Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps

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Introduction

Results from epidemiologic and animal studies suggest that dietary fat intake may play an important role in the development of colorectal cancer (1–6). Polyunsaturated fatty acids (PUFAs) are of increasing interest due to their potential role in inflammatory processes (5,7). Three n-3 PUFAs are required from the diet: ω-3-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), although ω-3-linolenic acid is also converted to EPA and DHA inefficiently in the body (8). Major sources of EPA and DHA are fatty fish, such as salmon, tuna and herring; ω-3-linolenic acid is found in seeds and seed oils such as flaxseed and canola oils (4,5). The n-6 PUFAs, including arachidonic, linoleic and gamma-linolenic acids, are more widely found in grains, meats, vegetable oils and eggs (3).

The n-6 PUFAs arachidonic acid is a major precursor for the cyclooxygenase (COX) and lipoygenase (ALOX) pathways. These pathways and their downstream products, prostaglandins and leukotrienes, are involved in inflammation, and have been implicated in carcinogenesis (9–14). COX-1 and -2 are the main targets of non-steroidal anti-inflammatory drugs (NSAIDs), which prevent colorectal neoplasia (9,15–17).

N-3 and n-6 PUFAs are generally considered to have opposite effects on inflammatory processes (Figure 1)(18). EPA competes with arachidonic acid as a substrate for COX and ALOX metabolism, producing 3-series prostaglandins (such as prostaglandin E3, thromboxane A3 and prostaglandin I3) and 5-series leukotrienes (such as leukotriene B5) and inhibiting the formation of 2-series prostaglandins (such as prostaglandin E2, thromboxane A2 and prostaglandin I2) and 4-series leukotrienes (such as leukotriene B4). The 3-series prostaglandins and 5-series leukotrienes have reduced inflammatory potential compared with the 2-series prostaglandins and 4-series leukotrienes (5,7,19), thus possibly reducing the risk of colorectal neoplasia. In addition, n-3 and n-6 PUFAs compete for enzymes in the desaturation and elongation pathways that convert linoleic acid into arachidonic acid and ω-3-linolenic acid into DHA and EPA (19), providing another level at which n-3 PUFAs may suppress n-6 PUFA metabolism and potentially reduce carcinogenesis.

Epidemiologic studies of n-3 PUFAs in relation to colorectal cancer risk have been inconclusive (20–27). We hypothesized that this may be due to differential effects of n-3 PUFA intake among genetically defined subgroups, specifically that high intakes of n-3 PUFAs would be associated with lower risk of colorectal neoplasia only among those with genetic variants that produce higher levels of or more active prostaglandins and leukotrienes. Because NSAIDs and n-3 PUFAs inhibit the formation of 2-series prostaglandins and 4-series leukotrienes, and are thus associated with reduced inflammation, we expected that results for n-3 PUFAs would be similar to those observed previously for gene–drug interactions between polymorphisms in prostaglandin synthesis and NSAID use (28–30). To investigate this hypothesis, we have evaluated potential effect modification between genetic polymorphisms in these pathways and fish intake, a proxy for intake of EPA and DHA, in relation to risk of colorectal adenomas or hyperplastic polyps.

Methods

Study subjects

Recruitment for this case–control study has been described previously (31–34). Briefly, cases with adenomatous and/or hyperplastic polyps and polyp-free controls were recruited through a large, multi-clinic gastroenterology practice in metropolitan Minneapolis. All patients aged 30–74 years who were scheduled for colonoscopy from April 1991 to April 1994 were screened for eligibility and recruited prior to colonoscopy to blind study personnel to the diagnosis. Indications for colonoscopy have been published previously and included screening, diagnostic purposes, family history and others (34). This study was approved by the internal review board of the University of Minnesota and written informed consent was obtained.

Eligible study participants were 30- to 74-year-old English-speaking residents of the Twin Cities metropolitan area with no known genetic syndrome associated with increased risk of colon neoplasia and no individual history of cancer (except non-melanoma skin cancer), prior colorectal polyps or inflammatory bowel disease. Patients whose colonoscopy did not reach the cecum were ineligible; removed polyps were examined histologically using standard diagnostic criteria (35).

Cases were identified with a first diagnosis of adenomatous (N = 522) or hyperplastic polyp (N = 194) at the time of colonoscopy; controls were polyp-free at colonoscopy (N = 626). Information on diet, use of aspirin or other NSAIDs, anthropometry, demographics and medical information (including family history of cancer) was obtained by questionnaire. The participation rate for all colonoscopy participants was 68%.

