Polymorphisms in ATP-binding cassette transporter genes and interaction with diet and life style factors in relation to colorectal cancer in a Danish prospective case-cohort study

TINE ISKOV KOPP1,2,3, VIBEKE ANDERSEN4,5,6, ANNE TJONNELAND2 & ULLA VOGEL7

1National Food Institute, Technical University of Denmark, Søborg, Denmark, 2Danish Cancer Society Research Center, Copenhagen, Denmark, 3Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark, 4Unit for Diagnostic and Clinical Research, Hospital of Southern Jutland, Aabenraa, Denmark, 5Institute of Regional Health Research-Center Sønderjylland, University of Southern Denmark, Odense, Denmark, 6Medical Department, Regional Hospital Viborg, Viborg, Denmark, and 7National Research Centre for the Working Environment, Copenhagen, Denmark

Abstract

Background and aims. The ATP-binding cassette (ABC) transporter family transports various molecules across the enterocytes in the gut protecting the intestine against potentially harmful substances. Moreover, ABC transporters are involved in mucosal immune defence through interaction with cytokines. The study aimed to assess whether polymorphisms in ABCB1, ABCC2 and ABCG2 were associated with risk of colorectal cancer (CRC) and to investigate gene–environment (dietary factors, smoking and use of non-steroidal anti-inflammatory drugs) and gene–gene interactions between previously studied polymorphisms in IL1B and IL10 and ABC transporter genes in relation to CRC risk. Materials and methods. We used a Danish prospective case-cohort study of 1010 CRC cases and 1829 randomly selected participants from the Danish Diet, Cancer and Health cohort. Incidence rate ratios were calculated based on Cox’ proportional hazards model. Results. None of the polymorphisms were associated with CRC, but ABCB1 and ABCG2 haplotypes were associated with risk of CRC. ABCB1/rs1045642 interacted with intake of cereals and fiber (p-Value for interaction (Pint) = 0.001 and 0.01, respectively). In a three-way analysis, both ABCB1/rs1045642 and ABCG2/rs2231137 in combination with IL10/rs3024505 interacted with fiber intake in relation to risk of CRC (Pint = 0.0007 and 0.009). Conclusions. Our results suggest that the ABC transporters P-glycoprotein/multidrug resistance 1 and BRCP, in cooperation with IL-10, are involved in the biological mechanism underlying the protective effect of fiber intake in relation to CRC. These results should be replicated in other cohorts to rule out chance findings.

Key Words: ATP-binding cassette transporter, colorectal cancer, cytokines, diet, gene–environment interaction, genetic epidemiology, inflammation, non-steroidal anti-inflammatory drug, polymorphism

Introduction

Colorectal cancer (CRC) is one of the most frequent cancer types in the world. Environmental factors, such as alcohol, smoking, obesity and high meat intake, have major impact on the carcinogenesis in combination with inheritable genetic factors [1–3]. Modulation of the exposure to food carcinogens in the intestines by enzymes involved in transport or metabolism of the carcinogenic substances may modify risk of CRC. Identifying gene–environment interactions may provide insight into the underlying mechanism of action because an interaction places gene or pathways and environmental factors in the same carcinogenic pathway [4].

The ATP-binding cassette transporter family (ABCs) transports various molecules across extra-
and intracellular membranes controlling absorption, distribution, metabolism and excretion of a wide variety of exogenous and endogenous substrates including numerous drugs, but also natural food constituents such as flavonoids, lipids and mycotoxins [5–8] and “human-made” food constituents like pesticides [9], insecticides [9], the carcinogens polycyclic aromatic hydrocarbons (PAHs) [10] and heterocyclic amines (HCAs) [11–13] including 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine [10,11,14]. Moreover, the ABC transporters are likely implicated in mucosal immune defence since the expression of the ABC transporters is modified by several cytokines [15–18]. Some studies also suggest that cytokines are substrates for the ABC transporters [19–21]. MDR1a −/− mice develop colitis spontaneously due to a defective intestinal epithelial barrier [22].

The three ABC transporter proteins P-glycoprotein (Pgp)/multidrug resistance 1 (MDR1) (encoded by ABCB1), multidrug-resistance-associated protein 2 (encoded by ABCC2) and breast cancer resistance protein (BCRP) (encoded by ABCG2) are all efflux transporters. They are expressed in various tissues including the apical surface of enterocytes where they serve to restrict the body from exposure to potentially harmful substances [5,7,8,23]. Abnormal expression of ABCB1, ABCC2 and ABCC2 has been detected in adenoma and carcinoma tissues indicating that these changes are early events in the adenoma-carcinoma sequence [24,25]. Genetic variations in these ABC transporters have various effects on their expression, mRNA stability, protein folding, intracellular localization, degradation, substrate binding, and/or transport kinetics [5,8,26,27], resulting in variation in the exposure of the intestinal epithelial barrier to harmful substances.

