Published in final edited form as:
Cancer Epidemiol. 2010 June ; 34(3): 291–297. doi:10.1016/j.canep.2010.02.010.

Functional polymorphisms to modulate luminal lipid exposure and risk of colorectal cancer

Ikuko Kato[a,b], **Susan Land**[a,c], **Adhip P. Majumdar**[a,d,e], **Jill Barnholtz-Sloan**[f], and **Richard K. Severson**[g]

[a] Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA

[b] Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

[c] Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

[d] Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan, USA

[e] John D. Dingell Veterans Affair Medical Center, Detroit, Michigan, USA

[f] Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

[g] Department of Family Medicine and Public Health Sciences, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract

PURPOSE—Fat absorption may play a crucial role in colorectal carcinogenesis by determining intra-colonic exposure to potentially carcinogenic lipid metabolites.

METHODS—We conducted a population-based case-control study that included 1163 cases and 1501 controls to examine whether individuals who carry genetic variants associated with lower lipid absorption have a higher risk of colorectal cancer. Using Taqman assay, we determined *FABP2* alanine (A)/threonine (T) polymorphism at codon 54 in exon-2 and *APOE* isoforms. Multivariable odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression models, assuming *FABP2* A54 and *APO non-E*4 as high risk alleles.

RESULTS—We found no associations with either of the polymorphisms. The OR associated with *FABP2 A54* homozygotes compared with the others was 1.01 (95% CI; 0.86–1.45) and that for non-*ApoE4* carriers compared with carries was 0.95 (95% CI; 0.80–1.13). However, there was a statistically significant negative interaction between total fat intake and *FABP2 AA* genotypes (P=0.025), indicating that the risk of colorectal cancer associated with this polymorphism is higher in the subjects with lower fat intake.

Address for reprints and correspondence: Dr. Ikuko Kato, Karmanos Cancer Institute, 110 East Warren Avenue, Detroit, MI 48201, USA, telephone 1-313-578-4206, fax 1-313-578-4306, katoi@karmanos.org.

Conflict of interest
We declare no conflict of interest.

CONCLUSIONS—These results suggest that these SNPs may not be useful in predicting colorectal cancer risk in populations with high fat intake.

Keywords

Colorectal cancer; Case-control study; Lipid absorption; Single nucleotide polymorphism (SNP)

1. Introduction

The association between fat intake and colorectal cancer has been mixed in epidemiologic studies and intervention trials [1–3], despite unequivocal data in experimental animals indicating high fat diets increase the occurrence of colorectal tumors [4,5]. These observations suggest that genetic susceptibilities may modify the effects of a high fat diet and thus that only a fraction of individuals is in fact susceptible to colorectal cancer when they consume a high fat diet. One of the mechanistic bases on which dietary fat increases the risk of colorectal cancer is intracolonic exposure of colorectal epithelial cells to potentially carcinogenic substances that are generated from lipid and its metabolites with fecal bacterial activities [6–8]. Accordingly, fat absorption may play a crucial role in colorectal carcinogenesis by determining luminal lipid concentrations, and thus exposure to such carcinogenic metabolites.

In this context, it is interesting to note an inverse association between circulating cholesterol levels and colorectal cancer that have been reported by several prospective cohort studies [9]. Some of these studies showed that the association was confined to the first few years of follow-up, suggesting that the association might be due to a consequence of preclinical cancer. This inverse association was not confirmed for colorectal adenomas [10–11]. Importantly, others with longer follow-up demonstrated a persistent association which was not explained by other confounding factors [9,12–16]. Collectively, these studies may suggest that albeit positive correlations between total fat intake and both coronary heart disease (CHD) and colorectal cancer at the population level, colorectal cancer and CHD occur in different subsets of the population with high fat intake. Hence a key to these two pathways is intestinal lipid absorption that points dietary fat either to the systemic circulation or to the colorectal lumen.

Recent genetic studies have discovered common functional polymorphisms on genes that play an important role in intestinal lipid absorption. In this population-based case-control study, we address whether individuals with genetic variants leading to lower lipid absorption have a higher risk of colorectal cancer. Specifically we focus on the following two polymorphisms: *FABP2* (intestinal fatty acid binding protein) alanine (A)/threonine (T) substitution at codon 54 in exon-2 [17]; and *APOE* isoforms (*E2*, *E3* and *E4*), which are defined by combinations of amino acid (cysteine/arginine) substitutions at codons 112 and 158 [18]. These have been selected based on their well-characterized phenotypes in lipid absorption [17,19,23], their adjunct effects on bile acid secretion [21–24], sources of known colorectal tumor promoters [25], and limited data on cancer to date. Our primary intention to study these two polymorphisms exclusively was to use them as a potential surrogate for protein functions rather than as a genetic marker for variability of the entire genomic region, because these phenotypes are not easy to study in vivo for a large number of human subjects and are prone to intra-individual variability.

