P, Ding E, Driscoll T, Ekwueme D, Endries AY, Farvid M, Farzadfar F, Fernandes J, Fischer F, G/Hiwot TT, Gebru A, Gopalani S, Hailu A, Horino M, Horita N, Husseini A, Huybrechts I, Inoue M, Islami F, Jakovljevic M, James S, Javanbakht M, Jee SH, Kasaeian A, Kedir MS, Khader YS, Khang YH, Kim D, Leigh J, Linn S, Lunevicius R, El Razek HMA, Malekzadeh R, Malta DC, Marcenes W, Markos D, Melaku YA, Meles KG, Mendoza W, Mengiste DT, Meretoja TJ, Miller TR, Mohammad KA, Mohammadi A, Mohammed S, Moradi-Lakeh M, Nagel G, Nand D, Le Nguyen Q, Nolte S, Ogbo FA, Oladimeji KE, Oren E, Pa M, Park EK, Pereira DM, Plass D, Qorbani M, Radfar A, Rafay A, Rahman M, Rana SM, Søreide K, Satpathy M, Sawhney M, Sepanlou SG, Shaikh MA, She J, Shiue I, Shore HR, Shrime MG, So S, Soneji S, Stathopoulou V, Stroumpoulis K, Sufiyan MB, Sykes BL, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tessema GA, Thakur JS, Tran BX, Ukwaja KN, Uzochukwu BSC, Vlassov VV, Weiderpass E, Wubshet Terefe M, Yebyo HG, Yimam HH, Yonemoto N, Younis MZ, Yu C, Zaidi Z, Zaki MES, Zenebe ZM, Murray CJL, Naghavi M. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol*. 2017 Apr 1;3(4):524-548. doi: 10.1001/jamaoncol.2016.5688. Erratum in: *JAMA Oncol*. 2017 Mar 1;3(3):418. PMID: 27918777; PMCID: PMC6103527.

46. Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, Norat T. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. Version 2. *BMJ*. 2011 Nov 10;343:d6617. doi: 10.1136/bmj.d6617. PMID: 22074852; PMCID: PMC3213242.

47. Vieira AR, Abar L, Chan DSM, Vingeliene S, Polemiti E, Stevens C, Greenwood D, Norat T. Foods and beverages and colorectal cancer risk: a systematic review and meta-analysis of cohort studies, an update of the evidence of the WCRF-AICR Continuous Update Project. *Ann Oncol*. 2017 Aug 1;28(8):1788-1802. doi: 10.1093/annonc/mdx171. PMID: 28407090.
48. Tieri M, Ghelfi F, Vitale M, Vetrani C, Marventano S, Lafranconi A, Godos J, Titta L, Gambera A, Alonzo E, Sciacca S, Riccardi G, Buscemi S, Del Rio D, Ray S, Galvano F, Beck E, Grosso G. Whole grain consumption and human health: an umbrella review of observational studies. *Int J Food Sci Nutr*. 2020 Jan 21:1-10. doi: 10.1080/09637486.2020.1715354. Epub ahead of print. PMID: 31964201.
49. Pellatt AJ, Slattery ML, Mullany LE, Wolff RK, Pellatt DF. Dietary intake alters gene expression in colon tissue: possible underlying mechanism for the influence of diet on disease. Version 2. *Pharmacogenet Genomics*. 2016 Jun;26(6):294-306. doi: 10.1097/FPC.0000000000000217. PMID: 26959716; PMCID: PMC4853256.
50. Ministerio de Agricultura, Alimentación y Medio Ambiente, Gobierno de España. Informe del Consumo de Alimentación en España 2014. Available from: https://www.mapa.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2014_tcm30-104149.pdf; [25/02/2020]
51. Federación Española del Corazón (FEC). Encuesta de consumo de pescado azul en España 2015. Available from: https://fundaciondelcorazon.com/prensa/notas-de-prensa/2773-la-poblacion-adulta-espanola-suspende-en-el-consumo-de-pescado-azul-recomendado-.html [25/02/2020]
52. Aglago EK, Huybrechts I, Murphy N, Casagrande C, Nicolas G, Pischon T, Fedirko V, Severi G, Boutron-Ruault MC, Fournier A, Katzke V, Kühn T, Olsen A, Tjønneland A, Dahm CC, Overvad K, Lasheras C, Agudo A, Sánchez MJ, Amiano P, Huerta JM, Ardanaz E, Perez-Cornago A, Trichopoulou A, Karakatsani A, Martimianaki G, Palli D, Pala V, Tumino R, Naccarati A, Panico S, Bueno-de-Mesquita B, May A, Derksen JWG, Hellstrand S, Ohlsson B, Wennberg M, Van Guelpen B, Skeie G, Brustad M, Weiderpass E, Cross AJ, Ward H, Riboli E, Norat T, Chajes V, Gunter MJ. Consumption of Fish and Long-chain n-3 Polyunsaturated Fatty Acids Is Associated With Reduced Risk of Colorectal Cancer in a Large European Cohort. *Clin Gastroenterol Hepatol*. 2020 Mar;18(3):654-666.e6. doi: 10.1016/j.cgh.2019.06.031. Epub 2019 Jun 25. PMID: 31252190.

