MARINE N-3 FATTY ACID INTAKE, GLUTATHIONE S-TRANSFERASES (GST) POLYMORPHISMS AND COLORECTAL CANCER RISK: THE SINGAPORE CHINESE HEALTH STUDY

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ABSTRACT

The role of marine n-3 polyunsaturated fatty acids (PUFAs) in colorectal carcinogenesis has been investigated in many epidemiological studies; however, the epidemiological evidence is inconclusive. A potential explanation is due to competing products of n-3 PUFAs metabolism. Anti-inflammatory eicosanoids, products of n-3 PUFAs metabolism through cyclooxygenase (COX) enzymes, could inhibit inflammatory responses, which have a protective effect against colorectal cancer. Alternatively, malondialdehyde (MDA) and 4-hydroxy-2-hexenal (4-HHE), lipid peroxidation products of marine n-3 PUFAs, could be mutagenic. It has been suggested that glutathione *S*-transferases (GSTs) are involved in removing lipid peroxidation products. Therefore, we investigated whether GST genotypes (i.e., *GSTT1, GSTM1*) modified the marine n-3 PUFAs-colorectal cancer association using a nested case-control study within the Singapore Chinese Health Study. With 469 incident colorectal cancer cases and 1,167 noncases, we observed the effect modification of combined *GSTT1* and *GSTM1* positive genotypes with marine n-3 PUFAs on colorectal cancer (p for interaction < 0.01), and with the ratio of marine n-3 to n-6 PUFAs on colorectal cancer (p for interaction = 0.01). An inverse association of marine n-3 PUFAs with colorectal cancer was observed among those with high activity GST genotypes (i.e., combined *GSTM1* and *GSTT1* positive genotype) [Odds ratio (OR) for Q4 vs. Q1 = 0.57,

95% CI = 0.32-1.01, *p* for trend <0.05]; however, a positive association was observed among those with one or more GST null genotypes [OR for Q4 vs. Q1 = 1.49, 95% CI = 1.00-2.23, *p* for trend = 0.01]. Among those with one or more GST null genotypes, a positive association was also shown for the ratio of marine n-3 to n-6 PUFAs and colorectal cancer [OR for Q4 vs. Q1 = 1.64, 95% CI = 1.09-2.37, *p* for trend < 0.01], although no statistically significant association was observed for high activity GST genotypes. Our results suggest the role of *GSTT1* and *GSTM1* in the association between marine n-3 PUFAs and colorectal cancer. This finding provided a point to consider *GST* genotypes in the marine n-3 PUFAs-colorectal cancer association in the population. It is important for further public health intervention program to consider this interaction while intervening on the population.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 EPIDEMIOLOGY OF COLORECTAL CANCER

Colorectal cancer is the third most common cancer in men (746,000 new cases per year) and the second in women (614,000 new cases per year) worldwide.[1] There is a 10-fold variation in incidence across regions worldwide, regardless of sex, where highest age-standardized rate per 100,000 is in Australia/New Zealand (44.8 in men and 32.2 in women), whereas the lowest rate is in Western Africa (4.5 in men and 3.8 in women).[1] Approximately 55% of cases occur in more developed regions.[1] The highest incidence rates are among western regions (i.e., Australia/New Zealand, Europe, and Northern America), whereas the lowest incidence rates are among populations in south and central Asia and Africa.[2] This difference indicates the association between the incidence of colorectal cancer and economic growth. Increasing incidence among populations as they transition from developing to developed economies, like Singapore since independence in 1965, also reflects the relationship between colorectal cancer and economic growth. In Singapore, for men, the incidence for colorectal cancer increased from 27.2 to 37.2 and 40.9 per 100,000 per year for the time period of 1975-1979, 1990-1994, and 2000-2004, respectively. Similar trends were seen in women, from 21.7 to 30.1 to 29.3 per 100,000 per year for the same three time periods.[3] In 2014, the rates have dropped slightly to 38.2 in men and 26.7 in women. However, colorectal cancer remains the most common cancer in men and second most common in women among Singapore Chinese, comprising 17.8% and 13.9% of total

cancers during the period 2010-2014 in men and women, respectively.[3] Given that economic growth correlates with urbanization and adoption of western lifestyle and behavior (e.g., diets high in red meat, low in vegetables and fiber, and less active lifestyles)[4,5], it is a special opportunity to investigate the association between different aspects of lifestyle (e.g., dietary factors) and colorectal cancer risk among countries such as Singapore during the transition period.

1.2 RISK AND PREVENTIVE FACTORS FOR COLORECTAL CANCER

1.2.1 Non-dietary factors

Family History. Family history is a well-established risk factor for colorectal cancer.[6] The estimates of relative risk (RR) for those who had a first-degree relative with colorectal cancer was 2.25 [95% confidence interval (CI) = 2.00-2.53] compared to those without a first degree relative with colorectal cancer, according to a 2001 meta-analysis of 27 studies (20 case-control and 7 cohort studies).[7] In addition, the RR of colorectal cancer was greater for those first-degree relatives of cases diagnosed at younger ages: 3.87 (95% CI = 2.40-6.22) among first-degree relatives of cases diagnosed before age 45, 2.25 (95% CI = 1.85-2.72) among first-degree relatives of cases diagnosed between ages 45 and 59, and 1.82 (95% CI = 1.47-2.25) among first-degree relatives of cases diagnosed after 59 year-old, compared with those without a first degree relative having colorectal cancer.[7] The increased risk among people with family history may reflect inherited genetic susceptibility in the occurrence of this cancer.