2. Materials and methods

2.1 Study population

Eligible study subjects were residents in the Metropolitan Detroit Tri-County (Wayne, Oakland and Macomb) area, between 45 and 80 years of age at time of ascertainment, with a working telephone and no prior history of any invasive cancer, in-situ colorectal cancer or colectomy. Eligible colorectal cancer cases were histologically diagnosed between January 1, 2003 and September 30, 2005, and were identified through a rapid case-reporting system implemented in the Metropolitan Detroit Cancer Surveillance System (MDCSS), a founding member of the National Cancer Institute=s (NCI) Surveillance, Epidemiology, and End Results (SEER)-cancer registries, which allowed access to patients within 3–4 months from their diagnosis. A total of 3 746 potentially eligible cases were identified. Among those, physician consent was not obtained for 385. Of the remaining, 110 subjects were further excluded because no subject contact information was available or other administrative reasons. An additional 47 cases were found to be ineligible before enrollment, leaving a total of 3 204 subjects. Among these 1 335 consented to the study (41.7%).

Population controls were identified though random digit dialing (RDD). These controls were frequency matched to the cases by 5-year age group, gender, county of residence and race (African American (AA) vs non-AA), which were projected based on the data from preceding years. The RDD telephone numbers were generated by Survey Sampling (Fairfield, CT) with pre-screening for business and non-working numbers. RDD interviewers were instructed to call each selected telephone number up to 9 times at different times of the day and week including evenings and weekends, in order to obtain a household census to identify potentially eligible study subjects. Each telephone contact was recorded on electronic files. A total of 36 936 unique RDD telephone numbers were surveyed. 36% of the numbers were excluded, 33% were screened for household census information, and 31% were not able to be screened. The excluded numbers were business, public or disconnected numbers, out of study area, dataline (modem, fax etc.), or from other miscellaneous reasons. The numbers not screened were due to no answers throughout 9 attempts and refusals to give any household information and communication problems in either language or hearing ability. Among the remaining numbers screened for household census, 47% were ineligible primarily due to ages of household members. Of the eligible households (53%), 75% agreed to receive a study invitation letter and the rest refused to provide a mailing address. To balance the age distribution of cases and controls, we selected 2 831 for enrollment and 1 682 completed the study (59.4%). Of the total of 3 017 participants, 135 controls were considered to be ineligible according to their age and medical history in the questionnaires, and 130 cases were also considered ineligible because the final MDCSS reports did not confirm the eligibility or because of previous cancer or colectomy reported in the completed questionnaires. Thus, a total of 2 752 (1 205 cases and 1 547 controls) study subjects were included in this study.

2.2 Data and specimen collection

The cases and controls were interviewed over the telephone using structured questionnaires regarding their usual diet and other risk factors for colorectal cancer for the time- period preceding cancer diagnosis (approximately 2 years prior to the interview). Dietary intake was assessed by a validated semi-quantitative food frequency questionnaire (FFQ) [26,27], Block 98.2 (Block Dietary Data Systems, Berkeley, CA). Energy-adjusted nutrient intake was calculated by means of the residual method described by Willett and Stampfer [28]. The study participants provided one of the following types of biospecimens; 1) peripheral blood through home phlebotomy service, 2) buccal cells collected by commercial mouthwash liquid and 3) archived (grossly normal) tissue blocks. 71 % of the controls provided blood

samples while the rest mouthwash samples. The cases chose the phlebotomy option less often than the controls (66%) and 7% tissue blocks. This study was approved by Wayne State University Human Investigation Committee and all subjects gave written informed consent to participate in the study.

2.3. Laboratory analyses

All laboratory assays were performed at the Wayne State University Applied Genomics Technology Center. DNA was isolated with a Gentra Autopure under standard conditions. TaqMan genotyping assays were employed to determine the three relevant single nucleotide polymorphisms (SNP), rs1799883, rs429358 and rs7412, using an Applied Biosystems 7900 (Foster City, CA). For quality control, 10% of the assays were repeated and amplicons for each of the assays were sequenced. For added sensitivity, DNA isolated from low yield samples was preamplified in an outer PCR reaction using oligonucleotide primers designed with Primer Express software (Applied Biosystems). When Hardy-Weinberg equilibrium was tested for the cases and controls separately (based on a chi square test), all SNPs were found to be in equilibrium using p=0.05 as the threshold.

2.4. Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) for colorectal cancer associated with *FABP2* and *APOE* polymorphisms were estimated using an unconditional logistic regression model [29], adjusting for selected covariates. The ORs were calculated for high risk (low absorption) alleles (i.e., *FABP2* A54 and *APOE*2 or *E3*) according to functional data available in the literature [17,19–23] using homozygous low risk (i.e., *FABP2* T54 and *APOE* 4) alleles (dominant model) or homozygous low risk alleles + heterozygotes (recessive model) as the reference group, as well as for the combinations of these two polymorphisms based on the sum of the presumed high risk alleles. The gene-dose effect was also tested with the logit of risk according to the number of presumed high risk alleles.

