Polyunsaturated Fatty Acids, DNA Repair Single Nucleotide Polymorphisms and Colorectal Cancer in the Singapore Chinese Health Study

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Key Words

Colorectal cancer, Singapore • PARP • Polyunsaturated fatty acids • n–3 Polyunsaturated fatty acids

Abstract

Animal and in vitro studies support a role for polyunsaturated fatty acids (PUFAs) in colon carcinogenesis; however, the epidemiological evidence is inconclusive. Recently, we investigated their role within the Singapore Chinese Health Study, a population-based cohort of Singapore Chinese men and women. We reported that a high intake of marine n–3 PUFAs was associated with an increased risk of colorectal cancer (CRC). Oxidation of PUFAs incorporated into cell membranes generates lipid hydroperoxides, which can be mutagenic. In this report, we investigated whether single nucleotide polymorphisms (SNPs) in DNA repair genes modified the effect of PUFAs on CRC risk using a nested case-control study within the Singapore Chinese Health Study. We genotyped 1,181 controls and 311 cases (180 colon and 131 rectal cancer) for SNPs in the *XRCC1* (Arg194Trp, Arg399Gln), *OGG1* (Ser326Cys), *PARP* (Val762Ala, Lys940Arg), and *XPB* (Asp312Asn, Lys751Gln) genes. We observed that the *PARP* Val762Ala SNP modified the association between marine

n–3 PUFA and rectal cancer risk, with no evidence of interaction among colon cancer (heterogeneity test $p = 0.003$). Our results suggest a positive association between high intake of marine n–3 PUFA and rectal cancer risk among carriers of at least one *PARP* codon 762 Ala allele (odds ratio = 1.7, 95% confidence interval = 1.1–2.7).

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Introduction

Animal and in vitro studies support a role for polyunsaturated fatty acids (PUFAs) in colon carcinogenesis. In particular, the ratio of high intake of n–6 PUFAs, such as linoleic acid (18:2, LA) and arachidonic acid (20:4, AA) to low intake of n–3 PUFAs, such as α -linolenic acid (18:3, ALA), docosahexanoic acid (22:6, DHA), and eicosapentaenoic acid (20:5, EPA), are linked to increased

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colon tumor formation [1]. Findings from epidemiological studies of PUFAs and CRC risk are generally inconclusive, although a 2007 meta-analysis of four prospective cohort studies reported a trend for an inverse association between diets high in n-3 PUFA and CRC incidence [2].

Recently, we reported that within the Singapore Chinese Health Study, a population-based cohort of Singapore Chinese, diets high in marine n-3 PUFA were positively associated with an increased risk of CRC, especially for advanced tumors among subjects with diets high in n-6 PUFA (hazard ratio = 1.82, 95% confidence interval (CI) 1.23–2.68; *p* for trend <0.01) [3]. Singapore Chinese are historically at low risk for CRC; however, CRC incidence rates have doubled in the past three decades [4]. This change might be partially attributable to a transition from a traditional Chinese diet (i.e. high in fish and cruciferous vegetables) and lifestyle towards a more Western diet (i.e. high in red meat and saturated fat).

Dietary PUFAs are incorporated into cell membranes where they can be oxidized to lipid hydroperoxides, such as peroxy radicals and hydroxy-alkenals. These compounds are highly reactive and can form adducts with macromolecules, such as DNA and proteins [5, 6]. Specifically, lipid peroxidation of n-6 PUFAs generates the mutagenic compounds, malondialdehyde and 4-hydroxynonenal [7], whereas n-3 PUFA generates mainly 4-hydroxy-2-hexenal, of which less is known regarding mutagenic potential [8]. However, 4-hydroxy-2-hexenal has been reported to be toxic for mitochondria function [9]. In contrast with these potential mutagenic effects for n-3 PUFA-derived adducts, there is some support from in vitro data for a role of n-3 PUFA-derived lipid hydroperoxides in colon cancer growth inhibition and increased apoptosis [6, 10–12], and experiments in rodents have shown an increase in apoptosis linked to diets high in marine n-3 PUFAs [13, 14]. Therefore, it is still unclear whether high levels of n-3 PUFAs prevent or promote colorectal carcinogenesis.