The formation of PAHs and HCAs during cooking of meat at high temperatures could explain the higher risk of CRC associated with red meat consumption [2,3,28]. We have previously found interaction between two polymorphisms in ABCB1 and red meat intake in relation to risk of CRC [29] which could be caused by a change in binding affinity or transport activity for PAHs and HCAs. Moreover, we found interaction between use of non-steroidal anti-inflammatory drugs (NSAID) and an ABCB1 polymorphism which is in line with studies showing that several NSAIDs modulate expression of ABC transporters [30–35] or function as substrates for the ABC transporters [30]. In another study using the same study group as the present, we found interaction for 959 CRC cases and 1799 sub-cohort members. In total, 120 with missing genotype data were excluded. All information on diet and lifestyle were collected at study entry. Blood samples and questionnaire data on diet and lifestyle were collected at study entry. Informed consent was obtained from all patients for being included in the study.

Methods

Studied subjects

The Diet, Cancer and Health Study is an ongoing Danish cohort study designed to investigate the relation between diet, lifestyle and cancer risk [37]. The cohort consists of 57,053 persons, recruited between December 1993 and May 1997. All the subjects were born in Denmark, and the individuals were 50–64 years of age and had no previous cancers at study entry. Follow-up and endpoints

The present study used a case-cohort design. Follow-up was based on population-based cancer registries. Between 1994 and 31th December 2009, 1010 CRC cases were diagnosed. A sub-cohort of 1829 persons was randomly selected within the full cohort at time of entry into the cohort in agreement with the case-cohort study design [38] and, thus, without respect to time and disease status. Due to the used design, with a priori sampling of the sub-cohort, 28 persons were both cases and sub-cohort, and these persons were kept in the analyses. In total, 120 with missing genotype data were excluded. All information on genotypes and diet and lifestyle factors was available for 959 CRC cases and 1799 sub-cohort members. The present study group was previously described [36,39,40].

cytokines control expression of the ABC transporters, identification of gene–gene and gene–environment interactions may indicate pathways implicated in colorectal carcinogenesis.

In the present study, we aimed to attempt to reproduce our previous findings on meat intake, use of NSAID and ABCB1 polymorphisms in relation to CRC in an updated prospective cohort of 1010 CRC cases and 1829 randomly selected participants from the Danish Diet, Cancer and Health Study. Additionally, we wanted to investigate whether other dietary and life style factors interact with other ABC transporter polymorphisms in relation to CRC risk and to investigate gene–gene interactions between the previously studied polymorphisms in IL1B and IL10 and ABC transporter genes in relation to different dietary factors and CRC risk to delineate a possible role of ABC transporters in colorectal carcinogenesis.
Dietary and lifestyle questionnaire

Information on diet, lifestyle, weight, height, medical treatment, environmental exposures and other socio-economic factors were collected at enrolment using questionnaires and interviews and has been described in details elsewhere [36,41–43]. In short, the food-frequency questionnaire, diet consumption was assessed in 12 categories of predefined responses, ranking from ‘never’ to ‘eight times or more per day’. The daily intake was then calculated by using FoodCalc [37]. Smoking status was classified as never, past or current. Persons smoking at least one cigarette daily during the last year were classified as smokers. NSAID use (“Aspirin”, “Paracetamol”, “Ibuprofen” or “Other pain relievers”) was assessed as ≥2 pills per month during 1 year at baseline.

Genotyping

Buffy coat preparations were stored at minus 150°C until use. DNA was extracted as described [44]. The DNA was genotyped by LGC KBioscience (LGC KBioscience, Hoddesdon, United Kingdom) by PCR-based KASP™ genotyping assay (http://www.lgcgenomics.com/). To confirm reproducibility, genotyping was repeated for 10% of the samples yielding 100% identity.

\( \text{ABCB1/rs1045652 [45–48], ABCB1/rs1128503 [49], ABCG2/rs2231142 [50–52], ABCG2/rs2231137 [53], ABCG2/rs2622604 [53], ABCC2/rs2273669 [54], ABCC2/rs17222723} \) were all selected based on their documented functionality from a literature search; and \( \text{ABCG2/rs3789243} \) was chosen based on its association with inflammatory bowel disease [56], CRC [29] and low mRNA levels in morphologically normal intestinal tissue from patients with adenoma [24]. \( \text{ABCB1/rs1045652, ABCB1/rs3789243, ABCG2/rs2231142, ABCG2/rs2273669 and ABCC2/rs717620} \) have also been determined and were all selected based on their documented functionality from a literature search; and \( \text{ABCG2/rs3789243} \) was chosen based on its association with inflammatory bowel disease [56], CRC [29] and low mRNA levels in morphologically normal intestinal tissue from patients with adenoma [24].

\( \text{ABCB1/rs1045652, ABCB1/rs3789243, ABCG2/rs2231142, ABCG2/rs2273669 and ABCC2/rs717620} \) have been studied previously in a subset of the present cohort [29,57]. \( \text{IL1B/rs1800872, IL10/rs3024505, IL1B/rs4848306, IL1B/rs1143623 and IL1B/rs1143627} \) have also been determined and reported in the same cohort as the present [36] and were only included in interaction analyses with ABC transporter polymorphisms based on findings from the study by Andersen et al. 2013.

Statistical analysis

Deviation from Hardy-Weinberg equilibrium was assessed using a Chi-square test.