Cigarette Smoking. Results from a 2009 meta-analysis of 36 prospective studies (2 nested case-control and 34 prospective cohort studies), showed that compared with nonsmokers, current

smokers had a RR of 1.15 (95% CI = 1.00-1.32), and former smokers had a RR of colorectal cancer of 1.20 (95% CI = 1.04-1.38).[8] Additionally, among ever smokers, the dose of cigarette consumption (RR = 1.38 per 40 cigarettes/day), smoking duration (RR = 1.20 for a 40-year increase in duration), and earlier age of initiation (RR = 1.04 for every 10-year earlier age when start smoking) were associated with an increased risk of colorectal cancer.[8] The magnitude of association was greater for rectal cancer than colon cancer.[8]

Similar results have been observed from the Singapore Chinese Health Study. Compared with never smokers, number of cigarettes per day (RR for ≥13 cigarettes/day = 1.71, 95% CI = 1.28-2.28), age at starting to smoke (RR for <15 year-old = 2.34, 95% CI = 1.63-3.36), and number of years of smoking (RR for ≥40 years = 1.85, 95% CI = 1.36-2.52) were significantly associated with rectal cancer; however, no statistically significant association has been found for colon cancer.[9] A smoking exposure index was created where 'heavy' smoker was defined as those who started to smoke before 15 years of age and smoked 13 or more cigarettes per day; all other ever smokers were classified as 'light' smokers.[9] Number of years of smoking was not included in the index, because it was highly correlated with age at starting to smoke. Compared with never smokers, heavy and light smokers were at increased risk for rectal cancer with RRs of 2.64 (95% CI = 1.77-3.96) and 1.43 (95% CI = 1.10-1.87), respectively, whereas a statistically significant association was not found for colon cancer.[9]

Alcohol Consumption. A 2011 meta-analysis of 19 studies (12 case-control studies and 7 cohort studies) showed that moderate drinkers (2-3 drinks/day) and heavy drinkers (≥4 drinks/day), compared with non-/occasional drinkers, had RRs of 1.21 (95% CI = 1.13-1.28) and 1.52 (95% CI = 1.27-1.81) for colorectal cancer, respectively.[10] There was a dose-dependent relationship. Compared with nondrinkers, the RRs of 10, 25, 50, and 100 g/day of alcohol intake were 1.07

(95% CI = 1.04-1.10), 1.18 (95% CI = 1.12-1.25), 1.38 (95% CI = 1.28-1.50), and 1.82 (95% CI = 1.41-2.35), respectively. Among Singapore Chinese, heavy drinking was positively associated with colorectal cancer risk [RR for <7 drinks/week vs. nondrinkers = 0.96 (95% CI = 0.72-1.25); RR for ≥7 drinks/week vs. nondrinkers=1.84 (95% CI = 1.31-2.58), *p* for trend = 0.0004].[9]

Obesity. Based on a 2009 meta-analysis, every 5 kg/m^2 increase in BMI is associated with a 24% higher risk of colon cancer in men (RR = 1.24, 95% CI = 1.20-1.28), and a 9% higher risk of colon cancer in women (RR = 1.09, 95% CI = 1.04-1.14).[11] The magnitude of RR was weaker for rectal cancer, which was 1.09 (95% CI = 1.06-1.12) for men and 1.02 (95% CI = 0.99-1.04) for women.[11] This positive association could be explained by the relationship between obesity and insulin resistance.[12,13] Higher blood insulin levels, as a result of obesity-induced insulin resistance, may stimulate the growth of colorectal tumor cells.[14] Although obesity is associated with higher risk of colorectal cancer, there is some evidence suggesting that a low BMI is not associated with decreased risk. In the Singapore Chinese Health Study, the mean BMI is low (i.e., 23.1 kg/m^2) and only 10.4% are with BMI ≥27.5 kg/m^2. Compared with normal BMI (i.e., 21.5≤BMI<24.5), the HR for BMI <18.5 kg/m^2 was 1.03 (95% CI = 0.80-1.32) and the HR for BMI ≥27.5 kg/m^2 was 1.25 (95% CI = 1.01-1.55).[15] These results suggest that normal weight may be more preferable than underweight or overweight in terms of risk of developing colorectal cancer.[15]

Diabetes Mellitus. According to a meta-analysis of 17 cohort studies, compared with those without DM, the RR for those who with a history of DM was 1.28 (95% CI = 1.19-1.39).[16] Obesity may be a confounder of this association, because of its role in inducing the insulin resistance syndrome as stated above[12]; however, the association between DM and colorectal cancer remained statistically significant after adjusting for BMI in several prospective studies.[17-

[19] Among Singapore Chinese, a history of physician-diagnosed diabetes was associated with 50% increased risk for colorectal cancer among men (RR = 1.5, 95% CI = 1.2-2.1) and a 40% increased risk among women (RR = 1.4, 95% CI = 1.0-1.9), after adjusting for covariates including BMI.[20]

Treatments for diabetes are also associated with colorectal cancer risk. Insulin treatment may increase the risk of colorectal cancer among those who have been diagnosed with DM: among type 2 DM patients, long-term insulin treatment has found increasing the risk of colorectal cancer (OR = 1.21 with 95% CI = 1.03-1.42 for each incremental year of insulin therapy).[21] This relationship is not surprising, considering the growth promoting effect of insulin. Treatment with metformin may reduce the incidence of cancer, including colorectal cancer.[22-24] The interplay between DM and its treatment may need further investigation.

Physical activity. Generally, the increase in total physical activity is associated with a decreased risk of colorectal cancer.[25] Its effect on colorectal cancer could be related to its influence on insulin sensitivity and insulin level.[13] The intensity and duration of physical activity may have different effects on colorectal cancer risk. A meta-analysis of 19 cohort studies showed that, compared with sedentary activity, those who were highly active had a reduction in risk of colorectal cancer [RRs for highest vs. none for men for occupational and recreational activities were 0.79 (95% CI = 0.72-0.87) and 0.78 (95% CI = 0.68-0.91); RRs highest vs. none for women for occupational and recreational physical activities were 1.12 (95% CI = 0.85-1.47) and 0.71 (95% CI = 0.57-0.88)], but no statistically significant result has been shown for total physical activity.[26] According to another 2015 meta-analysis (10 prospective studies), a potential dose-response has been shown for the association between leisure time physical activity and colorectal cancer risk: compared with those who were inactive, people had 10, 20 and 40

metabolic equivalents of energy hours per week (MET-h/wk) has a reduction in colorectal cancer risk by 8% (95% CI=0.85-1.00), 15% (95% CI=0.79-0.92), and 14% (95% CI=0.80-0.94), respectively.[27] As people are very likely to change their physical activity patterns at different stages of life, one case-control study in Australia investigated if being physical activity over multiple life stages would be more beneficial than those who were always no/low active or only active in specific age periods. The results showed that people who were vigorously physically active throughout their lifetime had a lower risk for both of distal colon cancer and rectal cancer, compared with those who were always no/low active or only active in specific age periods; the association of vigorously physical activity and proximal colon cancer was not statistically significant.[28] Therefore, physical activity is beneficial, but the magnitude of this protective effect may vary by type (e.g., occupational or recreational physical activity) and how long people keep it as a habit through lifetime. Among Singapore Chinese, strenuous activity or vigorous work was associated with lower risk of colon cancer [RR for $\geq$ 1.5 hours/week vs. <1.5 hours/week strenuous physical activity and/or vigorous work = 0.61 (95% CI = 0.42-0.88)], but the association of rectal cancer was not statistically significant.[29]