We have followed-up our PUFA findings from the Singapore Chinese Health Study with analyses taking into account variants in genes that are relevant for the proposed mechanism of action of PUFAs. We hypothesized that genes that play key roles in pathways that repair PUFA-induced damage might modify the effect of these fatty acids on CRC. Therefore, as a first step in addressing this hypothesis, we investigated the potential modifying role of single nucleotide polymorphisms (SNPs) in genes that participate in the base excision repair pathway (*XRCC1*: Arg194Trp and Arg399Gln; *OGG1*: Ser326Cys;

and *PARP*: Val762Ala, Lys940Arg) which repairs lipid hydroperoxide-induced oxidative DNA base modifications and single-strand breaks [15], and the nucleotide excision repair pathway (*XPB*: Asp312Asn, Lys751Gln), which repairs bulky adducts induced by smoking- and lipid hydroperoxide acetaldehyde-induced adducts [8, 16]. These SNPs were selected based on putative impact on protein function and/or previous evidence of cancer risk associations.

Methods

Study Subjects

The design of the Singapore Chinese Health Study has been described [17]. Briefly, the cohort was drawn from permanent residents or citizens of Singapore who resided in government-built housing estates (86% of the Singapore population resided in such facilities during the enrollment period). The age eligibility criterion was 45–74 years. We restricted recruitment to the two major dialect groups of Chinese in Singapore, the Hokkiens and the Cantonese. Between April 1993 and December 1998, 63,257 subjects (about 85% of eligible subjects) were recruited. At recruitment, information on lifestyle factors and diet during the last year was obtained through in-person interviews.

Details on the characteristics of the subjects included in this nested case-control set have been recently reported [18]. Briefly, incident cancer cases among cohort members were identified through record linkage with the nationwide Singapore Cancer Registry database [19]. Blood or buccal cells were collected from a random 3% sample of cohort participants between April 1994 and July 1999 for a total of 1,194 subjects. In addition, 52% of incident CRC cases donated blood/buccal cell samples for genetic analyses. For this study we included 310 cases with available DNA samples available (colon cancer = 180, rectum cancer = 130), and 1,176 subjects free of cancer. The study was approved by the Institutional Review Boards of the University of Minnesota, the University of Southern California, and the National University of Singapore.

Baseline Exposure Assessment

At recruitment, an in-person interview was conducted in the home of the subject by a trained interviewer using a structured questionnaire. The questionnaire requested information on demographics, lifetime use of tobacco (cigarettes and water-pipe), current physical activity, reproductive history (women only), occupational exposure, medical history, and family history of cancer. Information on current diet, including alcohol consumption, was assessed via a 165-item food frequency questionnaire (FFQ) that has been validated against a series of 24-hour dietary recall interviews [17] and selected biomarker studies [20, 21] conducted on random subsets of cohort participants. The FFQ listed 14 seafood items, including fresh fish (fish ball or cake, deep fried fish, pan or stir fried fish, boiled or steamed fish), fresh shellfish (shrimp or prawn, squid or cuttlefish), dried/salted fish (salted fish, ikan bilis, dried fish, other dried seafoods such as dried shrimp, dried oyster, dried cuttlefish), and canned fish (canned

tuna, canned sardine). The average portion weight (without bone) for fresh fish was approximately 60 g and for fresh shellfish, dried/salted fish, and canned fish approximately 35, 10, and 60 g, respectively. The island of Singapore is situated one degree north of the equator. Thus, fatty acid composition for fish intake was derived from the commonly consumed warm water, or lean fish species. The Singapore Food Composition Table, developed in conjunction with this cohort study, allows for the computation of intake levels of roughly 100 nutritive and non-nutritive compounds per study subject [17], including levels of 25 fatty acids. To adjust for energy intake, PUFA variables were expressed in percentage of total energy (grams per 1,000 kcal). We determined 'high' and 'low' intake categories by using the median nutrient density among controls in the entire cohort. At baseline we also asked about supplement use (e.g. vitamins/minerals, cod liver oil). We did not collect information on over-the-counter or prescription drug use.