53. Spencer EA, Key TJ, Appleby PN, Dahm CC, Keogh RH, Fentiman IS, Akbaraly T, Brunner EJ, Burley V, Cade JE, Greenwood DC, Stephen AM, Mishra G, Kuh D, Luben R, Mulligan AA, Khaw KT, Rodwell SA. Meat, poultry and fish and risk of colorectal cancer: pooled analysis of data from the UK dietary cohort consortium. *Cancer Causes Control.* 2010 Sep;21(9):1417-25. doi: 10.1007/s10552-010-9569-7. Epub 2010 May 1. Erratum in: *Cancer Causes Control*. 2011 Sep;22(9):1351. PMID: 20437091.
54. Rao CV, Hirose Y, Indranie C, Reddy BS. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Res*. 2001 Mar 1;61(5):1927-33. PMID: 11280748.
55. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am J Clin Nutr*. 2004 Jun;79(6):935-45. doi: 10.1093/ajcn/79.6.935. PMID: 15159222.
56. Castelló A, Amiano P, Fernández de Larrea N, Martín V, Alonso MH, Castaño-Vinyals G, Pérez-Gómez B, Olmedo-Requena R, Guevara M, Fernandez-Tardon G, Dierssen-Sotos T, Llorens-Ivorra C, Huerta JM, Capelo R, Fernández-Villa T, Díez-Villanueva A, Urtiaga C, Castilla J, Jiménez-Moleón JJ, Moreno V, Dávila-Batista V, Kogevinas M, Aragonés N, Pollán M; MCC-Spain researchers. Low adherence to the western and high adherence to the mediterranean dietary patterns could prevent colorectal cancer. *Eur J Nutr*. 2019 Jun;58(4):1495-1505. doi: 10.1007/s00394-018-1674-5. Epub 2018 Mar 26. PMID: 29582162.
57. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am J Clin Nutr.* 2004 Jun;79(6):935-45. doi: 10.1093/ajcn/79.6.935. PMID: 15159222.
58. Jones P, Cade JE, Evans CEL, Hancock N, Greenwood DC. The Mediterranean diet and risk of colorectal cancer in the UK Women's Cohort Study. *Int J Epidemiol*. 2017 Dec 1;46(6):1786-1796. doi: 10.1093/ije/dyx155. PMID: 29025095.
59. Donovan MG, Selmin OI, Doetschman TC, Romagnolo DF. Mediterranean Diet: Prevention of Colorectal Cancer. *Front Nutr*. 2017 Dec 5;4:59. doi: 10.3389/fnut.2017.00059. PMID: 29259973; PMCID: PMC5723389.
60. Jafari Nasab S, Bahrami A, Rafiee P, Hekmatdoust A, Ghanavati M, Rashidkhani B, Sadeghi A, Asadzadeh Aghdaei H, Naja F, Hejazi E. Healthy Eating Index-2010 and Mediterranean-Style Dietary Pattern Score and the risk of colorectal cancer and adenoma: a case-control study. *Nutr Cancer*. 2019 Nov 5:1-10. doi: 10.1080/01635581.2019.1683212. Epub ahead of print. PMID: 31687849.