Covariates were first selected from established risk factors for colorectal cancer in the literature [1,30,31]. They were tested one at a time in a model that included basic demographic variables, age, gender and educational levels, and those showing an association at the 10% level were included to estimate multivariable ORs. As a result, the final multivariable model included age, gender, educational level, source of DNA, total calcium, fiber, red meat intake, family history of colorectal cancer, regular (>= 3 times per week for 6 months or longer) non-steroidal anti-inflammatory drug (NSAID) use, body mass index (body weight (kg)/body height $(m)^2$), and physical activity index in their 30s, which was the weighted sum of time the subject spent per 24 hours as described previously [32]. The source of genomic DNA (blood vs. others) was included because it is a potential indicator for volunteer bias that may not be necessarily captured by other covariates, because DNA quality is clearly different between blood and other sources, which affects intensities of signals in Taqman genotyping assays and thus potentially the discrimination of alleles and because there was a statistically significant difference in the proportion of the subjects who chose blood between the cases and the controls. Height, history of diabetes, smoking, number of alcoholic drinks per day, use of postmenopausal hormone replacement therapy and race were also assessed and were not included in the final model. After exclusion of subjects with missing covariates, the final analytical samples in this study consisted of 1163 cases ands 1501 controls.

In addition, the ORs were calculated by level of total fat intake dichotomized at the median in the entire study population (96 g per day) for all cancer cases and for subsites, distal (from the descending colon to the rectum) and proximal (from the cecum to the splenic flexure), in view of varied fat concentrations by subsite of the large intestine. The

interactions between the polymorphisms (in the recessive model) and fat intake (below or above median) were tested by including their multiplicative interaction terms. All statistical analyses were performed using SAS version 9.

3. Results

Table 1 presents demographic characteristics of the colorectal cancer cases and the population controls and the ORs and 95% CI for selected colorectal cancer risk factors. The matching variables (age, race and county (data not shown)) were well balanced between the cases and controls except for gender, but there was a significant excess in the proportion of female controls (P< 0.001). This excess was primarily due to the facts that female controls had been accrued quicker than male controls and that the target number of the corresponding cases was reduced in the middle of the study. In addition, the control group had a higher educational level than the cases (P< 0.001, data not shown). The risk of colorectal cancer was inversely associated with regular NSAID use, menopausal hormone use and high physical activity in their 30s and positively associated with history of diabetes, higher body mass index and family history of colorectal cancer. Among other covariates, dietary calcium and fiber showed an inverse association and red meat intake yielded a positive association (data not shown).

FABP2 A54T and *APOE* genotypes were determined for a total of 2659 and 2661 subjects respectively and thus their combined genotypes were available for 2656 subjects. Because of the unbalanced gender matching, we first calculated bi-variable (gender-adjusted) ORs, instead of univariable ORs. As shown in Table 2, none of the bi-variable ORs (OR1) associated with *FABP2 A54*, *APO-non-E4* or their combination were significantly different from unity. Adjustment for other covariates had only modest effects on the risk estimates (OR2), resulting in a slight reduction in the ORs (OR2), which ranged from 0.90 (*non-ApoE4 carriers* compared with *E4* homozygotes) to 1.01 (*FABP2 AA* compared with *AT+TT*). There were no significant trends with the number of *FABP2 A54* or *APO non-E4* alleles or the sum of those. When *apoE2* carriers (N=427) were separated from non-*ApoE4* (*E2/E2* and *E2/E3*) carriers and carriers (*E2/E4)*, their multivariable OR compared with non-*E2* carriers was also less than unity (0.97, 95%CI: 0.78–1.20) (data not shown). There were no differences in the main effects of the polymorphisms by subsite (data not shown).

Next we assessed the interactions with fat intake (Table 3). There was a statistically significant negative interaction between total fat intake and *FABP2 AA* genotypes (P=0.025), and a similar non-significant negative interaction with *non-APOE4* (P=0.400), indicating that the risk of colorectal cancer associated with these genotypes is higher in the subjects with lower fat intake (below median) rather than those with high intake (above median). These negative interactions were more pronounced for proximal colon cancer for the both polymorphisms and that for *APOE* was also statistically significant (P=0.044).

4. Discussion

The present study failed to support our working hypothesis that individuals carrying common genetic variants that have been associated with lower intestinal lipid absorption have a higher risk of colorectal cancer. *T54* containing FABP2 protein has been shown to exert 2-fold greater affinity for long chain fatty acids (LCFA) than A54 containing protein [17] and that Caco-2 cell expressing the T54 form can transport LCFAs and secrete triglycerides to a 2 fold greater degree than Caco-2 cell expressing the A54 form [19]. The T54 genotype has been associated with higher plasma levels of triglycerides [20,33,34] and of free fatty acids [35], a higher postprandial lipemic response [36,37] to an oral fat load and lower secretion of total fecal bile acid [24]. *ApoE* polymorphism has been extensively

studied in relation to blood lipid levels as well as to risk of cardiovascular disease and of Alzheimer>s disease [38]. Despite the fact that apoE is not synthesized to a significant extent in the gut, *ApoE* influences intestinal cholesterol absorption. Cholesterol absorption is higher in *E4* carriers than non carriers, and correspondingly, fecal bile acid output is lower in *E4* carries than non-*E4* carriers [21–23]. Others have shown that rare *E2* carries absorb dietary cholesterol more efficiently than *E3* homozygotes [39,40]. Overall, Apo ε4 allele carriers are more responsive to dietary fat modification than non-carriers [41,42].