Genotyping

We have previously reported on the 7 SNPs we considered in this study [18] as follows: *XRCC1* Arg194Trp (rs1799782; Trp prevalence = 0.295), *XRCC1* Arg399Gln (rs25487; Gln prevalence = 0.267), *PARP* Val762Ala (rs1136410; Ala prevalence = 0.435), *PARP* Lys940Arg (rs3219145; Arg prevalence = 0.022), *OGG1* Ser326Cys (rs1052133; Cys prevalence = 0.610), *XPD* Asp312Asn (rs1799793; Asn prevalence = 0.042), and *XPD* Lys751Gln (rs13181; Gln prevalence = 0.074). Briefly, we genotyped them using TaqMan assays (Applied Biosystems, Inc., Foster City, Calif., USA), including a 5% randomly selected duplicate samples, for which we observed 100% concordance.

Data Analysis

Genetic and exposure data were analyzed using standard methods for unmatched case-control studies, as we previously described [18]. We considered total n-6, n-3, and marine n-3 PUFAs. To adjust for energy intake, PUFA variables were expressed in percentage of total energy (grams per 1,000 kcal), and total energy intake was included in the adjusted models [17]. Analyses of PUFA or DNA repair genotypes or PUFA $\times$ DNA repair genotypes and CRC were done using unconditional logistic regression models that also included following covariates: age at recruitment (years), gender, dialect group (Hokkien, Cantonese), year of recruitment (1993-1995, 1996-1998), level of education (no formal education, primary school, secondary or higher education), body mass index (<20, 20-<24, 24-<28, 28+), history of diabetes (no, yes), family history of colorectal cancer (CRC) (no, yes), weekly vigorous physical activities (no, yes), smoking index ('heavy' = started to smoke before age 15 and smoked ≥ 13 cigarettes per day, 'light' = all non-heavy smokers, never), alcohol index (non-drinkers, <7, 7+ drinks per week), and total caloric intake (continuous). As we previously reported [3], inclusion of additional dietary variables (total meat, preserved meat, preserved fish, or nitrosamines), use of supplements (vitamins/minerals, cod liver oil), and dietary fiber did not materially change any of the study results. Thus those variables were not included in final multivariate regression models.

We tested for gene-environmental (G $\times$ E) interactions on a multiplicative scale by including the product term between the gene and PUFA variables in addition to the two main effect terms in regression models and using likelihood ratio tests. We con-

ducted all G $\times$ E analyses using dichotomous variables of PUFA intake using the median among the cohort as cutpoint. Heterogeneity of the gene-PUFA interaction odds ratios (ORs) across tumor site (colon vs. rectum) or stage (localized vs. advanced) was examined by using polytomous logistic regression models. To account for multiple testing, we controlled for the false discovery rate (FDR) at 5% using the Benjamini-Hochberg method [22]. All gene $\times$ diet interaction tests are corrected for testing 7 SNPs, for each exposure variable. All tests were two-sided and all analyses were done using the statistical software STATA version 8 (STATA Corp., College Station, Tex., USA).

Results

Demographic and descriptive statistics of the study population are shown in table 1. Cases and controls did not significantly differ by dialect group or level of physical activity. Compared to controls, cases had a higher proportion of men ($p < 0.001$), lower level of education ($p = 0.004$), and higher prevalence of family history of CRC ($p = 0.08$), diabetes ($p < 0.001$), heavy smoking ($p < 0.001$), and heavy drinking ($p = 0.02$). Consistent with our recently reported findings [3], within our nested case-control set we also observed a positive association between the highest quartile of marine n-3 PUFA and CRC risk (OR = 1.4, 95% CI = 0.9-2.0), albeit the trend was not statistically significant ($p = 0.179$). We observed no association for total PUFA, total n-6 PUFA or total n-3 PUFA.