61. Zhong Y, Zhu Y, Li Q, Wang F, Ge X, Zhou G, Miao L. Association between Mediterranean diet adherence and colorectal cancer: a dose-response meta-analysis. *Am J Clin Nutr*. 2020 May 2:nqaa083. doi: 10.1093/ajcn/nqaa083. Epub ahead of print. PMID: 32359135.
62. Real LM, Ruiz A, Gayán J, González-Pérez A, Sáez ME, Ramírez-Lorca R, Morón FJ, Velasco J, Marginet-Flinch R, Musulén E, Carrasco JM, Moreno-Rey C, Vázquez E, Chaves-Conde M, Moreno-Nogueira JA, Hidalgo-Pascual M, Ferrero-Herrero E, Castellví-Bel S, Castells A, Fernandez-Rozadilla C, Ruiz-Ponte C, Carracedo A, González B, Alonso S, Perucho M. A colorectal cancer susceptibility new variant at 4q26 in the Spanish population identified by genome-wide association analysis. *PLoS One*. 2014 Jun 30;9(6):e101178. doi: 10.1371/journal.pone.0101178. PMID: 24978480; PMCID: PMC4076321.
63. Houlston RS, Cheadle J, Dobbins SE, Tenesa A, Jones AM, Howarth K, Spain SL, Broderick P, Domingo E, Farrington S, Prendergast JG, Pittman AM, Theodoratou E, Smith CG, Olver B, Walther A, Barnetson RA, Churchman M, Jaeger EE, Penegar S, Barclay E, Martin L, Gorman M, Mager R, Johnstone E, Midgley R, Niittymäki I, Tuupanen S, Colley J, Idziaszczyk S; COGENT Consortium, Thomas HJ, Lucassen AM, Evans DG, Maher ER; CORGI Consortium; COIN Collaborative Group; COINB Collaborative Group, Maughan T, Dimas A, Dermitzakis E, Cazier JB, Aaltonen LA, Pharoah P, Kerr DJ, Carvajal-Carmona LG, Campbell H, Dunlop MG, Tomlinson IP. Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. *Nat Genet*. 2010 Nov;42(11):973-7. doi: 10.1038/ng.670. Epub 2010 Oct 24. PMID: 20972440; PMCID: PMC5098601.
64. Zhang B, Shrubsole MJ, Li G, Cai Q, Edwards T, Smalley WE, Ness RM, Zheng W. Association of genetic variants for colorectal cancer differs by subtypes of polyps in the colorectum. *Carcinogenesis*. 2012 Dec;33(12):2417-23. doi: 10.1093/carcin/bgs308. Epub 2012 Oct 1. PMID: 23027627; PMCID: PMC3510743.
65. Carvajal-Carmona LG, Zauber AG, Jones AM, Howarth K, Wang J, Cheng T; APC Trial Collaborators; APPROVe Trial Collaborators; CORGI Study Collaborators; Colon Cancer Family Registry Collaborators; CGEMS Collaborators, Riddell R, Lanas A, Morton D, Bertagnolli MM, Tomlinson I. Much of the genetic risk of colorectal cancer is likely to be mediated through susceptibility to adenomas. *Gastroenterology*. 2013 Jan;144(1):53-5. doi:

10.1053/j.gastro.2012.09.016. Epub 2012 Sep 19. PMID: 22999960; PMCID: PMC3572711.