Nonsteroidal Anti-inflammatory drugs (NSAIDs). The overall reduction in risk with NSAID use was suggested for both of colon and rectal cancer (colon: OR = 0.72, 95% CI = 0.62- 0.85; rectal: OR = 0.73, 95% CI = 0.61-0.88).[30] Regular use of aspirin was associated with a 27% decrease in colorectal cancer risk (RR = 0.73, 95% CI = 0.67-0.79) among observational studies.[31] According to a meta-analysis of four clinical trials, the hazard ratio of those who were assigned to aspirin versus those who were assigned to placebo was 0.76 (95% CI = 0.60-0.96) for colon cancer and 0.90 (95% CI = 0.64-1.30) for rectal cancer.[32] Nonaspirin NSAIDs (e.g., Ibuprofen) also had preventive effect against colorectal cancer (OR = 0.94, 95% CI = 0.90-

0.98).[33] The major mechanism of this preventive effect is ability of NSAIDs to inhibit the cyclooxygenase (COX) enzymes. COX enzymes are involved in the synthesis of prostaglandin (PG). The inhibition of COX enzymes would decrease the PG synthesis. The products of PG synthesis are involved in the progress of colorectal cancer.[34]

1.2.2 Dietary factors

Red meat and processed meat. In addition to the factors above, diet plays an important role in the development of colorectal cancer.[35] There is convincing evidence of the positive associations of increased red and processed meat consumption, and inverse association of increased dietary fiber with colorectal cancer. [36 36] In 2015, red meat was classified as "probably carcinogenic to humans" (Group 2A) and processed meat was classified as "carcinogenic to humans" (Group 1) by International Agency for Research on Cancer (IARC).[37] In a systematic review of prospective studies with results for meat intake and colorectal cancer risk, the RR per 100 g per day of red meat was 1.17 (95% CI=1.05-1.31), and the RR per 50 g per day of processed meat was 1.18 (95% CI=1.10-1.28).[38]

Dietary fiber. Dietary fiber intake was inversely associated with the risk for colorectal cancer. [36] Based on results from a meta-analysis of 25 prospective studies, the RR per 10 g per day intake of fiber was 0.90 (95% CI=0.86-0.94). The dietary fiber-colorectal cancer risk association varied by the food source of fiber. The RRs for each 10 g/day intake were 0.93 (95% CI = 0.82-1.05), 0.98 (95% CI = 0.91-1.06), 0.90 (95% CI = 0.86-0.94) and 0.62 (95% CI = 0.27-1.42) for fiber from fruit, vegetable, cereal, and legumes, respectively.[39] Fiber from legumes may be most strongly associated with decreased risk, although the RR is not statistically significant.

Fish intake. Fish intake has been suggested as a protective factor for colorectal cancer by World Cancer Research Fund and American Institute for Cancer Research.[36] One meta-analysis (20 prospective cohort studies) in 2014 demonstrated an inverse association for which fish intake decreased the colorectal cancer risk by 7% (RR for highest consumption vs. lowest consumption = 0.93, 95% CI = 0.87-0.99).[40] However, only 3 out of 20 studies showed a statistically significantly inverse association.[41-43] Kato et al. reported that fish and shellfish intake was inversely associated with colorectal cancer risk [RR for Q4 vs. Q1 = 0.49 (95% CI = 0.27-0.89)] among females in the U.S.[43] The inverse association between fish intake and colorectal cancer risk has also been reported by Hall et al. among U.S. men: compared to those who had fish less than 1 time/week, those who had fish 5 or more times/week had 37% lower risk [RR for ≥5 times/week vs. <1 time/week = 0.63 (95% CI = 0.42-0.95)].[41] Another study in 10 European countries showed the RR for those who had ≥80g/d fish was with 31% lower risk than those who had <10g/d fish.[42] None of other 17 cohort studies which were conducted across the U.S.[44-48], Europe[42,49-55], Australia[56], Japan[57,58], and China[59] showed a statistically significant result for the association between fish intake and colorectal cancer.

One possible reason for different results could be the association between fish intake and colorectal cancer varies by subsite. Based on a meta-analysis, the summary RR of colon cancer for highest vs. lowest fish intake is 0.95 (95% CI = 0.91-0.98), and the summary RR of rectal cancer is 0.85 (95% CI = 0.75-0.95).[40] However, colon and rectal cancer were not analyzed separately by most studies, and the prevalence of colon cancer was greater than rectal cancer. Another reason could be the different within-study variation in intake. Studies reporting statistically significant association had greater within-study variation. For instance, Hall et al. compared the risk of colorectal cancer for ≥5 times/week versus <1 time/week.[41] This variation

was greater than most studies using frequency.[44,50,53,56] The different dietary measurement tools used could be another reason.