DNA Repair SNPs, PUFA Intake, and CRC Risk

We have previously reported our analyses on the main effects of the 7 SNPs we considered in this study on CRC risk, for which we considered a dominant mode of action for all SNPs with the exception of *XRCC1* Arg399Gln for which we considered a recessive mode of action [18]. We observed a positive association between the *PARP* codon 940 Lys/Arg and Arg/Arg genotypes and CRC (OR = 1.8, 95% CI = 1.1-3.1) [18]. When considering all tumors combined, we did not observe evidence that any of the 7 SNPs modified the association between total n-3 PUFAs, total marine n-3 PUFAs, or total n-6 PUFAs and CRC risk (online suppl. table 1, www.karger.com/doi/10.1159/000308467). Moreover, we did not find statistically significant evidence that the interaction between each of the 7 SNPs and total n-6 or n-3 PUFAs differed by gender, stage of disease, or that the interaction with n-3 PUFA differed by n-6 PUFA intake levels.

When considering tumor subsites (colon vs. rectum) we observed evidence that the *PARP* Val762Ala SNP modified the association between marine n-3 PUFA and

Table 1. Demographics and descriptive statistics of cases and controls

	Controls	Cases		
	(n = 1,176) n (%)	colon (n = 180) n (%)	rectum (n = 130) n (%)	all cases (n = 310) n (%)
Mean age at interview (SD)	56.5 ± 8.1	61.6 ± 7.4	60.9 ± 7.6	61.3 ± 7.6
<i>Dialect group</i>				
Cantonese	571 (49)	83 (46)	56 (43)	139 (45)
Hokkien	605 (51)	97 (54)	74 (57)	171 (55)
<i>Gender</i>				
Female	667 (57)	87 (48)	46 (35)	133 (43)
Male	509 (43)	93 (52)	84 (65)	177 (57)
<i>Level of education</i>				
No formal education	317 (27)	61 (34)	33 (26)	94 (30)
Primary school	505 (43)	84 (47)	68 (52)	152 (49)
Secondary or higher education	354 (30)	35 (19)	29 (22)	64 (21)
<i>Family history of colorectal cancer</i>				
No	1,149 (98)	172 (96)	125 (96)	297 (96)
Yes	27 (2)	8 (4)	5 (4)	13 (4)
<i>Diabetes</i>				
No	1,067 (91)	149 (83)	112 (86)	261 (84)
Yes	109 (9)	31 (17)	18 (14)	49 (16)
<i>Weekly physical activity</i>				
No	768 (65)	120 (67)	88 (68)	208 (67)
Yes	408 (35)	60 (33)	42 (32)	103 (33)
<i>Smoking index</i>				
Never smokers	850 (72)	118 (65)	66 (50)	184 (60)
Light smokers	294 (25)	48 (27)	49 (38)	97 (31)
Heavy smokers	32 (3)	14 (8)	15 (12)	29 (9)
<i>Alcohol intake</i>				
Never drinkers	965 (82)	149 (83)	94 (72)	234 (78)
<7 drinks/week	164 (14)	17 (9)	25 (19)	42 (14)
7+ drinks/week	47 (4)	14 (8)	11 (9)	25 (8)
<i>Total n-6 PUFA intake¹</i>				
Low	560 (48)	78 (43)	65 (50)	143 (43)
High	616 (52)	102 (57)	65 (50)	167 (54)
<i>Total n-3 PUFA intake¹</i>				
Low	576 (49)	92 (51)	62 (48)	154 (50)
High	600 (51)	88 (49)	68 (52)	156 (50)
<i>Total marine n-3 PUFA intake¹</i>				
Low	580 (49)	87 (48)	56 (43)	143 (46)
High	596 (78)	93 (52)	74 (57)	167 (54)

¹ Fat intake values are expressed as nutrient densities and dichotomized into low and high using the median among controls.

CRC risk among rectal cancer cases but not colon cancer cases (heterogeneity test $p = 0.003$) (table 2). This heterogeneity by tumor subsite remained statistically significant after correcting for multiple testing using 5% FDR. Our results suggest a positive association between high intake of marine n-3 PUFA and rectal cancer risk only among carriers of one or two copies of the Ala allele (OR = 1.7, 95% CI = 1.1–2.7) (table 2). We did not find evidence of heterogeneity by tumor subsite of the marine n-3 PUFA interaction ORs for any of the other SNPs. Furthermore, we did not find statistically significant evidence that the interaction between each of the 7 SNPs and marine n-3 PUFA for CRC differed by gender, stage or by n-6 PUFA intake levels.