66. Ensembl genome database project [Internet]; 2018 https://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=1:221991106-221992106;v=rs6687758;vdb=variation;vf=3766274
67. Duan X, Gao Y, Yang H, Feng T, Jin T, Long Y, Chen C. Polymorphisms in the DUSP10 gene are associated with sex-specific colorectal cancer risk in a Han population. I*nt J Clin Exp Pathol*. 2015 Feb 1;8(2):2018-25. PMID: 25973098; PMCID: PMC4396290.
68. Bermudez O, Pagès G, Gimond C. The dual-specificity MAP kinase phosphatases: critical roles in development and cancer. *Am J Physiol Cell Physiol*. 2010 Aug;299(2):C189-202. doi: 10.1152/ajpcell.00347.2009. Epub 2010 May 12. PMID: 20463170.
69. Qian F, Deng J, Gantner BN, Flavell RA, Dong C, Christman JW, Ye RD. Map kinase phosphatase 5 protects against sepsis-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2012 May 1;302(9):L866-74. doi: 10.1152/ajplung.00277.2011. Epub 2012 Feb 3. PMID: 22307906; PMCID: PMC3362165.
70. Png CW, Weerasooriya M, Guo J, James SJ, Poh HM, Osato M, Flavell RA, Dong C, Yang H, Zhang Y. DUSP10 regulates intestinal epithelial cell growth and colorectal tumorigenesis. *Oncogene*. 2016 Jan 14;35(2):206-17. doi: 10.1038/onc.2015.74. Epub 2015 Mar 16. PMID: 25772234.
71. Keyse SM. Dual-specificity MAP kinase phosphatases (MKPs) and cancer. *Cancer Metastasis Rev*. 2008 Jun;27(2):253-61. doi: 10.1007/s10555-008-9123-1. PMID: 18330678.
72. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem J.* 2009 Mar 15;418(3):475-89. doi: 10.1042/bj20082234. PMID: 19228121.
73. Nomura M, Shiiba K, Katagiri C, Kasugai I, Masuda K, Sato I, Sato M, Kakugawa Y, Nomura E, Hayashi K, Nakamura Y, Nagata T, Otsuka T, Katakura R, Yamashita Y, Sato M, Tanuma N, Shima H. Novel function of MKP-5/DUSP10, a phosphatase of stress-activated kinases, on ERK-dependent gene expression, and upregulation of its gene expression in colon carcinomas. *Oncol Rep*. 2012 Sep;28(3):931-6. doi: 10.3892/or.2012.1862. Epub 2012 Jun 12. PMID: 22711061.