To our knowledge, there have been no published data on the relationship between *FABP2* polymorphism and colorectal cancer risk. *ApoE4* allele was associated with lower risk of proximal colonic adenoma and carcinoma [43] with OR for of 0.36 (95% CI: 0.14–0.89) and that for cancer of 0.35 (95% CI: 0.14–0.86) compared to non-*E4* carriers. A similar, but non-significant, risk reduction (OR=0.59) was observed among Japanese patients with proximal adenoma with the *E4* allele [44]. Alternatively, increased risk among *E2* carriers, particularly for male colorectal cancer, was reported by Watson et al [45]. These three studies were all hospital-based and involved a relatively small sample size. A subsequent much larger population-based case-control study in the US did not confirm these results [46]. Thus, to date the association between *Apo E* polymorphism and colorectal cancer has been very mixed and is not conflicting with our null result. In addition, allele frequencies of each polymorphism observed in our study were comparable to those previously reported for Caucasians and African Americans [20,47,48].

We realize that the overall null associations in the present study are possibly due to insufficient statistical power, as a growing number of pooled or meta-analyses of the association studied suggest that the main effects of common genetic polymorphism are generally very modest (~OR 1.10) [49–52]. If this is the case, our study was not adequately powered. We also acknowledge that it possible that the inclusion of more than 10 covariates reduced the statistical power. However, given the relative large sample size of the study and the fact that the sizes of the confident intervals did not differ materially between the minimally and fully adjusted ORs as presented in Table 2, we estimate that its potential effects on power was small.

A major limitation of this study is the number of genes analyzed, despite a growing number of genes and their functions that have recently been delineated in lipid transport/metabolic pathways. Those include other apolipoproteins such as *Apo B48, Apo AI, and Apo AIV*, other fatty acid and cholesterol transporters, such as *CD36, NPC1L1, FATP4, FABP1* and other lipid transfer proteins such as *MTP, ACAT2 and SAR1B* [53–56]. Furthermore, it is important to note that unabsorbed lipid is not a sole source of luminal lipid content. Lipids are excreted back to the intestinal lumen through bile or directly through intestinal epithelial cells using reverse cholesterol transport mechanism, which is also mediated by several transporters, e.g., *ABCG5, ABCG8, ABCB4* and *ABCA1* [53,54]. It has indeed become clear that direct reverse transport by intestinal epithelial cells exceeds that by the biliary pathway, resulting in more fecal sterol excretion than the sum of dietary cholesterol intake and biliary cholesterol secretion [57]. In addition, there is a large redundancy in functions of digestive enzymes and lipid binding and transfer proteins, leading to the extreme efficiency of lipid assimilation [55]. Thus, the overall impact of the SNPs examined in this study in determining luminal lipid content may be much smaller than we originally anticipated. Combinations of multiple functional SNPs in a number of genes in these pathways ways may modulate the risk of colorectal cancer, but their population-attributable risks should be much smaller because of very low frequencies of such combinations expected in the general population.

Due to the relative low participation rate, self-selection bias or volunteer bias is a concern [58]. In fact, higher proportions of college graduates and of those who chose to donate blood in the control group are indicative of this bias. Thus, we adjusted for these factors and others in calculating multivariable ORs. However, the associations with other established colorectal cancer risk factors as described above in the Results were consistent with those reported by others, which may indicate that the extent of selection bias is not substantial. In addition, we believe the effect of self-selection bias on genotype distributions of SNPs of interest is relatively limited, compared with that on environmental risk factors. Furthermore, selection bias has been demonstrated not to affect the assessment of gene- environmental interactions [59]. Differential recalls between the cases and controls (recall bias) is also a concern in any retrospective studies like this study. Cases who are aware of their own diagnosis may recall past exposures differently from controls. But the use of structured questionnaires (like our study) has been shown to reduce this bias [60].

On the other hand, the strengths of our study include high quality genotyping data (demonstrated by only 3 and 5 subjects with undetermined genotypes for the two polymorphisms of interest, respectively), and the population-based study design where controls are less likely to have medical conditions that have been associated with these polymorphisms. In addition, there was a strong rationale supporting functional effects of these polymorphisms on LCFA and cholesterol absorption, respectively, as summarized above.

The negative interaction between *FABP2* polymorphism and total fat intake was unexpected and possibly a chance finding due to multiple comparisons in the stratified analyses. However, Le Marchand and Wilkens point out that studies have more power to detect interactions than main effects if genetic effects are environment- specific [61]. Because *FABP2* expression in intestinal epithelial cells is enhanced by dietary fat content [55,62], this negative interaction may imply that the polymorphism (i.e., altered protein characteristics) exerts its function only when the basal gene expression level is low. In other words, a sufficient amount of protein expression may override differences in amino acid composition. If true, it suggests that these polymorphisms have little consequence in western countries where fat intake and colorectal cancer incidence are high. The main effect of this polymorphism needs to be re-evaluated in other populations with low fat intake.

In conclusion, the results of the present study do not indicate that these common genetic polymorphisms can be used in prescribing tailored dietary recommendation to reduce the risk of colorectal cancer. Thus, at this point it is safe to moderate fat intake regardless of individuals= genetic background in order to avoid possible consequences of high fat (energy-dense) diets, in view of the high and rising prevalence of overweight, obesity and its related morbidities in US adults and children [63].