1.3 DIETARY POLYUNSATURATED FATTY ACIDS (PUFAS) AND COLORECTAL CANCER

1.3.1 Dietary PUFAs

Long-chain polyunsaturated fatty acids (LC-PUFAs, fatty acids which contain 14-22 carbon atoms) could be classified into n-6 and n-3 PUFAs, according to the position of the first double bond from the methyl end group of fatty acid.[60] Linoleic acid (LA), an n-6 PUFA, and alpha-linolenic acid (ALA), an n-3 PUFA, are essential fatty acids that must be obtained from the diet.[61] LA and ALA are mainly found in vegetable oil and nuts.[62]

Blood concentrations of fatty acids reflect dietary intake and endogenous synthesis.[63] From LA and ALA, humans can synthesize other LC-PUFAs. Longer chain n-6 PUFAs, such as dihomo-γ-linolenic acid (DGLA) and arachidonic acid (AA), are synthesized from LA (Figure 1). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized from ALA through desaturation and elongation. A competitive relationship exists between the synthesis of longer n-3 and n-6 PUFAs from ALA and LA, because the same elongase and desaturase enzymes are involved in these processes.[64] However, the ability to elongate ALA to the longer chain EPA and DHA in human body is very limited, therefore EPA and DHA are mainly obtained through dietary sources.[65] EPA and DHA are mainly found in seafood, especially oily fish (e.g., salmon, tuna, mackerel, and herring).[62,66] Per 100g cooked fish or seafood would provide approximately 0.2g-2.0g EPA plus DHA.[62]

1.3.2 The association between dietary PUFAs and colorectal cancer

The relationship between dietary PUFAs and colorectal cancer risk differs by the specific PUFA structural classification (e.g., number and position of double bonds). The intake of n-6 PUFAs has been suggested to increase the risk of colorectal cancer, because AA could be converted by cyclooxygenase (COX) 1 and COX-2 enzymes into prostaglandins, of which Prostaglandin E_2 and Prostaglandin I_2 have been linked to colorectal cancer (Figure 2).[67] However, this association has not been found in epidemiological studies.[48,68-72] One study in the U.S. reported that the RR of colorectal cancer for n-6 PUFAs was 0.89 [RR for ≥12.0 g/d vs. <8.0 g/d = 0.89 (95% CI = 0.70-1.12)] for women, and 1.17 [RR for ≥14.0 g/d vs. <10.0 g/d = 1.17 (95% CI = 0.95-1.79)] for men.[68] Women's Health Study showed similar results: compared with the lowest quintile of intake (median = 3.8% total energy), highest quintile (median = 7.6% total energy) of n-6 PUFAs intake has a RR of 1.6 (95% CI = 0.98-2.60).[70] Among Chinese women, the association between total n-6 PUFAs intake and colorectal cancer was not statistically significant either [RR highest quintile vs. lowest quintile = 1.10 (95% CI = 0.57-2.12)].[69] Among Singapore Chinese, n-6 PUFAs were not statistically significantly associated with either of localized [RR for Q4 vs. Q1 = 0.92 (95% CI = 0.68-1.23)] and advanced [RR for Q4 vs. Q1 = 0.90 (95% CI = 0.70-1.59)] colorectal cancer.[71] Therefore, the association with n-6 PUFAs intake for colorectal cancer risk needs further investigation.

Dietary n-3 PUFAs are hypothesized to have a protective effect against colorectal cancer, due in part to their effect of inhibiting the expression of COX-2.[73] Another potential mechanism is through alteration in the cellular redox state and increasing oxidative stress.[74] The peroxidation process of PUFAs generates reactive oxygen species (ROS) such as the superoxide radical.[74] The elevation of intracellular ROS levels induced by n-3 PUFAs has been hypothesized

to induce cancer cell apoptosis.[75] Inconsistent results were found in epidemiological studies with respect to the relationship between the intake of total n-3 PUFAs and risk of developing colorectal cancer. Based on a systematic review, the RR for colorectal cancer incidence of the highest versus the lowest category was 0.91 (95% CI = 0.70-1.19) for total n-3 fatty acids intake (3 studies).[76]

The role of marine n-3 PUFAs (i.e., EPA and DHA) has been considered individually, given that the food sources of EPA and DHA are different from ALA (the most common n-3 PUFAs), and ALA is commonly used for energy. The association with marine n-3 PUFAs has been investigated by many studies across different countries (Table 1). Hall et al. reported a negative association between marine n-3 PUFAs and colorectal cancer risk [RR for Q4 vs. Q1 = 0.76 (95% CI = 0.59- 0.98), *p* for trend = 0.02] among men in the US.[41] A study in Japan found a negative association of marine n-3 PUFAs with colorectal cancer risk among women [RR for Q5 vs. Q1 = 0.60 (95% CI = 0.31-1.14), *p* for trend = 0.04].[72] In the Singapore Chinese Health Study, a positive association was reported between marine n-3 PUFAs intake and advanced colorectal cancer (Dukes C or D) [Hazard ratio (HR) for Q4 vs. Q1 = 1.33, 95%CI = 1.05-1.70, *p* for trend = 0.01].[71] Similar results were reported from a prospective study among a U.S. population (HR Tertile 3 vs. Tertile 1 = 1.44, 95% CI = 1.02-2.04, *p* for trend = 0.04).[77] According to a systematic review, the RR for colorectal cancer incidence of the highest versus the lowest category was 0.92 (95% CI = 0.74-1.13) for DHA, and 0.84 (95% CI = 0.69-1.01) for EPA.[78] Overall, the association of marine n-3 PUFAs and colorectal cancer is not supported by most epidemiologic studies. Null results have been reported by different countries and regions, including people had low (e.g., US[44,48,68,79,80]) and high (e.g., Sweden[81]) fish intake. There are some possible reasons. First, marine n-3 PUFAs has been suggested by animal data playing a

role in inhibiting colorectal tumor initiation.[82] Among Singapore Chinese, the positive association between marine n-3 PUFAs and colorectal cancer was only observed among people who had a follow-up period ≤ 5 years [RR for Q4 vs. Q1 = 1.35 (95% CI = 1.01-1.80)].[71] Among people who had a follow up period > 10 years, there is a potentially inverse association [RR for Q4 vs. Q1 = 0.77 (95% CI = 0.47-1.26)].[71] It is also possible that the inconsistent results for dietary marine n-3 PUFAs and colorectal cancer, despite convincing evidence from biological experiments, reflect that it is necessary to take another important factor, such as genetic susceptibility, into consideration.