74. Fang JY, Richardson BC. The MAPK signalling pathways and colorectal cancer. *Lancet Oncol*. 2005 May;6(5):322-7. doi: 10.1016/S1470-2045(05)70168-6. PMID: 15863380.
75. Han J, Sun P. The pathways to tumor suppression via route p38. *Trends Biochem Sci*. 2007 Aug;32(8):364-71. doi: 10.1016/j.tibs.2007.06.007. Epub 2007 Jul 10. PMID: 17624785.
76. Jiménez-Martínez M, Stamatakis K, Fresno M. The Dual-Specificity Phosphatase 10 (DUSP10): Its Role in Cancer, Inflammation, and Immunity. *Int J Mol* Sci. 2019 Apr 1;20(7):1626. doi: 10.3390/ijms20071626. PMID: 30939861; PMCID: PMC6480380.
77. Song N, Shin A, Jung HS, Oh JH, Kim J. Effects of interactions between common genetic variants and smoking on colorectal cancer. *BMC Cance*r. 2017 Dec 19;17(1):869. doi: 10.1186/s12885-017-3886-0. PMID: 29258461; PMCID: PMC5737484.
78. Ouyang W, Ma Q, Li J, Zhang D, Ding J, Huang Y, Xing MM, Huang C. Benzo[a]pyrene diol-epoxide (B[a]PDE) upregulates COX-2 expression through MAPKs/AP-1 and IKKbeta/NF-kappaB in mouse epidermal Cl41 cells. *Mol Carcinog*. 2007 Jan;46(1):32-41. doi: 10.1002/mc.20260. PMID: 16921490.
79. Gunathilake MN, Lee J, Cho YA, Oh JH, Chang HJ, Sohn DK, Shin A, Kim J. Interaction between physical activity, *PITX1* rs647161 genetic polymorphism and colorectal cancer risk in a Korean population: a case-control study. *Oncotarget*. 2018 Jan 10;9(7):7590-7603. doi: 10.18632/oncotarget.24136. Erratum in: *Oncotarget*. 2018 Oct 9;9(79):35027. PMID: 29484135; PMCID: PMC5800927.
80. Khoury-Shakour S, Gruber SB, Lejbkowicz F, Rennert HS, Raskin L, Pinchev M, Rennert G. Recreational physical activity modifies the association between a common GH1 polymorphism and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2008 Dec;17(12):3314-8. doi: 10.1158/1055-9965.EPI-08-0062. PMID: 19064544; PMCID: PMC2665206.
81. Dennis DL, Waring JL, Payeur N, Cosby C, Daudt HM. Making lifestyle changes after colorectal cancer: insights for program development. *Curr Oncol*. 2013 Dec;20(6):e493-511. doi: 10.3747/co.20.1514. PMID: 24311950; PMCID: PMC3851346.
82. Wang H, Burnett T, Kono S, Haiman CA, Iwasaki M, Wilkens LR, Loo LW, Van Den Berg D, Kolonel LN, Henderson BE, Keku TO, Sandler RS, Signorello LB, Blot WJ, Newcomb PA, Pande M, Amos CI, West DW, Bézieau S, Berndt SI, Zanke BW, Hsu L; Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), Lindor NM, Haile RW, Hopper JL, Jenkins MA, Gallinger S, Casey G;

Colon Cancer Family Registry (CCFR), Stenzel SL, Schumacher FR, Peters U, Gruber SB; Colorectal Transdisciplinary Study (CORECT), Tsugane S, Stram DO, Le Marchand L. Trans-ethnic genome-wide association study of colorectal cancer identifies a new susceptibility locus in VTI1A. *Nat Commun*. 2014 Aug 8;5:4613. doi: 10.1038/ncomms5613. PMID: 25105248; PMCID: PMC4180879.

83. Dunlop MG, Dobbins SE, Farrington SM, Jones AM, Palles C, Whiffin N, Tenesa A, Spain S, Broderick P, Ooi LY, Domingo E, Smillie C, Henrion M, Frampton M, Martin L, Grimes G, Gorman M, Semple C, Ma YP, Barclay E, Prendergast J, Cazier JB, Olver B, Penegar S, Lubbe S, Chander I, Carvajal-Carmona LG, Ballereau S, Lloyd A, Vijayakrishnan J, Zgaga L, Rudan I, Theodoratou E; Colorectal Tumour Gene Identification (CORGI) Consortium, Starr JM, Deary I, Kirac I, Kovacević D, Aaltonen LA, Renkonen-Sinisalo L, Mecklin JP, Matsuda K, Nakamura Y, Okada Y, Gallinger S, Duggan DJ, Conti D, Newcomb P, Hopper J, Jenkins MA, Schumacher F, Casey G, Easton D, Shah M, Pharoah P, Lindblom A, Liu T; Swedish Low-Risk Colorectal Cancer Study Group, Smith CG, West H, Cheadle JP; COIN Collaborative Group, Midgley R, Kerr DJ, Campbell H, Tomlinson IP, Houlston RS. Common variation near CDKN1A, POLD3 and SHROOM2 influences colorectal cancer risk. *Nat Genet*. 2012 May 27;44(7):770-6. doi: 10.1038/ng.2293. PMID: 22634755; PMCID: PMC4747430.
84. Abe M, Ito H, Oze I, Nomura M, Ogawa Y, Matsuo K. The more from East-Asian, the better: risk prediction of colorectal cancer risk by GWAS-identified SNPs among Japanese*. J Cancer Res Clin Oncol*. 2017 Dec;143(12):2481-2492. doi: 10.1007/s00432-017-2505-4. Epub 2017 Aug 28. PMID: 28849422.
85. Dunlop MG, Tenesa A, Farrington SM, Ballereau S, Brewster DH, Koessler T, Pharoah P, Schafmayer C, Hampe J, Völzke H, Chang-Claude J, Hoffmeister M, Brenner H, von Holst S, Picelli S, Lindblom A, Jenkins MA, Hopper JL, Casey G, Duggan D, Newcomb PA, Abulí A, Bessa X, Ruiz-Ponte C, Castellví-Bel S, Niittymäki I, Tuupanen S, Karhu A, Aaltonen L, Zanke B, Hudson T, Gallinger S, Barclay E, Martin L, Gorman M, Carvajal-Carmona L, Walther A, Kerr D, Lubbe S, Broderick P, Chandler I, Pittman A, Penegar S, Campbell H, Tomlinson I, Houlston RS. Cumulative impact of common genetic variants and other risk factors on colorectal cancer risk in 42,103 individuals. *Gut*. 2013 Jun;62(6):871-81. doi: 10.1136/gutjnl-2011-300537. Epub 2012 Apr 5. PMID: 22490517; PMCID: PMC5105590.
86. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*.