Acknowledgments

This research was supported by a grant from National Institutes of Health R01-CA93817. The authors thank the study participants for their generosity in donating time and biospecimens, the Metropolitan Detroit Cancer Surveillance System for rapid case-ascertainment and Ms. Barbara Rusin, Dr. Maria Samerson and Ms. Ann Bankowski for their excellent technical assistance.

References

1. WCRF/AICR. Food, Nutrition, physical activity and the prevention of cancer: global perspective. Washington DC: AICR; 2007. Colon and rectum; p. 280-8.

2. Alexander DD, Cushing CA, Lowe KA, Sceurman B, Roberts MA. Meta-analysis of animal fat or animal protein intake and colorectal cancer. Am J Clin Nutr. 2009; 89:1402–19. [PubMed: 19261724]
3. Beresford SA, Johnson KC, Ritenbaugh C, Lasser NL, Snetselaar LG, Black HR, et al. Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA. 2006; 295:643–54. [PubMed: 16467233]
4. Reddy BS. Dietary fat, calories, and fiber in colon cancer. Prev Med. 1993; 22:738–49. [PubMed: 8234214]
5. Tang ZC, Shivapurkar N, Frost A, Alabaster O. The effect of dietary fat on the promotion of mammary and colon cancer in a dual-organ rat carcinogenesis model. Nutr Cancer. 1996; 25:151–9. [PubMed: 8710684]
6. Babbs CF. Free radicals and the etiology of colon cancer. Free Rad Bio Med. 1990; 8:191–200. [PubMed: 2185144]
7. Stone WL, Papas AM. Tocopherols and the etiology of colon cancer. JNCI. 1997; 89:1006–14. [PubMed: 9230882]
8. Erhardt JG, Lim SS, Bode JC, Bode C. A diet rich in fat and poor in dietary fiber increases the in vitro formation of reactive oxygen species in human feces. J Nutr. 1997; 127:706–9. [PubMed: 9164990]
9. Law MR, Thompson SG. Low serum cholesterol and the risk of cancer: an analysis of the published prospective studies. Cancer Causes Control. 1991; 2:253–61. [PubMed: 1831389]
10. Demers RY, Neale AV, Demers P, Deighton K, Scott RO, Dupuis MH, et al. Serum cholesterol and colorectal polyps. J Clin Epidemiol. 1988; 41:9–13. [PubMed: 3335874]
11. Rumi G Jr, Szabo I, Vincze A, Matus Z, Toht G, Rumi G, et al. Decrease in serum levels of vitamin A and zeaxanthin in patients with colorectal polyps. Eur Gastroenterol Hepatol. 1999; 11:305–8.
12. Nomura AM, Stermmermann GN, Chyou P-H. Prospective studies of serum cholesterol levels and large-bowel cancer. JNCI. 1991; 83:1402–7.
13. Eichholzer M, Stähelin B, Gutzwiller F, Lin E, Bernasconi F. Association of low plasma cholesterol with mortality for cancer at various sites in men: 17-y follow-up of the prospective Basel study. Am J Clin Nutr. 2000; 71:569–74. [PubMed: 10648273]
14. Kreger BE, Anderson KM, Schayzkin A, Splansky GL. Serum cholesterol level, body mass index, and the risk of colon cancer. Cancer. 1992; 70:1038–43. [PubMed: 1515981]
15. Schuit AJ, van Dijk CEMJ, Dekker JM, Schouten EG, Kok FJ. Inverse association between serum total cholesterol and cancer mortality in Dutch Civil servants. Am J Epidemiol. 1993; 137:966–76. [PubMed: 8317454]
16. Gaard M, Tretli S, Urdal P. A prospective study of 62,173 Norwegian men and women. Scand J Gastroenterol. 1997; 32:162–8. [PubMed: 9051877]
17. Baier LJ, Sacchettuni JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, et al. An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation and insulin resistance. J Clin Invest. 1995; 95:1281–7. [PubMed: 7883976]
18. Brouwer DAJ, van Doormaal JJ, Muskiet FAJ. Clinical chemistry of common apolipoprotein E isoforms. J Chromatpgraph B. 1996; 678:23–41.
19. Baier LJ, Bogardus C, Sacchettini JC. A polymorphism in the human intestinal fatty avid binding protein alters fatty acid transport across Caco-2 cells. J Biol Chem. 1996; 271:10892–6. [PubMed: 8631905]
20. Georgopoulos A, Aras I, Tsai MY. Codon-54 polymorphism of the fatty acid-binding protein 2 gene is associated with elevation of lasting and postprandial triglyceride in type 2 diabetes. J Clin Endocrinol Metab. 2000; 85:3155–60. [PubMed: 10999802]
21. Kesäniemi YA, Ehnholm C, Miettinen TA. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. J Clin Invest. 1987; 80:578–81. [PubMed: 3611358]
22. Miettinen TA, Gylling H, Vanhanen H, Ollus A. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. Arterioscl Thomb. 1992; 12:1044–52.