The data were sampled according to the case-cohort design and the unweighted case-cohort approach was used for analyses [38]. Incidence rate ratios (IRR) for CRC were estimated by the Cox proportional hazards model. Age was used as the underlying time axis, which ensured that the estimation procedure was based on comparisons of individuals at the same age and the analyses were corrected for delayed entry, such that persons were considered under risk only from the age at enrolment in the cohort. Tests and 95% CI were based on Wald’s tests using the robust estimate of the variance-covariance matrix for the regression parameters in the Cox regression models [58] as previously described [29,36,41,57,59–65].

All models were adjusted for baseline values of suspected risk factors for CRC such as body mass index (kg/m², continuous), NSAID use (yes/no), use of hormone replacement therapy (never/past/current, among women), smoking status (never/past/current), intake of dietary fiber (g/day, continuous), and red meat and processed meat (g/day, continuous). Cereals, fiber, fruit and vegetables were also entered linearly. All analyses were stratified by gender, so that the basic (underlying) hazards were gender specific. For all the polymorphisms, IRR was calculated separately for heterozygous and homozygous variant allele carriers. For all the single-nucleotide polymorphisms (SNPs) except for \( \text{ABCG2/rs2622604, ABCG2/rs2273669 and ABCB1/rs1045642} \), all variant allele carriers were subsequently grouped for interaction analyses since no recessive effects were observed. For \( \text{ABCG2/rs2622604 and ABCC2/rs2273669} \), a recessive mode was used in the subsequent analyses. The frequencies of the \( \text{ABCB1/rs1045642} \) polymorphism vary across ethnical populations [66]. Mostly, the T-allele has been considered as the variant allele. In this study population, however, the T-allele is the most frequent and should therefore be considered as the wild type.

We also assessed weekly use of NSAID based on the results of a study of CRC within the Diet, Cancer and Health cohort [67] reporting that regular use of Aspirin or Non-Aspirin NSAID appears necessary to achieve a protective effect. However, there were no differences in risk estimates between monthly or weekly use, consequently, to maintain the statistical power in the strata; we used monthly NSAID use in the analyses.

Haplotypes of \( \text{ABCB1} \) and \( \text{ABCG2} \) were inferred manually as done previously [29,36,68,69].

The likelihood ratio test was used for interaction analyses between the studied polymorphisms and intake of meat, dietary fiber, cereals, fish, fruits, vegetables, alcohol, smoking status and NSAID use. In interaction analyses where the dietary factors were entered as categorical variables, tertile cutpoints
were based on the empirical distribution among cases divided by gender. The possible interactions were investigated using the likelihood ratio test. All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). A \( p < 0.05 \) was considered to be significant. Moreover, to test for multiple comparisons, Bonferroni correction was used.

**Ethics statement**

All participants gave verbal and written informed consent. The Diet, Cancer and Health study was approved by the National Committee on Health Research Ethics (journal nr. (KF) 01-345/93), and the Danish Data Protection Agency.

**Results**

Baseline characteristics of CRC cases and sub-cohort members are presented in Table I. Among sub-cohort members, the genotype distribution of the SNPs did not deviate from Hardy-Weinberg equilibrium (results not shown), except for the \( ABCC2/\) rs17222723 which was therefore excluded from further analyses.

Associations between polymorphisms and CRC

Risk estimates for the association between the studied SNPs and risk of CRC are presented in Supplementary Table I. No statistically significant associations between genotype distribution and risk of CRC were found. Haplotype analysis of the three \( ABCB1 \) polymorphisms revealed that the CCC/TTC combination (T-rs1045642C, C-rs1128503T, C-rs3789243T) was associated with a 41% increased risk of CRC (95% CI: 1.01–1.95) compared to the reference haplotype TTC/TTC (Table II). Having two copies of the CCC haplotype was associated with a 1.77-fold increased risk of CRC (95% CI: 1.00–3.13) (Table II). Conversely, having a GTC/GGT combination of the three \( ABCG2 \) polymorphisms (G-rs2231137A, G-rs2231142T and C-rs2622604T) was associated with a 39% lowered risk of CRC (IRR = 0.61; 95% CI: 0.44–0.86) compared to the reference haplotype GGC (Table III). We found no gene–gene interaction.
between ABC transporter polymorphisms and IL1B or IL10 SNPs in relation to CRC (results not shown).

Gene–environment analyses
Meat, fish, vegetables and alcohol. There was no interaction between ABC transporter SNPs and meat, fish, vegetables and alcohol intake in relation to risk of CRC (Table IV).

Cereals, fruits and fiber. We found interaction between ABCB1/rs1045642 and intake of both cereals and fiber in relation to risk of CRC and interaction between ABCG2/rs2231137 and fiber intake (Table IV).

ABCB1/rs1045642 wild type TT-carriers were at 13% increased risk of CRC per 50 g cereals per day (95% CI: 1.03–1.25), whereas variant C-carriers were not at increased risk by cereal intake (p-Value for interaction (Pint) = 0.001) (Table IV). However, in the tertile analyses (Supplementary Table II), a diet low in cereals was associated with a 1.37-fold increased risk of CRC among carriers of the variant C-allele (95% CI: 1.06–1.79) compared to homozygous wild type carriers. Thus, the higher risk of CRC associated with low intake of cereals among C-allele carriers was lowered following higher intake of cereals (Pint = 0.01) (Supplementary Table II).