2017 Jul 3;45(W1):W98-W102. doi: 10.1093/nar/gkx247. PMID: 28407145; PMCID: PMC5570223.

87. Cho YA, Lee J, Oh JH, Chang HJ, Sohn DK, Shin A, Kim J. Genetic Risk Score, Combined Lifestyle Factors and Risk of Colorectal Cancer. *Cancer Res Treat*. 2019 Jul;51(3):1033-1040. doi: 10.4143/crt.2018.447. Epub 2018 Oct 18. PMID: 30336659; PMCID: PMC6639208.
88. Phipps AI, Newcomb PA, Garcia-Albeniz X, Hutter CM, White E, Fuchs CS, Hazra A, Ogino S, Nan H, Ma J, Campbell PT, Figueiredo JC, Peters U, Chan AT. Association between colorectal cancer susceptibility loci and survival time after diagnosis with colorectal cancer. *Gastroenterology*. 2012 Jul;143(1):51-4.e4. doi: 10.1053/j.gastro.2012.04.052. Epub 2012 May 8. PMID: 22580541; PMCID: PMC3579620.
89. Hindorff LA, Gillanders EM, Manolio TA. Genetic architecture of cancer and other complex diseases: lessons learned and future directions. *Carcinogenesis*. 2011 Jul;32(7):945-54. doi: 10.1093/carcin/bgr056. Epub 2011 Mar 31. PMID: 21459759; PMCID: PMC3140138.
90. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Res*. 2007 Oct;17(10):1520-8. doi: 10.1101/gr.6665407. Epub 2007 Sep 4. PMID: 17785532; PMCID: PMC1987352.
91. Ortlepp JR, Lauscher J, Janssens U, Minkenberg R, Hanrath P, Hoffmann R. Analysis of several hundred genetic polymorphisms may improve assessment of the individual genetic burden for coronary artery disease. *Eur J Intern Med*. 2002 Dec;13(8):485-492. doi: 10.1016/s0953-6205(02)00182-6. PMID: 12446192.
92. Banqué M, Raidó B, Masuet C, Ramon JM. Food groups and nutrient intake and risk of colorectal cancer: a hospital-based case-control study in Spain. *Nutr Cancer.* 2012 Apr;64(3):386-92. doi: 10.1080/01635581.2012.657334. Epub 2012 Feb 27. PMID: 22369135.
93. Rosato V, Guercio V, Bosetti C, Negri E, Serraino D, Giacosa A, Montella M, La Vecchia C, Tavani A. Mediterranean diet and colorectal cancer risk: a pooled analysis of three Italian case-control studies. *Br J Cancer*. 2016 Sep 27;115(7):862-5. doi: 10.1038/bjc.2016.245. Epub 2016 Aug 18. PMID: 27537381; PMCID: PMC5046203.
94. Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Béghin L, Manios Y, Hallström L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA; HELENA Study Group. Development and evaluation of a self-administered computerized 24-h dietary recall method