Information about regular weekly fish intake over the year prior to polyp diagnosis or reference date was obtained using a food frequency questionnaire. Participants were asked four questions regarding the frequency with which they ate various types of fish, including canned tuna, fatty fish and white fish. The responses to these questions were combined to form a total fish intake index.
variable. This food frequency questionnaire was an adaptation of the Willett semi-quantitative food frequency questionnaire, which has been studied previously for validity and repeatability within the Nurses’ Health Study cohort (36), the Iowa Women’s Health Study cohort (37) and the Health Professionals Follow-up Study cohort (38). Regular aspirin and other NSAID use were ascertained as the average number of pills per week used and the duration of use. Study participants were provided with a list of 14 common aspirin brands and 24 common non-aspirin NSAIDs. Non-NSAIDs that should not be included in their responses (e.g. acetaminophen) were also listed.

Genotyping
Genotyping for the polymorphisms evaluated in this study has been described previously (28–30,39). Briefly, genomic DNA was extracted from peripheral white blood cells using the Puregene kit (Genta Systems, Minneapolis, MN). All genotyping was performed in the Core Laboratory of the Public Health Sciences Division of the Fred Hutchinson Cancer Research Center. COX-1 P17L, L15-L16del and L237M polymorphisms were genotyped by sequencing (29) and the ALOX5 
\[ \frac{1}{2} \] # 1700G \ 1700C \ \text{polymorphisms were genotyped using GeneScan (Applied Biosystems, Foster City, CA)} (30).

Statistical data analysis
Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) comparing cases (with adenomatous or hyperplastic polyps) to controls. Genotype, sex, race, body mass index, physical activity, intake of fiber, alcohol and kilocalories, hormone replacement therapy and smoking in pack-years) were included in the logistic regression models. All statistical tests were two-sided and p-values were considered significant at \( p \leq 0.05 \). Comparisons of ORs across genotypes were performed using the likelihood ratio test. For interactions, a multiplicative interaction term was included in the analysis model.

Results
Characteristics of the study subjects have been described previously (31–33). Briefly, adenoma cases were older than hyperplastic polyp cases and were more likely to be male (Table I). Regular aspirin or other NSAID use was somewhat more common among controls than among either adenoma or hyperplastic polyp cases [aspirin: OR 0.63, 95% CI 0.44–0.90; other NSAIDs: OR 0.50, 95% CI 0.31–0.82 (33)].

Fish intake (number of servings per week) was categorized into approximate tertiles. We investigated the association between fish intake and risk of colorectal adenomatous and hyperplastic polyps and further examined whether the association differed by polymorphisms in COX-1, COX-2, ALOX5 and PGIS. The main associations with fish intake are shown in Table II. Risk of colorectal adenomas or hyperplastic polyps did not differ by fish intake, although the results for hyperplastic polyps suggest that increasing fish intake may be associated with decreased risk (1–2 times per week versus <1 time per week: OR 0.91, 95% CI 0.58–1.41; >2 times per week versus <1 time per week: OR 0.70, 95% CI 0.41–1.20).

We determined whether the associations with fish intake differed by COX-1, COX-2, ALOX5 or PGIS genotype. Single-nucleotide polymorphisms in promoter and coding regions with probable or established functional impact were included in this study: COX-1 R8W, L15-L16del, P17L and L237M; COX-2 –765G>C and ALOX5 –1700G>A polymorphisms were genotyped by 5’ exonuclease (Taqman) assays (29) and the ALOX5 and PGIS repeat polymorphisms were genotyped using GeneScan (Applied Biosystems, Foster City, CA) (30).

Table I. Characteristics of the study population

<table>
<thead>
<tr>
<th>Location of largest adenoma</th>
<th>Controls, ( N = 626, \ N = 522, \ N = 194 )</th>
<th>Adenomatous polyps, ( n % )</th>
<th>Hyperplastic polyps, ( n % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>109 (21)</td>
<td>329 (63)</td>
<td>84 (16)</td>
</tr>
<tr>
<td>Distal</td>
<td>194 (37)</td>
<td>62 (12)</td>
<td>111 (57)</td>
</tr>
<tr>
<td>Rectal</td>
<td>243 (39)</td>
<td>328 (62)</td>
<td>58 (30)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>82 (13)</td>
<td>23 (4)</td>
<td>21 (11)</td>
</tr>
<tr>
<td>40–59</td>
<td>162 (26)</td>
<td>80 (15)</td>
<td>39 (20)</td>
</tr>
<tr>
<td>50–59</td>
<td>197 (32)</td>
<td>166 (32)</td>
<td>74 (38)</td>
</tr>
<tr>
<td>60–69</td>
<td>146 (23)</td>
<td>194 (37)</td>
<td>48 (25)</td>
</tr>
<tr>
<td>70+</td>
<td>39 (6)</td>
<td>63 (12)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>243 (39)</td>
<td>328 (62)</td>
<td>111 (57)</td>
</tr>
<tr>
<td>Regular use of aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>200 (32)</td>
<td>152 (29)</td>
<td>58 (30)</td>
</tr>
<tr>
<td>Regular use of non-aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>124 (20)</td>
<td>61 (12)</td>
<td>32 (17)</td>
</tr>
<tr>
<td>Regular use of aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>193 (37)</td>
<td>79 (41)</td>
<td>278 (45)</td>
</tr>
</tbody>
</table>

*Percentages may not add to 100% due to rounding and missing values.