We also observed statistically significant heterogeneity by subsite for the interaction between total n-3 PUFA and *XPD* Lys751Gln (colon vs. rectum heterogeneity $p = 0.03$); however, this interaction was compatible with chance when considering a 5% FDR (data not shown). Our data suggested an n-3 PUFA × *XPD* interaction for rectal but not colon cancer, that indicated that among carriers of two Gln alleles there was a positive association between high intake of n-3 PUFA and rectal cancer (OR = 2.5, 95% CI = 0.9–7.0), which was absent among carriers of two Lys alleles. However, the interaction p value within rectal cases did not reach statistical significance ($p = 0.069$). We observed no evidence of heterogeneity by tumor subsite for the interaction between any of the 7 SNPs and total n-6 PUFAs.

Discussion

Recent prospective analyses within the Singapore Chinese Health Study have shown that diets high in marine n-3 PUFA were positively associated with CRC risk [3]. Using a subset of this prospective cohort, we now report that the marine n-3 PUFA association with rectal cancer is confined to those who carry the *PARP* codon 762 Ala allele.

The *PARP* protein plays an important role in maintaining genomic stability, apoptosis, and regulating transcription [23]. In addition to the role of *PARP* in the BER pathway detecting single-strand breaks, *PARP* is also a co-activator of the β -catenin-TCF-4 complex, which aberrantly regulates growth and differentiation of intestinal epithelial cells [24]. *PARP* overexpression has been reported in colorectal tumors [24, 25]. The *PARP* Val762Ala SNP has been reported to impact *PARP* protein function, with the Ala allele having reduced enzy-

Table 2. PARP codon 762 × marine n-3 PUFAs × tumor subsite interaction

	Colon						Rectum						
	Val/Val			Val/Ala or Ala/Ala			Val/Val			Val/Ala or Ala/Ala			
	Co/Ca	OR ¹	95% CI	Co/Ca	OR ¹	95% CI	Co/Ca	OR ¹	95% CI	Co/Ca	OR ¹	95% CI	
Marine n-3 PUFA ²													
Low intake	172/19	1 ^{ref}		406/68	1.5	0.8-2.7	172/21	1 ^{ref}		406/35	0.6	0.3-1.1	
High intake	209/35	1.5	0.8-2.7	386/57	1.3	0.7-2.4	209/18	0.6	0.3-1.2	386/54	1.1	0.6-1.9	
Genotype-specific OR ³		1.5	0.8-2.7		0.9	0.6-1.3		0.6	0.3-1.2		1.7	1.1-2.8	
Marine n-3-PUFA × PARP Val762Ala interaction for colon p = 0.164 ⁴							Marine n-3 PUFA × PARP Val762Ala for rectum p = 0.016 ⁵						
Marine n-3-PUFA × PARP Val762Ala × tumor subsite interaction p = 0.003 ⁶													

^{ref} = Reference group.

¹ ORs obtained from polytomous logistic regression models adjusted for age at interview (continuous), year at interview (continuous), sex (dichotomous), dialect (Cantonese and Hokkien), level of education (tertiles), body mass index (categorical), family history of colorectal cancer (present, absent), total alcohol drinks/week (none, <7, >7), smoking index (never, light, heavy), physical activity (low, high), total caloric intake.

² Fat intake values are expressed as nutrient densities and dichotomized into low and high using the median among controls.

³ Genotype-specific OR: OR for high versus low PUFA intake within each genotypic subgroup.

⁴ p value for marine n-3 PUFA × genotype interaction test when only considering colon cases.

⁵ p value for marine n-3 PUFA × genotype interaction test among when only considering rectal cases.