Intake of 10 g fiber per day was associated with 20% reduced risk of CRC among carriers of the variant ABCB1/rs104562 C-allele (IRR = 0.80; 95% CI: 0.71–0.90), whereas wild type TT-carriers had no risk reduction at similar intake (Pint = 0.01) (Table IV). In the tertile analyses, a diet low in fiber was associated with a 1.3-fold increased risk of CRC carriers of the variant C-allele (95% CI: 1.0–1.7) compared to variant C-allele carriers with the highest fiber intake (P = 0.01) (Supplementary Table II).

ABCG2/rs2231137 exhibited similar, but weaker non-statistically significant interactions with fiber intake. Being a wild type GG-carrier was associated with a 10% reduced risk of CRC per 10 g fiber intake per day (IRR = 0.90; 95% CI: 0.82–0.99), whereas variant A-allele carriers had a 37% reduced risk of CRC (IRR = 0.63; 95% CI: 0.44–0.90) (Pint = 0.06) (Table IV). In the tertile analysis, only variant A-allele carriers with the highest fiber intake were associated with statistically significantly decreased risk of CRC of 53% (IRR = 0.47; 95% CI: 0.27–0.82; Pint = 0.01) (Supplementary Table II).

**Table II. Risk estimates for different combinations of ABCB1 haplotypes in relation to risk of CRC.**

| Haplotype | TTC       | CCT       | TCT       | CCC       | TCC       | CTC       | TTT       |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|           | (132/273) | (88/172)  | (12/30)   | (41/58)   | (5/7)     | (1/0)     | (0/1)     |
| TTC       | 1.00 (ref.) | 1.03 (0.85–1.25) | 1.13 (0.89–1.45) | 0.99 (0.73–1.34) | 0.90 (0.56–1.45) | 1.11 (0.67–1.83) | 1.04 (0.63–1.73) |
| CCT       | 0.99 (0.77–1.28) | 0.93 (0.71–1.22) | 1.18 (0.88–1.60) | *          | 1.30 (0.75–2.26) | 1.15 (0.68–1.93) |
| TCT       | 0.84 (0.49–1.42) | 0.63 (0.73–1.25) | 0.84 (0.49–1.42) | 1.41 (1.01–1.95) | 1.17 (1.00–1.33) | *          | 1.14 (0.43–2.98) |
| CCC       |           |           |           |           |           |           |           |
| TCC       |           |           |           |           |           |           |           |
| CTC       |           |           |           |           |           |           |           |
| TTT       |           |           |           |           |           |           |           |

IRR (95% CI) for CRC for different combinations of haplotypes. The numbers of cases and controls in each cell are listed. Variant alleles are bold. Adjusted for smoking status, alcohol, hormone replacement therapy status (women only), body mass index, use of non-steroidal anti-inflammatory drug, intake of red and processed meat, and dietary fiber.

*Haplotype is identical with another haplotype in the table.

*Haplotype sequence: T-rs1045642C, C-rs1128503T, C-rs3789243T.
**Table III.** Risk estimates for different combinations of *ABCG2* haplotypes in relation to risk of CRC.

| Haplotype<sup>a</sup> | GGC | GGT | GTC | AGC |
|-----------------------|-----|-----|-----|-----|
| GGC 1.00 (ref.) (327/583) | 1.02 (0.87–1.18) (295/534) | 0.94 (0.76–1.15) (117/231) | 0.94 (0.64–1.38) (29/56) |
| GGT 0.79 (0.58–1.08) (48/113) | 0.61 (0.44–0.86) (37/118) | 0.89 (0.52–1.50) (16/37) |
| GTC 1.19 (0.72–1.98) (14/23) | 1.10 (0.59–2.06) (9/13) |
| AGC 1.40 (0.18–10.73) (1/1) |

IRR (95% CI) for CRC for different combinations of haplotypes. The number of cases and controls in each cell is listed. Variant alleles are bold. Adjusted for smoking status, alcohol intake, hormone replacement therapy status (women only), body mass index, use of non-steroidal anti-inflammatory drug, intake of red and processed meat, and dietary fibre.
<sup>a</sup>Haplotype sequence: G-rs2231137A, G-rs2231142T, C-rs2622604T.

*ABCG2/rs2273697* interacted with fruit intake (*P*<sub>int</sub> = 0.01) such that carriers of the G-allele had a non-statistically significantly 2% decreased risk of CRC per 50 g per day (IRR = 0.98; 95% CI: 0.95–1.01), whereas homozygous carriers of the variant A-allele had a borderline statistically significantly 5% increased risk of CRC per 50 g fruit per day (IRR = 1.05; 95% CI: 0.99–1.11). In the tertile analysis, no interaction between *ABCG2/rs2273697* and fruit intake was detected (Supplementary Table II).