23. Gylling H, Aalto-Setälä K, Kontula K, Miettinen TA. Serum low density lipoprotein cholesterol level and cholesterol absorption efficiency are influenced by apolipoprotein B and E polymorphism and by the FH-Helsinki mutation of the low density lipoprotein receptor gene in familial hypercholesterolemia. Arterioscl Thromb. 1991; 11:1368–75. [PubMed: 1911722]
24. Hegele RA, Wolever TMS, Story JA, Connelly PW, Jenkins DJA. Intestinal fatty acid-binding protein variation associated with variation in the response of plasma lipoproteins to dietary fibre. Eur J Clin Invest. 1997; 27:857–62. [PubMed: 9373766]
25. Narisawa T, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation N-methyl-N′-nitro-N-nitrosoguanidine in rats. JNCI. 1974; 53:1093–7. [PubMed: 4427390]
26. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol. 1990; 43:1327–35. [PubMed: 2254769]
27. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc. 1992; 92:686–93. [PubMed: 1607564]
28. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol. 1986; 124:17–27. [PubMed: 3521261]
29. Breslow, NE.; Day, NE. IARC Scientific Publications No. 32. Lyon: IARC; 1980. Statistical Methods in Cancer Research, Vol I: The Analysis of Case-Control Studies.
30. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ, et al. Women's Health Initiative Investigators. Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med. 2004; 350:991–1004. [PubMed: 14999111]
31. 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. [PubMed: 19350627]
32. Kato I, Nomura A, Stemmermann GN, Chyou P-H. Prospective study of clinical gallbladder disease and its association with obesity, physical activity, and other factors. Dig Dis Sci. 1992; 37:784–90. [PubMed: 1563324]
33. Hegele RA, Connelly PW, Hanley AJG, Sun F, Harris SB, Zinman B. Common genomic variants associated with variation in plasma lipoproteins in young aboriginal Canadians. Arterio Thromb Vasc Biol. 1997; 17:1060–6.
34. Pihlajamäki J, Rissanen J, Heikkinen S, Karjalaunen L, Laakso M. A codon 54 polymorphism of the human intestinal fatty acid binding protein 2 gene is associated with dyslipidemias but not with insulin resistance in patients with familial combined hyperlipidemia. Arterio Thromb Vasc Biol. 1997; 17:1039–44.
35. Vidgren HM, Sipiläinen H, Heikkinen S, Laakso M, Uusitupa MI. Threonine allele in codon 54 of the fatty acid binding protein 2 gene does not modify the fatty acid composition of serum lipids in obese subjects. Eur J Clin Invest. 1997; 27:405–8. [PubMed: 9179548]
36. Ågren JJ, Valve R, Vidgren H, Laakso M, Uusitupa M. Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene. Arterio Thromb Vasc Biol. 1998; 18:1606–10.
37. Ågren JJ, Vidgren H, Valve R, Laakso M, Uusitupa M. Postprandial responses of individual fatty acids in subjects homozygous for the threonine- or alanine-encoding allele in codon 54 of the intestinal fatty acid binding protein 2 gene. Am J Clin Nutr. 2001; 73:31–5. [PubMed: 11124746]
38. Kesäniemi YA. Genetics and cholesterol metabolism. Curr Opin Lipidol. 1996; 7:124–31. [PubMed: 8818508]
39. Gylling H, Kontula K, Miettinen TA. Cholesterol absorption and metabolism and LDL kinetics in healthy men with different apoprotein E phenotypes and apoprotein B Xba I and LDL receptor Pvu II genotypes. Arterio Thromb Vasc Biol. 1995; 15:208–13.
40. Gylling H, Kontula K, Koivisto U-M, Miettinen HE, Miettinen TA. Polymorpohisms of the genes encoding apoproteins A-I, B, C-III, and E and LDL receptor, and cholesterol and LDL metabolism during increased cholesterol intake. Arterio Thromb Vasc Biol. 1997; 17:38–44.