⁶ p value for a test of whether the interaction observed among colon cases is different than the one observed among rectal cases.

matic activity [26]. To date, three studies have reported positive associations between the *PARP* Val762Ala SNP and various cancer types [27–29]. Recently, we reported a positive association between *PARP* Lys940Arg and CRC risk in Singapore (OR = 1.8, 95% CI = 1.1–3.1, p = 0.029) [18]. The data we report here suggests that among carriers of a *PARP* protein with reduced enzymatic activity, diets high in marine n-3 PUFAs might be harmful for the rectum. The fact that we do not observe a similar finding for total n-3 PUFAs suggests that the association is driven by the long-chain EPA and DHA present mostly in fish.

Experimental evidence supports a protective role for diets high in marine n-3 PUFAs in colorectal carcinogenesis [6, 30]; however, epidemiological findings are mostly null [31–34] and only one study reported inverse associations for the marine n-3 PUFAs EPA and DHA [35]. Our results from the Singapore Chinese Health Study [3] support an adverse effect of marine n-3 PUFA intake for CRC. In agreement with this, one study reported non-statistically significant positive associations between increasing levels of EPA and DHA and CRC risk among women [34], and two other studies reported modest positive associations between EPA and rectal cancer risk [31, 33]. Experimental studies suggest a dual role for n-3 PUFA, which could exhibit beneficial effects early in car-

cino-genesis but harmful effects during the later stages of tumor progression and metastasis [36]. Experimental studies in breast cancer cells have reported opposing effects for n-3 PUFA depending on total dietary levels [37], suggesting that there might be an upper limit for the protective effects conferred by diets high in n-3 PUFA. For example, n-3 PUFA-induced lipid hydroperoxides are known to prevent the accumulation of mutations through their pro-apoptotic effects, and thus have anti-carcinogenic properties. However, at high levels of n-3 PUFA intake, these induced lipid hydroperoxides may also contribute to DNA damage, and this, perhaps in combination with reduced expression of antioxidant genes and decreased DNA repair proficiency, may shift the balance from apoptosis to the accumulation of mutations and pro-carcinogenic events.

The strengths of this study are its population-based design and the availability of prospective data obtained via in-person interviews and an FFQ created for and validated in our study population. Specifically for PUFAs, we have unpublished pilot data among 50 cohort subjects that demonstrate a tight correlation between self-reported total dietary PUFAs and the sum plasma level of five PUFAs. Correlation coefficients for our FFQ and 24-hour recalls for total fat and saturated fat have been previously

reported [17]. Concern for survival bias arises from the fact that only 52% of CRC diagnosed within the cohort by April 2002 donated biospecimens for genetic analyses. For all major demographic variables and selected nutritional and lifestyle variables, controls and cases who consented to donate biospecimens do not differ from those who refused (data not shown). However, the prevalence of advanced tumors was lower among cases who donated biospecimens compared to those who did not (46.8 vs. 62%). Although the prevalence of the genotypes we investigated did not differ across cancer stages, we cannot completely discard the potential for survival bias. Another limitation is the relatively modest number of cancer cases ($n = 310$) in this nested case-control study, in particular for subgroup analyses. We recognize that our statistical power to obtain precise estimates for the interactions we investigated is limited. Specifically, when considering rectal cancer cases only, among which we find a strong interaction between *PARP* and $n-3$ PUFA from fish, we had 62% statistical power to detect the observed interaction OR (IOR = 2.8).

Based on our previous report on the roles of *PARP* and marine $n-3$ PUFAs on CRC risk [3, 18], and the growing evidence on the role of the *PARP* protein in colorectal

carcinogenesis, our findings suggest that diets high in marine $n-3$ PUFAs might increase risk for rectal cancer among subjects who have less efficient *PARP* function. Given the dual role of *PARP* in the repair of oxidative damage and apoptosis signaling, our findings could suggest that among individuals with deficient *PARP* function the reported beneficial pro-apoptotic effects of long-chain fatty acids might be outweighed by the mutagenic effects induced by $n-3$ PUFA-derived lipid hydroperoxides. Given the known role of *PARP* in CRC, our findings should be replicated in larger cohorts and if validated followed up with experimental studies focused on testing the proposed mutagenic role of marine $n-3$ PUFAs in rectal cancer.

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