We have previously found strong interaction between fiber intake and *IL10/rs3024505* [36,41]. Only homozygous wild type allele carriers had reduced risk of CRC by fiber intake. However, tertile analysis revealed that the variant allele carriers were at lower risk of CRC than homozygous wild type carriers in the tertile with the lowest fiber intake. Homozygous wild type allele carriers had a risk reduction by fiber intake, and in the tertile with the highest fiber intake, the risk of CRC was the same for both genotype groups. Therefore, we explored the interactions between *ABCB1/rs1045642* and fibers by assessing the interaction between *ABCB1/rs1045642*, *IL10/rs3024505* and fiber intake. We found strong interaction between *ABCB1/rs1045642* and *IL10/rs3024505* in relation to the protective effect of fiber intake (Table V). Homozygous carriers of the *ABCB1/rs1045642* wild type T-allele, who were also carriers of the variant IL10/rs3024505 T-allele, had a 1.31-fold increased risk of CRC per 10 g fiber per day (95% CI: 1.04–1.65), whereas carriers of the *ABCB1/rs1045642* C-allele, who were also homozygous wild type carriers of the *IL10/rs3024505* C-allele, had a 25% reduced risk of CRC for similar fiber intake (IRR = 0.75; 95% CI: 0.65–0.87) (*P*<sub>int</sub> = 0.0007) (Table V). Furthermore, similar interaction with *IL10/rs3024505* in relation to the protective effect of fiber intake was identified for *ABCG2/rs2231137* (Table VI). Wild type carriers of both the *IL10/rs3024505* and the *ABCG2/rs2231137* polymorphism had an 18% decreased risk of CRC per 10 g fiber per day (IRR = 0.82; 95% CI: 0.72–0.92). If the CC-carriers of the *IL10/rs3024505* polymorphism also were carriers of the variant *ABCG2/rs2231137* A-allele, the risk of CRC was additionally reduced for identical fiber intake (IRR = 0.51; 95% CI: 0.30–0.86) (*P*<sub>int</sub> = 0.009) (Table VI). We also assessed the effect of interactions between ABC polymorphisms, *IL10/rs3024505* and intake of cereals, and found similar but much weaker interactions (results not shown), suggesting that fiber has a stronger effect than cereals.

**NSAID use.** A pattern where NSAID use was associated with decreased risk of CRC among carriers of low activity variants of the ABC transporter genes was seen (Table VII). Increased risk of CRC was found among users of NSAID, who were homozygous carriers of either *ABCB1/rs1128503* C-allele (IRR = 1.21; 95% CI: 0.96–1.52; *P*<sub>int</sub> = 0.04) or *ABCG2/rs2273697* A-allele (IRR = 1.75; 95% CI: 1.12–2.72; *P*<sub>int</sub> = 0.06), whereas risk reduction was found for variant T-allele carriers of the *ABCG2/rs2231142* polymorphism among NSAID users (IRR = 0.74; 95% CI: 0.55–1.00; *P*<sub>int</sub> = 0.03) (Table VII). These results, though, were mostly only borderline statistically significant.

There was no interaction with smoking status and any of the ABC transporter polymorphisms in relation to CRC risk (Supplementary Table III).

**Discussion**

In the present study, we found no association between the ABC transporter polymorphisms and CRC risk, whereas *ABCB1* and *ABCG2* haplotypes were associated with risk of CRC. In the haplotype analysis of *ABCB1*, the CCC/TCT combination was associated with a 41% increased risk of CRC; and carriage of two copies of the *ABCB1* CCC haplotype further increased the risk. The *ABCG2* GTC/GGT haplotype combination was associated with reduced risk of CRC. We found interaction between *ABCB1/rs1045642* and intake of cereals and fiber. Moreover,
### Table IV. Interaction between dietary factors and the studied polymorphisms in relation to CRC risk.