41. Lopez-Miranda J, Ordovas JM, Mata P, Lichtenstein AH, Clevidence B, Judd JT, et al. Effect of apolipoprotein E phenotype in diet-induced lowering of plasma low lipoprotein cholesterol. J Lipid Res. 1994; 35:1965–75. [PubMed: 7868975]
42. Weggemans RM, Zock PL, Ordovas JM, Perdp-Botet J, Katan MB. Apoprotein E genotype and the response of serum cholesterol to dietary fat, cholesterol and cafestol. Atherosclerosis. 2001; 154:547–55. [PubMed: 11257255]
43. Kervinen K, Servik H, Mäkelä J, Lehtola J, Niemi M, Kairaluoma MI, et al. Is the development of adenoma and carcinoma in proximal colon related to apolipoprotein E phenotype? Gastroenterology. 1996; 110:1785–90. [PubMed: 8964404]
44. Shinomiya S, Sasaki J, Kiyohara C, Tsuji E, Inoue H, Marugame T, et al. Apolipoprotein E genotype, serum lipids, and colorectal adenomas in Japanese men. Cancer Lett. 2001; 164:33–40. [PubMed: 11166913]
45. Watson MA, Gay L, Stebbings WS, Speakman CT, Bingham SA, Loktionov A. Apolipoprotein E gene polymorphism and colorectal cancer: gender-specific modulation of risk and prognosis. Clin Sci. 2003; 104:537–45. [PubMed: 12529167]
46. Slattery ML, Sweeney C, Murtaugh M, Ma KN, Potter JD, Levin TR, et al. Associations between apoE genotype and colon and rectal cancer. Carcinogenesis. 2005; 26:1422–19. [PubMed: 15817610]
47. Lei HH, Coresh J, Shuldiner AR, Boerwinkle E, Brancati FL. Variants of the insulin receptor substrate-1 and fatty acid binding protein 2 genes and the risk of type 2 diabetes, obesity, and hyperinsulinemia in African Americans. The Atherosclerosis Risk Community Study. Diabetes. 1999; 48:1868–72. [PubMed: 10480621]
48. Pablos-Mdez A, Mayeux R, Nagi C, Shea S, Berglund L. Association of APO E polymorphism with plasma lipid levels in a multicentric elderly population. Arterio Thromb Vasc Biol. 1997; 17:3534–41.
49. Masson LF, Sharp L, Cotton SC, Little J. Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. Am J Epidemiol. 2005; 161:901–15. [PubMed: 15870154]
50. Kamangar F, Cheng C, Abnet CC, Rabkin CS. Interleukin-1B polymorphisms and gastric cancer risk--a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2006; 15:1920–8. [PubMed: 17035400]
51. Raimondi S, Paracchini V, Autrup H, Barros-Dios JM, Benhamou S, Boffetta P, et al. Meta- and pooled analysis of GSTT1 and lung cancer: a HuGE-GSEC review. Am J Epidemiol. 2006; 164:1027–42. [PubMed: 17000715]
52. Cote ML, Chen W, Smith DW, Benhamou S, Bouchardy C, Butkiewicz D, et al. Meta- and pooled analysis of GSTP1 polymorphism and lung cancer: a HuGE-GSEC review. Am J Epidemiol. 2009; 169:802–14. [PubMed: 19240225]
53. Kruit JK, Groen AK, van Berkel TJ, Kuipers F. Emerging roles of the intestine in control of cholesterol metabolism. World J Gastroenterol. 2006; 12:6429–39. [PubMed: 17072974]
54. Williams KJ. Molecular processes that handle -- and mishandle -- dietary lipids. J Clin Invest. 2008; 118:3247–59. [PubMed: 18830418]
55. Storch J, Corsico B. The emerging functions and mechanisms of mammalian fatty acid-binding proteins. Annu Rev Nutr. 2008; 28:73–95. [PubMed: 18435590]
56. Mansbach CM 2nd, Gorelick D. Development and physiological regulation of intestinal lipid absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G645–50. [PubMed: 17627968]
57. van der Velde AE, Vrins CL, van den Oever K, Kunne C, Oude Elferink RP, Kuipers F, et al. Direct intestinal cholesterol secretion contributes significantly to total fecal neutral sterol excretion in mice. Gastroenterology. 2007; 133:967–75. [PubMed: 17854600]
58. Sackett DL. Bias in analytic research. J Chron Dis. 1979; 32:51–63. [PubMed: 447779]
59. Morimoto LM, White E, Newcomb PA. Selection bias in the assessment of gene -environment interaction in case-control studies. Am J Epidemiol. 2003; 158:259–63. [PubMed: 12882948]
60. Teschke K, Smith JC, Olshan AF. Evidence of recall bias in volunteered vs. prompted responses about occupational exposures. Am J Ind Med. 2000; 38:385–8. [PubMed: 10982978]

61. Le Marchand L, Wilkens LR. Design considerations foe Genomic association studies: importance of gene-environment interactions. Cancer Epidemiol Biomarkers Perv. 2008; 17:263–7.

62. Glatz JF, van Nieuwenhoven FA, Luiken JJFP, Schaap FG, van der Vusse GJ. Role of membrane-associated and cytoplasmic fatty acid-binding proteins in cellular fatty acid metabolism. Prostagrad Leuk Essen Fat Acids. 1997; 57:373–8.

63. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. Gastroenterology. 2007; 132:2087–102. [PubMed: 17498505]

Table 1

Demographic characteristics of colorectal cancer cases and population controls and odds ratios (OR) and 95% confidence intervals (CI) associated with selected colorectal cancer risk factors