1.3.3 The mechanistic rationale for a relationship between dietary PUFAs and colorectal cancer development

The endogenous metabolism of LC-PUFAs involves multiple pathways.[73] COX-1 and COX-2 enzymes are involved in the final stage of LC-PUFA metabolism into prostaglandins and leukotrienes.[73] The products of AA through the COX pathway are pro-inflammatory eicosanoids, while the products of EPA/DHA are eicosanoids with anti-inflammatory properties.[73] PGE_2 and PGI_2, the products of AA via COX pathway, have been involved in colorectal cancer carcinogenesis.[83] EPA has an inhibitory effect on the COX enzyme, thus reducing the synthesis of pro-inflammatory AA products.[73]

In a parallel, competing lipid peroxidation pathway, LC-PUFAs are excellent targets for oxidation. Both n-3 and n-6 PUFAs are susceptible to oxidation by reactive oxygenated species.[73] With more doubled bonds, marine n-3 PUFAs, especially DHA, are more susceptible to oxidation than AA.[84] With their difference in structure, the profiles of their lipid peroxidation products might be different. The lipid peroxidation products of AA include malondialedehyde

(MDA) and 4-hydroxy-2-nonenal (4-HNE), while the lipid peroxidation products of EPA and DHA are MDA and 4-hydroxy-2-hexenal (HHE).[85]

The lipid peroxidation products such as MDA have high capability of reaction with proteins and DNA that leads to the formation of adducts.[86] Given that DNA is the target molecule for carcinogens, DNA adducts may contribute to the etiology of human cancers.[87] The role of 4-HNE in inducing significant DNA damage has been demonstrated by both of *in vivo*[88] and *in vitro*[89] studies. 4-HNE has been suggested as the most toxic product of lipid peroxidation, and its carcinogenic effect in normal cells has also been demonstrated.[73,86] The function of 4-HHE has been less investigated, but it might have mutagenicity.[88] In general, higher levels of these chemicals may increase the colorectal cancer risk.

The inconsistent results in epidemiologic studies investigating the relationship between dietary n-3 and n-6 PUFAs and colorectal cancer risk may be influenced by the unmeasured heterogeneity of genetic susceptibility of the lipid peroxidation pathway. For example, in Singapore Chinese, single nucleotide polymorphisms (SNPs) in DNA repair genes *PARP* Val762Ala modified the effect of marine n-3 PUFAs on colorectal cancer risk among rectal cancer cases (p-interaction=0.016) but not colon cancer cases.[90] This study suggested that high intake of marine n-3 PUFAs might increase risk for rectal cancer among subjects with less efficient PARP function.[90]

1.3.4 Glutathione S-transferase (GST) enzyme

The GST enzymes can be divided into five main classes: alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT) and zeta (GSTZ), which belong to a super family of phase II detoxification enzymes. They have been linked to the metabolism of a wide range of chemicals,

some of which are associated with higher risk of cancer.[91,92] Additionally, they are polymorphic enzymes with interindividual variations in enzymatic level and activity.[93] The genes coding for the enzymes GSTM, GSTT and GSTP are polymorphic, and the genotypes of *GSTM1 null* and *GSTT1 null* result in the absence of GSTM1 and GSTT1 expression, and *GSTP1 AB/BB* leads to a decreased enzymatic activity of protein.[91,94,95] These genotypes lead to a low activity in GST enzymes. Considering the role of GST enzymes in metabolism, the association of *GST* genotypes with colorectal cancer has been investigated by many studies in Asian populations: nonsignificant associations have been found by most studies[96-99], but an increased colorectal cancer risk for *GSTT1 null vs. GSTT1 positive* has also been reported.[100]

The role of *GST* genotypes in modifying the association of some factors and colorectal cancer has been suggested. For example, it has been suggested that isothiocyanates (ITCs) have an interaction with GST genotypes. The effect of ITCs, a chemical in cruciferous vegetable that may have chemopreventive activity against cancer, depends on an individual GST genotype, as found by A.Seow et al. in Singapore Chinese. An inverse association between ITCs and colorectal cancer has been shown among those who possessed low activity GST genotypes [OR high (>median) versus low (≤median) intake of ITCs = 0.43, 95% CI = 0.20-0.96] (low activity GST genotypes have been defined as *GSTM1* and *GSTT1 null*), but absent among people with high activity GST genotypes (OR high vs. low intake of ITCs = 0.92, 95% CI = 0.64-1.32).[99] In addition, GST genotypes have been reported interacting with cigarette smoking in terms of colorectal cancer risk.[92] Subjects who possessed more low activity GST genotypes would be more susceptible to smoking-induced colorectal carcinogenesis.[92] The ORs for heavy smokers vs. never smokers were 5.43 (95% CI = 2.22-13.23), 2.43 (95% CI = 1.01-5.86), and 1.34 (95%

CI = 0.38-4.76) for those who possessed 2+, 1 and 0 low activity GST genotypes (*GSTM1 null, GSTT1 null, GSTP1 AB/BB*).[92]

1.4 A RATIONALE FOR GST GENOTYPE MODIFICATION ON THE RELATIONSHIP BETWEEN DIETARY PUFAS AND RISK OF COLORECTAL CANCER

The above evidence suggests a gene-environment interaction of *GST*s on the association between dietary PUFAs and colorectal cancer, given that harmful lipid peroxidation product, especially 4-HNE, could undergo detoxification through the process of glutathione-associated metabolism by GST enzymes.[86,101,102]

One related study which was a nested-case control study conducted in the Netherlands reported that the presence of *GSTM1* gene may modify the association of fish consumption and risk for colorectal cancer.[50] According to their result, among those with *GSTM1 positive*, the OR of colorectal cancer for fish intake >4 times per month versus 0-1 times was 0.5 (95% CI = 0.2-1.1). The OR for those who had *GSTM1* null was 0.9 (95% CI = 0.4-1.7). Although not statistically significant, there was a trend that the inverse association of fish intake with colorectal cancer was shown among those who possessed positive *GSTM1* genotype, but not among those with *GSTM1 null*.[50]

As marine n-3 PUFAs have been considered as the main contributor to the effect of fish intake on colorectal cancer, a reasonable hypothesis is that, among individuals with high activity GST genotypes (i.e., *GSTT1 positive, GSTM1 positive, GSTP1 AA*), the inverse association between marine n-3 PUFAs and colorectal cancer risk would be stronger than among those who possessed low activity GST genotypes (i.e., *GSTT1 null, GSTM1 null, GSTP1 AB/BB*). The

association of n-6 PUFAs with colorectal cancer could also be different for those who possessed high activity GST genotypes from those who possessed low activity GST genotypes: an inverse association would be shown among those who possessed high activity GST genotypes, as the harmful products of n-6 PUFAs could be eliminated by GST enzymes. No previous studies have investigated the possible interplay between marine n-3 PUFAs and *GSTs* (i.e., *GSTM1*, *GSTT1* and *GSTP1*) for colorectal cancer.