for adolescents in Europe. *Int J Obes (Lond).* 2008 Nov;32 Suppl 5:S26-34. doi: 10.1038/ijo.2008.180. PMID: 19011650.

95. Sotos-Prieto M, Christophi C, Black A, Furtado JD, Song Y, Magiatis P, Papakonstantinou A, Melliou E, Moffatt S, Kales SN. Assessing Validity of Self-Reported Dietary Intake within a Mediterranean Diet Cluster Randomized Controlled Trial among US Firefighters. *Nutrients*. 2019 Sep 19;11(9):2250. doi: 10.3390/nu11092250. PMID: 31546768; PMCID: PMC6769698.
96. Anderson AS, Caswell S, Wells M, Steele RJ. Obesity and lifestyle advice in colorectal cancer survivors - how well are clinicians prepared? *Colorectal Dis*. 2013 Aug;15(8):949-57. doi: 10.1111/codi.12203. PMID: 23480570.
97. Berstad P, Løberg M, Larsen IK, Kalager M, Holme Ø, Botteri E, Bretthauer M, Hoff G. Long-term lifestyle changes after colorectal cancer screening: randomised controlled trial. *Gut*. 2015 Aug;64(8):1268-76. doi: 10.1136/gutjnl-2014-307376. Epub 2014 Sep 2. PMID: 25183203.
98. Wright SJ, Gibson D, Eden M, Lal S, Todd C, Ness A, Burden S. What are colorectal cancer survivors' preferences for dietary advice? A best-worst discrete choice experiment. *J Cancer Surviv*. 2017 Dec;11(6):782-790. doi: 10.1007/s11764-017-0615-2. Epub 2017 Apr 20. PMID: 28429186; PMCID: PMC5671542.

5. CONCLUSIONS

On the basis of the results obtained in this doctoral thesis, the following conclusions were derived:

1. The diet of the studied group of CRC patients postsurgery from the CRCSP of the Osakidetza/Basque Health Service is inadequate in many respects, including nutrients and food intakes. The inadequacy of some nutrients was associated with male gender, excess of weight, smoking and low educational level, and the low adherence to the MD was pronounced in those with a low educational level.

2. The cases of the case-control study were more likely than controls to have overweight/obesity, a higher frequency of consumption of salt added for cooking, a lower intake of vitamin B_2 and Ca/P ratio, and to have not adequate intakes of SFA and cholesterol. Thus, some environmental factors such as diet, and weight status could influence on the aetiology of CRC in this population group from the CRCSP.

3. High consumption of high-fat cheeses was associated with CRC risk, whereas, a high intake of fibre-containing foods, especially whole grains, and fatty fish, and adherence to the MD pattern was associated with a lower risk for CRC, in the case-control sample from the CRCSP. In addition, according to tumour location, cases with the proximal location had higher adherence to MD pattern than those with the distal location.

4. Most SNPs analysed in the case-control sample from the CRCSP were not associated with risk of CRC. Only one of the 48 SNPs analysed, rs6687758, was associated with risk of CRC, in this population (on crude analysis). Moreover, there were significant associations between smoking status, physical activity, the rs6687758SNP and CRC risk. On the other hand, the results of the GRS showed that the risk alleles were more frequent in cases than controls and this score was associated with this type of cancer in crude analysis.

Alegria Lertxundi, I. (2017)

eman ta zabal zazu
Universidad del País Vasco
Euskal Herriko Unibertsitatea