		Cases		Controls			
Characteristics		**No[a]**	**%**	**No[a]**	**%**	**OR [b]**	**95% CI**
Age	45–49	84	7.22%	145	9.66%	-	-
	50–54	180	15.48%	206	13.72%	-	-
	55–59	192	16.51%	277	18.45%	-	-
	60–64	175	15.05%	233	15.52%	-	-
	65–69	166	14.27%	233	15.52%	-	-
	70–74	179	15.39%	204	13.59%	-	-
	75+	187	16.08%	203	13.52%	-	-
Gender	Female	584	50.21%	861	57.36%	-	-
	Male	579	49.79%	640	42.64%	-	-
Race	Whites	814	69.99%	1038	69.15%	-	-
	Blacks	305	26.23%	398	26.52%	-	-
	Other	44	3.78%	65	4.33%	-	-
Regular NSAIDs use[c]	No	890	76.53%	1038	69.15%	1.00	-
	Yes	273	23.47%	463	30.85%	0.69	0.58–0.82
Menopausal hormones	Never	426	72.95%	591	68.64%	1.00	-
	Ever	158	27.05%	270	31.36%	0.81	0.64–1.02
Physical activity in 30s	=< median	599	51.50%	719	47.90%	1.00	-
	>median	564	48.50%	782	52.10%	0.86	0.74–1.00
Alcohol intake	<1 drink/day	924	80.00%	1228	81.81%	1.00	-
	>+1 drinks/day	231	20.00%	273	18.19%	1.03	0.84–1.25
Cigarette smoking	Never	477	41.12%	664	44.41%	1.00	-
	Ever	683	58.88%	831	55.59%	1.09	0.93–1.27
Diabetes	No	972	84.16%	1300	86.61%	1.00	-
	Yes	183	15.84%	201	13.39%	1.23	0.99–1.53
Body mass index	=< median	544	46.78%	784	52.23%	1.00	-
	> median	619	53.22%	717	47.77%	1.24	1.06–1.44
Family history of colorectal cancer	No	1003	86.47%	1352	90.43%	1.00	-

Characteristics		Cases No[a]	Cases %	Controls No[a]	Controls %	OR [b]	95% CI
	Yes	160	13.79%	149	9.97%	1.46	1.15–1.86
Total		1163		1501			

[a] Total number of cases and controls vary slightly due to missing values.

[b] OR was adjusted for gender except menopausal hormones, analysis of which was is limited to women

[c] NSAID: non-steroidal anti-inflammatory drug (>= 3 times per week for 6 months or longer use)

[d] Weight(kg)/height(m)^2 with median 27.46

Table 2

Odds ratio (OR) and 95% confidence intervals (CI) associated with FABP2-54 and APOE polymorphisms and their combinations

Genes	Genotypes	No of Cases	No of Controls	OR1[a]	95% CI	OR2[b]	95% CI
FABP2	T/T	82	106	1.00	-	1.00	
	A/T	434	564	1.00	0.73–1.38	0.98	0.71–1.35
	A/A	642	831	1.04	0.74–1.38	0.99	0.72–1.36
				p=0.917 [c]		p=0.953	
	T/T+A/T	516	670	1.00	-	1.00	-
	A/A	642	831	1.01	0.86–1.18	1.01	0.86–1.18
APOE	E4/E4	34	42	1.00	-	1.00	-
	E4/E2, E4/E3	318	398	0.99	0.61–1.60	0.95	0.58–1.55
	E3/E3, E2/E2, E2/E3	808	1061	0.95	0.60–1.50	0.90	0.56–1.45
				p=0.594		p=0.518	
	E4 carrier	352	440	1.00	-	1.00	-
	Non-E4 carrier	808	1061	0.95	0.81–1.13	0.95	0.80–1.13
No of FABP2-A + APO-E4 alleles	0–2	231	294	1.00	-	1.00	-
	3	477	617	0.98	0.80–1.21	0.97	0.78–1.21
	4	447	590	0.97	0.79–1.20	0.96	0.77–1.19
				p=0.789		p=0.714	

[a] OR1; adjusted for gender

[b] OR2: adjusted for age, gender, specimen type (blood vs. others), total calcium, fiber and red meat intake, physical activities in their 30s, body mass index, family history of colorectal cancer, highest education achieved, and NSAID use.

[c] P-values for a linear trend with the number of presumed high risk alleles

Table 3

Interactions between total fat intake and FABP2 and APOE polymorphisms among total subjects and by cancer subsite

		Fat intake =<median[a]				Fat intake > median				
Polymorphisms		**No. of subjects**		**OR [b]**	**95% CI**	**No. of subjects**		**OR [b]**	**95% CI**	**P-values[c]**
FABP2		TT+AT	AA			TT+AT	AA			
	Controls	345	420	1.00	-	325	411	1.00	-	
	All cases[d]	227	335	1.22	0.97–1.54	289	307	0.85	0.68–1.06	0.025
	Proximal	88	140	1.35	0.99–1.85	121	131	0.85	0.63–1.14	0.033
	Distal	135	195	1.19	0.91–1.56	166	176	0.85	0.65–1.11	0.082
Apo E4		Carrier	Non-carrier			Carrier	Non-carrier			
	Controls	249	516	1.00	-	191	545	1.00	-	
	All cases[d]	181	381	1.02	0.80–1.30	171	427	0.88	0.68–1.13	0.400
	Proximal	65	165	1.24	0.88–1.74	81	171	0.75	0.54–1.04	0.044
	Distal	115	213	0.90	0.68–1.19	90	254	0.99	0.74–1.34	0.684

[a] Energy-adjusted median total fat intake was 96 g

[b] Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for age, gender, specimen type (blood vs. others), total calcium, fiber and red meat intake, physical activities in their 30s, body mass index, family history of colorectal cancer, highest education achieved, and NSAID use

[c] p-values for interaction

[d] Distal: from the descending colon to the rectum, proximal: from the cecum to the splenic flexure, excluding 6 cases with colorectal NOS