This potential modification of the marine n-3 PUFAs-colorectal cancer risk association by *GST* genotype warrants investigation. The proposed analyses in the Singapore Chinese Health Study will not only help explain the inconsistent results from previous studies investigating the association between marine n-3 PUFAs and colorectal cancer, but also provide insight into the influence of n-3 PUFA metabolism that could be translated into dietary prevention strategies. In summary, it is hypothesized that individuals with high GST enzymatic activity are protected against the harmful effects of the lipid peroxidation products. In other words, higher dietary intake of n-3 or n-6 PUFAs may be more beneficial or less harmful, respectively, among individuals with high activity than those with low enzymatic activity. In addition, the inconsistent results found in epidemiological studies regarding the association of marine n-3 PUFAs and colorectal cancer may be due to the unaccounted heterogeneity in the enzymatic activity of GSTs in the study population.

2.0 METHOD

2.1 STUDY POPULATION

A nested case-control study was designed within the Singapore Chinese Health Study. The details of the Singapore Chinese Health Study, a prospective population-based cohort, have been described in detail elsewhere.[103] Briefly, the Singapore Chinese Health Study enrolled 63,257 Chinese women and men between 45 and 74 years of age who were permanent residents or citizens of Singapore who reside in government-build housing estates (~86% of Singaporeans resided in such facilities at the time) from April 1993 to December 1998. The study participants were drawn from the two major dialect groups of Chinese in Singapore, Hokkien and Cantonese. At enrollment baseline interviews were conducted by trained interviewers with a structural questionnaire in subjects' homes. Information on demographics, use of tobacco, physical activity, medical history as well as diet was collected.

The selection process of noncases from the entire cohort was shown in Figure 3. Between April 1994 and December 1999, approximately 3% cohort participants were selected randomly, and were asked to give blood and single-void urine specimens. Biospecimen collection and storage procedure have been described in detail previously.[99,104] For those who refused to give blood sample, buccal cells were an option. Totally, 1,194 subjects gave their specimens. Of these subjects, 27 subjects who had a history of colorectal cancer at recruitment (n=5) or developed

first colorectal cancer (n=22) by December 31, 2005 were excluded from the noncases. The remaining 1,167 subjects were included as noncases in this study.

We identified incident colorectal cancer cases through the nationwide cancer registry[105]. As of December 31, 2005, 1,005 colorectal cancer cases had occurred among the cohort participants. Among the 480 cases that provided either blood or buccal samples the following were excluded from this study: those with adeno-/carcinoma *in situ* (n=4), carcinoid tumors (n=5) or borderline malignancy (n=2). Of the 469 colorectal cancer cases included in the analysis, 271 were located in the colon (C180-189) and 198 were in the rectal/rectosigmoid junction (C199 and C209). Stage at diagnosis was available for 92% of cases, where localized disease was defined as having either Dukes A (n=67) or B (n=142), and advanced disease was either Dukes C (n=141) or D (n=81).

2.2 EXPOSURE ASSESSMENT

2.2.1 Dietary assessment

Dietary information was obtained using a 165-item food frequency questionnaire (FFQ) that was developed for, and validated in the study population, as previously described.[106] The FFQ collected the average frequency and quantity of consuming each food item during last year. The FFQ included 14 seafood items commonly consumed by Chinese in Singapore, 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). Fatty acid composition was computed via linkage to the Singapore Food

Composition Database.[106] The FFQ was validated using 2 x 24-hour dietary recalls from a random sample of 858 cohort participants during April 1994-March 1997. For each dietary component, the correlation coefficient and linear regression slope between the food frequency and 24-hour recall intakes for subjects within each of the four gender-dialect subgroups were calculated according to the method described by Willett and associates.[107] The correlation coefficients between FFQ- and 24-h recall-based intakes for energy-adjusted total fat intake for Cantonese men, Cantonese women, Hokkien men, and Hokkien women were 0.44, 0.47, 0.41, and 0.34, respectively.[106]

2.2.2 *GSTM1*, *GSTT1*, and *GSTP1* genotype

Genomic DNA was isolated using a PureGene Blood Kit (Gentra Systems, Minneapolis, MN) or a QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). Genotyping for *GSTM1*, *GSTT1* and *GSTP1* was performed using the fluoro-genic 5'-nuclease assay (TaqMan Assay).[108] The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

The oligonucleotide primers for amplification of the polymorphic region of *GSTP1* were GC070 for (5'-CCTGGTGGACATGGTGAATG-3') and GC070 rev (5'-TGCTCACACCATAGTTGGTGTAGATGA-3'). In addition, the fluorogenic MGB oligonucleotide probes used to detect each of the alleles were GC070F (5'-TGCAAATACGTCTCCCT-3') labeled with 6-FAM and GC070V (5'-TGCAAATACATCTCCCT-3') labeled with VIC (Applied Biosystems). PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95ºC for 10 min followed by 50 cycles of 95ºC for 25 s and 60ºC for 1

min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System (Applied Biosystems) and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus. Any samples that were outside the parameters defined by the controls were identified as non-informative and were retested.

Genotyping of the *GSTT1* and *GSTM1* loci consisted of separate assays for *GSTT1*, *GSTM1* and the albumin (ALB) control gene. The oligonucleotide primers for amplification of the *GSTT1*, *GSTM1* and ALB genes were GC003for (5'-GTGCAAACACCTCCTGGAGAT-3') and GC003rev (5'-AGTCCTTGGCCTTCAGAATGA-3'), GC004for (5'-CTTGGAGGAACTCCCTGAAAAG-3') and GC004rev (5'-TGGAACCTCCATAACACGTGA-3'), GC005for (5'-CGATTTTCTTTTTAGGGCAGTAGC-3') and GC005rev (5'-TGGAAACTTCTGCAAACTCAGC-3'), respectively. Fluorescent oligonucleotide probes, for detection of PCR reaction products, were synthesized to contain the dye 6-FAM (BioSearch Technologies, Novato, CA). The probes for the *GSTT1*, *GSTM1* and ALB genes were GC003FAM (5'-ATGCTGCCCATCCCTGCCC-3'), GC004FAM (5'-AAGCGGCCATGGTTTGCAGG-3') and GC005FAM (5'-CGCCTGAGCCAGAGATTTCCCA-3'), respectively. PCR amplification using ~10 ng of genomic DNA was performed in an ABI 7900HT Sequence Detection System (Applied Biosystems) with an initial step of 95ºC for 10 min followed by 50 cycles of 95ºC for 25s and 60ºC for 1 min. The fluorescence profile of each well was measured in real-time during the PCR amplification and the results analyzed with Sequence Detection Software (Applied Biosystems). Any sample with a fluorescence signal that crossed a threshold of 0.2 ΔRn before cycle 40 was considered positive for the loci analyzed. Samples negative for both *GSTT1* and *GSTM1* must be