|         | Red and processed meat per 25 g/day | Fish per 25 g/day | Dietary cereal per 50 g/day | Alcohol per 10 g/day |
|---------|-------------------------------------|-------------------|-----------------------------|----------------------|
| **ABCBI** |                                     |                   |                             |                      |
| rs1045642 | 1.01 (0.97–1.05)                     | 1.01 (0.96–1.05)  | 0.19 (0.91–1.01)            | 1.01 (0.97–1.13)     |
| rs1128503 | 1.04 (1.00–1.07)                     | 1.04 (1.01–1.08)  | 0.96 (0.90–1.04)            | 1.05 (0.97–1.13)     |
| rs3789243 | 1.01 (0.96–1.04)                     | 1.00 (0.96–1.05)  | 0.33 (0.91–1.01)            | 1.05 (1.03–1.25)     |
| **ABCBI** |                                     |                   |                             |                      |
| rs23231142 |                                  |                   |                             |                      |
| rs2323137 | 1.02 (1.00–1.05)                     | 1.03 (1.00–1.10)  | 0.90 (0.92–1.00)            | 1.04 (0.97–1.11)     |
| rs2622604 | 1.02 (0.99–1.05)                     | 1.02 (0.99–1.05)  | 0.39 (0.89–1.01)            | 1.04 (0.97–1.11)     |
| rs2373697 | 1.01 (0.96–1.06)                     | 1.01 (0.97–1.07)  | 0.94 (0.75–1.17)            | 1.00 (0.95–1.06)     |
| **ABCBI** |                                     |                   |                             |                      |
| rs1045642 | 0.99 (0.85–1.15)                     | 1.01 (0.87–1.18)  | 0.01 (0.98–1.01)            | 1.01 (0.95–1.06)     |
| rs1128503 | 0.79 (0.70–0.88)                     | 0.80 (0.71–0.90)  | 0.96 (0.93–0.99)            | 1.01 (0.95–1.06)     |
| rs3789243 | 0.81 (0.68–0.95)                     | 0.82 (0.70–0.97)  | 0.35 (0.97–1.03)            | 1.01 (0.95–1.06)     |
| rs23231142 |                                  |                   |                             |                      |
| rs2323137 | 0.89 (0.80–1.00)                     | 0.90 (0.81–1.01)  | 0.97 (0.94–1.00)            | 1.01 (0.95–1.06)     |
| rs2622604 | 0.95 (0.84–1.07)                     | 0.95 (0.84–1.08)  | 0.12 (0.99–1.02)            | 1.01 (0.95–1.06)     |
| rs2373697 | 0.96 (0.73–0.96)                     | 0.83 (0.74–0.93)  | 0.96 (0.93–0.99)            | 1.01 (0.95–1.06)     |
| **ABCBI** |                                     |                   |                             |                      |
| rs1045642 | 0.86 (0.78–0.94)                     | 0.87 (0.79–0.95)  | 0.70 (0.94–0.99)            | 1.00 (0.95–1.06)     |
| rs1128503 | 0.90 (0.74–1.09)                     | 0.90 (0.74–1.10)  | 0.96 (0.92–1.01)            | 1.00 (0.95–1.06)     |
| rs3789243 | 0.89 (0.81–0.98)                     | 0.90 (0.82–0.99)  | 0.06 (0.95–0.99)            | 1.00 (0.95–1.06)     |
| rs23231142 |                                  |                   |                             |                      |
| rs2323137 | 0.59 (0.41–0.85)                     | 0.65 (0.44–0.90)  | 0.92 (0.85–1.01)            | 0.98 (0.81–1.04)     |
|          | Fruit per 50 g/day | Vegetables per 50 g/day | Fiber per 10 g/day | Physical activity | Alcohol intake | Smoking status | Hormone replacement therapy | Body mass index | Use of NSAID | Meat intake | Fish intake | Vegetable intake |
|----------|-------------------|-------------------------|-------------------|------------------|-----------------|----------------|----------------------------|----------------|-------------|-------------|-------------|------------------|
| rs2622604|                   |                         |                   |                  |                 |                |                             |                 |             |             |             |                  |
| CC+TC   | 0.89 (0.82–0.97)  | 0.90 (0.83–0.98)        | 0.89 (0.82–0.97)  | 0.89 (0.82–0.97) |                 |                |                             |                 |             |             |             |                  |
| CT+TC   | 0.77 (0.53–1.12)  | 0.78 (0.53–1.13)        | 0.77 (0.53–1.12)  | 0.77 (0.53–1.12) |                 |                |                             |                 |             |             |             |                  |
| rs1128503|                   |                         |                   |                  |                 |                |                             |                 |             |             |             |                  |
| TT      | 0.77 (0.53–1.12)  | 0.78 (0.53–1.13)        | 0.77 (0.53–1.12)  | 0.77 (0.53–1.12) |                 |                |                             |                 |             |             |             |                  |
| CT      | 0.90 (0.83–0.98)  | 0.91 (0.88–0.99)        | 0.90 (0.83–0.98)  | 0.90 (0.83–0.98) |                 |                |                             |                 |             |             |             |                  |
| rs2273697|                   |                         |                   |                  |                 |                |                             |                 |             |             |             |                  |
| GG+AG   | 0.90 (0.83–0.98)  | 0.91 (0.88–0.99)        | 0.90 (0.83–0.98)  | 0.90 (0.83–0.98) |                 |                |                             |                 |             |             |             |                  |
| AG+AA   | 0.90 (0.83–0.98)  | 0.91 (0.88–0.99)        | 0.90 (0.83–0.98)  | 0.90 (0.83–0.98) |                 |                |                             |                 |             |             |             |                  |

aCrude adjusted for age and sex.
bAdditional adjustment for smoking status, alcohol intake, hormone replacement therapy status (women only), body mass index, use of Non-steroidal anti-inflammatory drug, intake of red and processed meat, and dietary fiber.
cp-Value for interaction for the adjusted estimates.
dAmong current drinkers only.

The risk ABCB1 haplotype (CCC) encompasses two high activity alleles (T-rs1045642C and C-rs1128503T), whereas the third (C-rs3789243T) has no known functionality, suggesting that inherent high activity of Pgp/MDR1 is associated with increased risk of CRC. The biological effect of the ABCG2 haplotype with the GTC/GGT combination is less clear. The reference ABCG2 haplotype (GGC) consists of three high activity alleles (G-rs2231137A, G-rs2231142T, C-rs2622604T), whereas the protective haplotype combination encompasses a combination of both high and low activity alleles. The found associations could thus be caused by LD with other neighbouring functional SNPs.

We were not able to reproduce the previously identified interactions between ABCB1/rs1045642 and ABCB1/rs3789243 and meat intake in this updated study where we had more than doubled both the number of cases and the number of members of the comparison group.