In a previous study of COX-1 polymorphisms in relation to risk of colorectal polyps, we reported an analogous interaction between the P17L polymorphism and current, regular aspirin or other NSAID use: among those who were wild type, regular NSAID use was associated with a decreased risk, whereas among those with at least one variant allele, low fish intake was associated with low risk and increased with higher fish intake (\( p \)-interaction = 0.08). No other statistically significant interactions between fish intake and genotypes were observed. However, the increase in adenoma risk with fewer PGIS repeats appeared strongest among those who consumed fish more than twice per week (\( p \)-trend = 0.02).

In a previous study of COX-1 polymorphisms in relation to risk of colorectal polyps, we reported an analogous interaction between the P17L polymorphism and current, regular aspirin or other NSAID use: among those who were wild type, regular NSAID use was associated with a decreased risk, whereas among those with at least one variant allele, no risk reduction was observed for NSAID users (28). To further explore the risks associated with fish intake, NSAID use and genotype, we combined fish intake and regular NSAID use into one category (low risk), low fish intake and no aspirin/NSAID use in a second category (high risk) and all other combinations in a third category (intermediate) and assessed the multiplicative interaction with P17L genotype.

Low-risk NSAID/fish intake was associated with a modestly reduced risk of adenomas compared with those in the high-risk category.
Fish intake, prostaglandin synthesis polymorphisms and polyp risk

Table II. Risk of adenomatous and hyperplastic polyps associated with weekly fish intake

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fish intake</th>
<th>Controls, (N)</th>
<th>Adenomas, Cases (N)</th>
<th>OR (95% CI)</th>
<th>Hyperplastic polyps, Cases (N)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;1 time per week</td>
<td>134</td>
<td>114</td>
<td>1.00 (ref.)</td>
<td>47</td>
<td>1.00 (ref.)</td>
</tr>
<tr>
<td></td>
<td>1–2 times per week</td>
<td>313</td>
<td>262</td>
<td>1.04 (0.75–1.46)</td>
<td>100</td>
<td>0.91 (0.58–1.41)</td>
</tr>
<tr>
<td></td>
<td>&gt;2 times per week</td>
<td>158</td>
<td>142</td>
<td>1.01 (0.68–1.50)</td>
<td>43</td>
<td>0.70 (0.41–1.20)</td>
</tr>
</tbody>
</table>

*Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use (females), caloric intake, BMI and fiber intake; ref., reference.

Table III. Association between fish intake and risk of adenomatous polyps, stratified by genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fish intake</th>
<th>Controls/controls OR (95% CI)</th>
<th>Adenomas/controls OR (95% CI)</th>
<th>Hyperplastic polyps/controls OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;1 time per week</td>
<td>99/107</td>
<td>1.00 (ref.)</td>
<td>224/270</td>
</tr>
<tr>
<td></td>
<td>1–2 times per week</td>
<td>14/27</td>
<td>0.41 (0.19–0.87)</td>
<td>35/41</td>
</tr>
<tr>
<td></td>
<td>&gt;2 times per week</td>
<td>76/115</td>
<td>1.00 (ref.)</td>
<td>171/272</td>
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<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R8W</td>
<td></td>
<td>77/129</td>
<td>0.91 (0.38–2.2)</td>
<td>28/39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63/94</td>
<td>1.00 (ref.)</td>
<td>124/202</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
<td>–765G&gt;C</td>
<td>62/89</td>
<td>1.22 (0.61–2.41)</td>
<td>61/92</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>57/86</td>
<td>1.00 (ref.)</td>
<td>124/202</td>
</tr>
<tr>
<td></td>
<td>GC or CC</td>
<td>27/35</td>
<td>1.22 (0.61–2.41)</td>
<td>61/92</td>
</tr>
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<td></td>
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</tbody>
</table>

*Multivariate adjustment for age, sex, smoking (pack-years), alcohol, hormone use (females), caloric intake, BMI and fiber intake; ref., reference.

Discussion

The present results indicate that genetic variability in the prostaglandin pathway may alter the anti-inflammatory effects of marine fatty acid intake. We hypothesized that high fish intake would be associated with lower risk of colorectal neoplasia predominantly among those with genetic variants that probably influence prostaglandin production. We observed no association between fish intake and risk of colorectal adenomas or hyperplastic polyps. However, risk of adenomas appeared to differ by COX-1 P17L genotype: among those with the PP genotype, higher fish intake was associated with a modest decrease in risk. Among those with PL or LL genotype, higher fish intake was not associated with a decreased risk. The P17L variant has been associated with increased inhibition of platelet PGF2α production by aspirin (40) and with decreased COX-1 inhibition by COX-2-selective NSAIDs (41), suggesting that this polymorphism may modify NSAID pharmacology.