positive for ALB to be called; otherwise, the sample was designated non-informative and retested. All analyses were carried out by laboratory personnel who were blinded to the case-control status of the specimens.

2.3 STATISTICAL ANALYSIS

An unconditional logistic regression model was used to obtain odds ratio (ORs) and corresponding 95% confidence intervals (CIs) for associations of dietary PUFAs and *GST* genotypes with colorectal cancer. PUFA intake was adjusted for energy intake using the residual method.[107] Study participants were grouped into quartiles of PUFA intake based on the distribution among the entire cohort. *P* values for trend were determined using ordinal values of the quartiles (0, 1, 2, 3).

The following covariates were individually assessed as potential confounders: sex, age at interview (yr), interview year (1993-1995, 1996-1998), dialect group (Cantonese, Hokkien), BMI (<20, 20-23.9, 24-27.9, ≥28 kg^2/m)[15], education (no formal education, primary level, and secondary level or higher), family history of colorectal cancer (yes first degree relative, no), diabetes at baseline (yes, no), smoking (never, <13 cigarette/day or started smoking at age of 15 or older, ≥13 cigarette/day and started smoking at age younger than 15)[9], alcohol consumption (never, <7 drinks per week, ≥7 drinks per week)[9], and weekly vigorous work or strenuous sports (yes, no)[29]. For each covariate, associations were evaluated with marine n-3 PUFAs (quartile variable) using chi-square test (p-value<0.05), and with colorectal cancer using unadjusted logistic regression (p-value of any category of a covariate<0.05). Smoking (never, <13 cigarette/day or started smoking at age of 15 or older, ≥13 cigarette/day and started smoking at

age younger than 15) and alcohol consumption (never, <7 drinks per week, ≥7 drinks per week) were statistically significantly associated with colorectal cancer risk. Female gender and BMI (<20, 20-23.9, 24-27.9, ≥28 kg^2/m) had statistically significant associations with marine n-3 PUFAs intake. Additional covariates were included in the final models, because their relationship with colorectal cancer risk has been previously described in the Singapore cohort, or based on a review of the literature. Therefore, all covariates listed above were included in all logistic regression models.

Considering the potential overlap in GST enzyme function, two variables were created to represent the composite GST profiles to assess their combined effect modification on the association between marine n-3 PUFAs and colorectal cancer risk. *GSTM1* and *GSTT1* genotype variables were categorized as null or positive. *GSTP1* genotype variables were categorized as AB/BB for "low activity" and AA for "high activity".

To evaluate effect modification of PUFA-colorectal cancer associations by *GST* genotypes, stratified analyses were conducted by individual *GST* genotypes (i.e., *GSTT1*, *GSTM1*, and *GSTP1*), and by the number of null/low activity alleles. The potential GST-PUFAs interaction was tested on a multiplicative scale using models that included a GST-PUFA product term, the corresponding GST and PUFA variables, in addition to the covariates included in the main model. Gender was also evaluated as a potential effect modifier of the PUFA-colorectal cancer associations.

All colorectal cancer analyses were repeated by subsite for colon and rectum, separately, and by stage at diagnosis (i.e., localized vs. advanced). Statistical analysis was conducted with the SAS software version 9.3 (SAS Institute, Cary, NC). All *p* values were two-sided.

3.0 RESULTS

Compared with noncases, colorectal cancer cases were older (percentage of 45-54: 22.2% versus 46.3%) and more likely to be male (58.5% versus 43.3%). The distribution of BMI and education level was similar among cases and noncases (Table 2). More cases had a positive family history of colorectal cancer and diabetes at baseline than noncases. Heavy smokers had a 3-fold higher risk of colorectal cancer compared with never smokers, and alcohol consumption (7 or more drinks per week versus none) was also associated with a statistically significant increased risk.

There were no differences by increasing level of marine n-3 PUFA intake for age, education level, or smoking (Table 3). However, lower intake of marine n-3 PUFAs was seen for males, for lower BMI, for having diabetes versus no diabetes history, for drinkers versus nondrinkers, and for more frequent physical activity versus none. The dietary intake of most foods and nutrients increased with increasing marine n-3 PUFAs intake.

The prevalence of *GSTM1 positive, GSTT1 positive,* and *GSTP1 AA* genotypes among noncases was 55%, 59%, and 66%, respectively (Table 4). There was no association between *GSTT1* or *GSTM1* genotype and colorectal cancer risk. There was a trending positive association with *GSTP1* AA versus AB/BB genotype, but it did not reach statistical significance. Neither the number of combined null genotypes in *GSTT1* and *GSTM1*, nor the number of combined null/low activity genotypes in *GSTT1*, *GSTM1*, and *GSTP1* were associated with colorectal

cancer risk. No association was observed for colon or rectal cancer risk with individual or combined GST genotypes (Table S1).

For colorectal cancer risk, there was a positive association and statistically significant trend with increasing quartiles of the marine n-3 to n-6 PUFA ratio (Table 5). The association for highest versus lowest quartile strengthened and became statistically significant for rectal cancer risk. The ORs (95% CIs) of colorectal cancer for 2nd, 3rd, and 4th versus 1st quartile were 1.28 (0.92, 1.78), 1.58 (1.14, 2.20), 1.40 (1.00, 2.00) (p for trend = 0.03). By stage at diagnosis, the positive association of the marine n-3 to n-6 PUFA ratio with colorectal cancer was only shown for advanced-stage colorectal cancer (Table S2). No associations were observed for colorectal, colon or rectal cancer risk with total PUFAs, n-6 PUFAs, n-3 PUFAs, or marine n-3 PUFAs. There were also no associations for PUFAs-colorectal cancer risk in analyses among men (Table S3). Among women, a statistically significant trend with increasing the ratio of marine n-3 to n-6 PUFA and colorectal cancer was observed (p for trend < 0.05) (Table S4).