It is well documented that dietary fiber protects against CRC [70]. In the intestine, plant polysaccharides from ingested fibers increase stool mass which lowers the exposure of the enterocytes to carcinogens through reduction in transit time [71]. Moreover, dietary fibers are fermented by the commensal bacteria to short-chain fatty acids, which are important for the colonic integrity and because of anti-tumorigenic and anti-inflammatory properties [71]. Butyrate is one of the most important short-chain fatty acids since it is the primary colonic energy source [72,73]. Butyrate has been shown to induce Pgp/MDR1 expression [74] and is a substrate for BCRP [75]. We found interaction between ABCB1/rs1045642 and fiber intake such that carriers of the high activity C-allele were at reduced risk of CRC by fiber intake. However, since C-allele carriers were at increased risk of CRC in the tertile with low fiber intake, the fiber intake only appeared to reduce the excess risk associated with being C-allele carrier. Moreover, the observed interaction between ABCB1, IL10 and fiber intake (Table V) suggests that Pgp/MDR1 (encoded by ABCB1), IL-10 and fiber intake are part of a common pathway protecting against CRC. Likewise, the found interaction with ABCG2 (Table VI) suggests that BRCP (encoded by...
Table V. IRR for CRC in relation to combinations of ABCB1/rs1045642 and IL10/rs3024505 genotypes per 10 g fiber per day.

| Genotype | IL10/rs3024505 | IL10/rs3024505 | p-Valuea |
|----------|----------------|----------------|---------|
|          | CC             | CT+TT          | CC      | CT+TT |
|          | IRR (95% CI)a  | IRR (95% CI)a  | IRR (95% CI)b | IRR (95% CI)b |
| ABCB1/rs1045642 |                |                |         |       |
| TT       | 202/382        | 93/178         | 0.83 (0.69–1.01) | 1.28 (1.02–1.61) |
| TC+CC    | 430/789        | 198/383        | 0.74 (0.64–0.86) | 0.89 (0.74–1.08) |

Genotype data of the IL10/rs3024505 polymorphism is obtained from Andersen et al. [34].

aCrude – adjusted for age and sex.

bIn addition, adjusted for smoking status, alcohol intake, hormone replacement therapy status (women only), body mass index, use of Non-steroidal anti-inflammatory drug and intake of red and processed meat.

p-Value for comparison of the adjusted risk estimates.

Table VI. IRR for CRC in relation to combinations of ABCG2/rs2231137 and IL10/rs3024505 genotypes per 10 g fiber per day.

| Genotype | IL10/rs3024505 | IL10/rs3024505 | p-Valuea |
|----------|----------------|----------------|---------|
|          | CC             | CT+TT          | CC      | CT+TT |
|          | IRR (95% CI)a  | IRR (95% CI)a  | IRR (95% CI)b | IRR (95% CI)b |
| ABCG2/rs2231137 |                |                |         |       |
| GG       | 587/1108       | 269/528        | 0.81 (0.71–0.91) | 1.05 (0.50–1.23) |
| GA+AA    | 42/82          | 21/40          | 0.48 (0.28–0.81) | 0.78 (0.48–1.26) |

Genotype data of the IL10/rs3024505 polymorphism is obtained from Andersen et al. [34].

aCrude – adjusted for age and sex.

bIn addition, adjusted for smoking status, alcohol intake, hormone replacement therapy status (women only), body mass index, use of Non-steroidal anti-inflammatory drug and intake of red and processed meat.

p-Value for comparison of the adjusted risk estimates.

Table VII. Interaction between NSAID use and the studied polymorphisms in relation to CRC risk.

|         | NSAID use       | NSAID use       | NSAID use       | p-Valuec |
|---------|-----------------|-----------------|-----------------|---------|
|         | n_case/nsub-cohort | n_case/nsub-cohort | n_case/nsub-cohort |       |
|         | No               | Yes             | Yes             |         |
|         | IRR (95% CI)a    | IRR (95% CI)b   | IRR (95% CI)b   | Activity (references) |

**ABC1**

|          | n1045642         | n1128503        | rs3789243       | rs2231142 |
|----------|------------------|-----------------|----------------|-----------|
| TT       | 215/391          | 435/804         | 192/384         | 458/819   |
| TC+CC    | 85/172           | 200/371         | 108/189         | 178/364   |
| n_case   | 85/172           | 200/371         | 108/189         | 178/364   |
| n_sub-cohort | 391/804       | 371/435        | 189/108         | 364/178   |
| IRR      | 0.89 (0.70–1.14) | 1.05 (0.86–1.25) | 1.21 (0.96–1.52) | 1.00 (0.92–1.30) |
| p-Value  | 0.25             | 0.04            | 0.70            | 0.03      |
| Activity | T = [45–48]      | T = [49]        | Unknown         | A = [53]  |