We have previously reported that the colorectal adenoma risk reduction associated with regular NSAID use differed by COX-1 P17L genotype (28): wild-type individuals benefited more from regular NSAID use than those with at least one variant allele. In the present study, we observed a similar, though statistically non-significant relationship with fish intake: fish intake was associated with lower risk among wild-type individuals and the association was stronger when both fish intake and NSAID use were considered together. This is consistent with chemopreventive NSAID use or n-3 PUFA intake being beneficial only among those with higher baseline prostaglandin production. However, the reported interaction requires confirmation in larger studies.

Although previous studies of n-3 PUFA intake in animal models suggest a protective role in colorectal carcinogenesis (4,42–44), results from human studies of both fish and n-3 PUFA intake have been mixed, with most (20–24), but not all (24–27), reporting no association.
PGIS interaction (i.e. lowest risk in the C0/C21 region were consistent with the previously observed NSAID a priori not previously observed gene–NSAID interactions, thus interactions of doses (15), which suggests that the observed COX-1 and COX-2 isoforms to varying degrees. Aspirin preferentially inhibits COX-1 at commonly used NSAID use interaction is biologically plausible. However, recent studies suggest that our understanding of NSAID targets and possible genetic effects is still incomplete. For example, Fries et al. (41) have recently reported that COX-1 polymorphisms affect the selectivity of COX-2 inhibitors. Taken together, these results underscore the need for a better understanding of the role of polymorphisms in prostaglandin synthesis in chemoprevention and pharmacogenetics.

Only the COX-1 P17L polymorphism was found to interact with fish intake in relation to risk of colorectal adenomas in this study. For most of the other polymorphisms investigated here, with the exception of COX-2 –765G>C and the PGIS promoter polymorphism, we did not previously observe gene–NSAID interactions, thus interactions between these polymorphisms and fish intake were a priori less probable (29,30). The risk estimates for the COX-2 –765G>C fish stratification were consistent with the previously observed NSAID interaction (i.e. lowest risk in the ‘variant genotype/high fish intake’ group), but the interaction did not reach statistical significance. For PGIS, the highest risk was seen in the group with <6/>6 repeats and high fish intake (OR 3.98, 95% CI 1.42–11.14). Again, this runs parallel to our previous results for the PGIS–NSAID interaction, but the stratified groups were too small to yield stable results. Fish intake was not independently associated with polyp risk in this population, which limited our statistical power.

Our study has several limitations. First, fish intake could not be separated by type; fish vary in their content of EPA and DHA, thus separation into types may have given a more precise estimate of n-3 PUFA consumption (19). Second, this study took place in a midwestern population where access to fatty fish may be limited and therefore the variation in fish intake in this population may be lower than that observed elsewhere. Third, fish intake does not capture all n-3 PUFA intake, given that α-linolenic acid, a major n-3 PUFA in seeds and plant oils, is a minor component of fish lipids (47). However, α-linolenic acid is inefficiently converted to DHA and EPA in humans and is thought to be a less important source of n-3 PUFAs than fish (5). The misclassification of total n-3 PUFA intake due to these measurement issues is likely to be non-differential and would have resulted in an attenuation of the association between fish and colorectal polyp risk. Fourth, because several of the polymorphisms examined in this study were rare (<5% minor allele frequency), we may not have had adequate power to detect associations that truly exist, especially among hyperplastic polyps, where the sample size was small.

This study has several strengths. First, controls were known to be polyp-free at the time of the study. Because colorectal polyps are highly prevalent in older adults, population-based controls would have included some undiagnosed polyp cases and, thus, misclassification of the disease endpoint and attenuation of any true associations. Second, we chose to examine polymorphisms in genes that were likely to interact with fish fatty acids. Because n-3 PUFAs compete with n-6 PUFAs at several points in the production of prostaglandins and leukotrienes, it is likely that genetic variation in these pathways alters the effects of n-3 fatty acid consumption on risk of colorectal neoplasia. Further, we chose to investigate polymorphisms in prostaglandin and leukotriene synthesis that are likely or proven to be functionally relevant (40,41,48–54).

In summary, we report here that fish intake may reduce risk of colorectal adenomas, but only among those who are wild type for COX-1 P17L. This result could provide insight into the potential role of n-3 PUFAs in carcinogenesis. However, our results should be replicated in larger studies of colorectal neoplasia and in studies with a more precise estimation of n-3 PUFA intake.

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Conflict of Interest Statement: None declared.

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