**ABC2**

|          | rs2231137        | rs2262604       | rs1273697       | rs717620 |
|----------|------------------|-----------------|-----------------|---------|
| GG       | 503/943          | 46/85           | 608/1119        | 37/86   |
| TG+TT    | 444/425          | 17/38           | 270/509         | 16/39   |
| n_case   | 503/943          | 46/85           | 608/1119        | 37/86   |
| n_sub-cohort | 425/444         | 138/46         | 509/270         | 39/16   |
| IRR      | 1.10 (0.95–1.29) | 0.73 (0.55–0.99) | 1.00 (0.84–1.22) | 0.74 (0.55–1.00) |
| p-Value  | 0.03             | 0.30            | 0.70            |         |
| Activity | T = [50–52]      | A = [53]        | T = [53]        |          |

**ABC3**

|          | rs2273697        | rs717620        | rs717620        | rs717620 |
|----------|------------------|-----------------|-----------------|---------|
| GG+AG    | 625/1159         | 24/55           | 244/528         | 1/3     |
| AA       | 25/45            | 19/16           | 104/631         | 1/3     |
| n_case   | 625/1159         | 24/55           | 244/528         | 1/3     |
| n_sub-cohort | 1159/625       | 55/24           | 528/244         | 3/1     |
| IRR      | 0.98 (0.85–1.13) | 1.77 (1.15–2.72) | 1.01 (0.70–1.44) | 1.75 (1.12–2.72) |
| p-Value  | 0.06             | 0.35            | 0.14            | A = [54] |

aCrude – adjusted for age and sex.

bIn addition, adjusted for smoking status, alcohol intake, hormone replacement therapy status (women only), body mass index, intake of red and processed meat, and dietary fiber.

p-Value for interaction for the adjusted estimates.

Abbreviations: ABC = ATP-binding cassette; NSAID = Non-steroidal anti-inflammatory drug.
ABCG2) is also involved in the protective effect of fiber mediated by IL-10. Cytokines have been shown to modulate expression of ABC transporter genes [15–18] and also suggested to function as substrates [20,21]. However, the present study does not clarify how the exact underlying mechanism is; only that IL-10, Pgp/MDR1 and BCRP are part of the same biological mechanism underlying the protective effect of dietary fiber on CRC risk. A possible implication of inflammation and bacteria in CRC is supported by the findings that functional polymorphisms in IL1B, IL10, PTGS2, TLR4 and NFKB1 were also associated with risk of CRC in the present cohort, and interacted with several dietary factors [36,39].

Consumption of cereals has also been associated with a protective effect in relation to CRC, mostly due to the high content of fiber [76]. However, in this study, T-allele carriers of the ABCB1/rs1045642 polymorphism were at an increased risk of CRC per 50 g intake of cereal per day. Cereals are often contaminated with mycotoxins produced by fungi of the genera Aspergillus, Fusarium and Penicillium that grow on food crops [77]. These mycotoxins are very toxic and carcinogenic [77]. Mycotoxins or their metabolites are substrates to Pgp/MDR1 [78–80] and other ABC transporters [81,82] and are known to interact with several drugs [6]. It is possible that carriage of the low activity T-allele of ABCB1/rs1045642 causes detrimental enterocytic accumulation of mycotoxins from cereals.

Several studies have shown that NSAIDs modulate expression of the ABC transporters [30–35] and possibly functions as substrates [30]. The present study supports these findings and suggests that carriers of polymorphisms that are associated with lower ABC transport activity benefit from the anti-inflammatory effect of the NSAIDs. Conversely, carriers of high activity ABC transporter polymorphisms, have a tendency toward increased risk of CRC if they use NSAID, which indicates that the drugs do not exert their anti-inflammatory effect in these persons due to enhanced efflux of the drugs. Indeed, several clinical studies report that drug resistance is caused by high activity variants of these transporter genes [26,46,83–85]. However, the results from the present study are not statistically strong and are based on small numbers in each stratum. They should therefore be interpreted with caution.

This prospective cohort has limited statistical power for studying gene-environment interactions. On the other hand, the study design has some advantages. Study participants were middle age (50–64 years) at entry which reduces the likelihood of substantial change in dietary patterns during follow up. However, if it happens, it is not expected to result in differential misclassification. The Danish cohort is very homogenous eliminating population specific genetics and dietary patterns seen in larger multicentre studies. Intake of meat in this cohort is very high, which makes it suitable for testing the effect of meat on colorectal carcinogenesis. Consumption of alcohol among women is the highest in Europe [86], which also makes it suitable for mechanistic investigating. We are well aware of the risk of change findings due to the large number of tests. None of the results withstood Bonferroni correction and could therefore theoretically be due to change. However, since all our tests are based on a priori hypotheses and previous findings, we believe that the risk of chance findings is somewhat reduced [87].

Conclusions

In conclusion, we found that ABCB1 and ABCG2 haplotypes were associated with risk of CRC and interaction between ABCB1/rs1045642 and intake of fiber and cereals. We also found three-way interactions between both ABCB1/rs1045642 and ABCG2/rs2231137, and IL10/rs3024505 in relation to the protective effect of fiber intake. This suggests that the ABC transporters Pgp/MDR1 and BCRP in cooperation with IL-10 are involved in the biological mechanism underlying the protective effect of fiber intake in relation to CRC. These associations and interactions should be sought replicated in other cohorts.

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Supplementary material available online

Supplementary Table I–III