Stratified analyses by *GSTT1* and *GSTM1*genotypes for dietary PUFAs and colorectal cancer risk are shown in Table 6. A statistically significant positive association and trend was observed for marine n-3 to n-6 PUFA ratio among *GSTT1* null genotype, but not among *GSTT1* positive genotype (p for interaction = 0.02). Similarly, a statistically significant trend with marine n-3 to n-6 PUFA ratio was only observed among *GSTT1* positive genotype (p for trend = 0.04). A statistically significant trend with increasing marine n-3 PUFA and colorectal cancer risk was observed only among *GSTM1* null genotype (p for interaction = 0.02). *GSTT1*, and *GSTM1* genotypes did not modify relationships between the other PUFA intake and colorectal cancer risk. By subsite, *GSTM1* genotype modified the association of total PUFAs, n-6 PUFAs, and n-3 PUFAs with colon cancer, but not rectal cancer (Table S5). *GSTT1* modified the

association of the ratio of marine n-3 to n-6 PUFAs with rectal cancer. In terms of *GSTP1*, a positive association was observed for marine n-3 PUFAs and colorectal cancer risk among *GSTP1* AA (high activity) genotype (OR for highest versus lowest quartile = 1.39; 95% CI: 0.95, 2.04; *p* for trend = 0.04; *p* for interaction = 0.03) (Table S6). *GSTP1* genotype did not modify relationships between the other PUFA intake and colorectal cancer risk. By subsite, *GSTP1* genotype modified the association of n-3 PUFAs as well as marine n-3 PUFAs with colon cancer, but not rectal cancer.

To further explore the potential modification by GST genotypes on the marine n-3 PUFA and the marine n-3 to n-6 PUFA ratio associations with colorectal cancer, we combined *GSTT1* and *GSTM1* genotypes into two categories [i.e., 0 null genotypes and ≥ 1 genotype(s)] (Table 7). Inverse associations were observed with marine n-3 PUFA intake and colorectal cancer risk, depending on the combined number of *GSTT1* and *GSTM1* null genotypes. For marine n-3 PUFAs, a positive association was observed among those with one or more null GST genotypes, while a statistically significant inverse association was observed among those with zero null GST genotypes (*p* for interaction < 0.01). For the ratio of marine n-3 to n-6 PUFAs, a statistically positive association was observed among those with one or more null GST genotypes, and no association among those with zero null GST genotypes (*p* for interaction < 0.01). The relationships between marine n-3 PUFAs and marine n-3 to n-6 PUFA ratio with colorectal cancer risk by GST genotype were clearest for localized disease (Table 8).

4.0 DISCUSSIONS

Using data from the prospective Singapore Chinese Health Study, we conducted a nested case-control study to evaluate the potential modifying effects of GST genotypes on the dietary PUFAs-colorectal cancer risk association. The main findings include statistically significant interaction between marine n-3 PUFA intake and combined *GSTT1* and *GSTM1* genotype on colorectal cancer risk. A trend of increasing risk with marine n-3 PUFA intake was observed among those with one or more GST null genotypes, while a trend of decreasing risk was observed among those with GST positive genotypes. Similarly, a statistically significant association with marine n-3/n-6 PUFAs and colorectal cancer risk was only present among those with one or more GST null genotypes. Our findings support a modifying role for combined *GSTM1* and *GSTT1* genotypes on the relationship between marine n-3 PUFAs and colorectal cancer risk.

The association between marine n-3 PUFAs and colorectal cancer has been investigated in the U.S.[41,48,68,79,80], Finland[49], Singapore[71], and Japan[72]. Null findings have been reported for U.S.[48,68,79] as well as Finland[49] population. However, another two studies of U.S. population[41,80] and one study of Japanese[72] showed an inverse association between marine n-3 PUFAs and colorectal cancer. Our previous study among the Singapore Chinese found a positive association between marine n-3 PUFAs and colorectal cancer (OR Q4 vs. Q1 = 1.22, 95% CI = 1.02-1.45, *p* for trend = 0.03).[71]

The role of marine n-3 PUFAs in colorectal cancer development is inconclusive. The potential protective effect of marine n-3 PUFAs (i.e., EPA and DHA) on colorectal cancer development is based on the inhibiting effect of the marine n-3 PUFAs on COX-2 expression. COX-2 metabolizes the n-6 PUFA arachidonic acid into downstream pro-inflammatory eicosanoids with established relevance for colorectal carcinogenesis.[83] However, it is possible for marine n-3 PUFAs having an adverse effect. Marine n-3 PUFAs undergo oxidation and generate lipid peroxidation products, MDA and 4-HHE.[109] MDA has a high affinity for DNA and the resulting [86] MDA-DNA adducts may play a role in the development of colorectal cancer.[110] 4-HHE demonstrates carcinogenic potential *in vitro* by inducing DNA double-strand breaks.[88]

Glutathione-associated metabolism is one important pathway in human body to metabolize these lipid peroxidation products, and other chemicals related to colorectal carcinogenesis.[86,101,102] GST gene polymorphisms have been suggested modifying the cigarette smoking-colorectal cancer risk association[92], and the association with ITCs from cruciferous vegetable for colorectal cancer risk[99]. The effect modification of GST genotypes on the marine n-3 PUFAs-colorectal cancer risk association has been less investigated. One study in the Netherlands showed that *GSTM1* genotype modified the association with fish consumption for colorectal cancer. This nested case-control study of 102 cases and 537 controls was conducted among Dutch population aged 20-59 years at baseline. Dietary information was collected using a FFQ at baseline. Cases were identified via the Netherlands Cancer Registry during an 11-year follow-up. An inverse association between fish consumption and colorectal cancer was observed among *GSTM1* positive. Among *GSTM1* positive, those who having fish >4 times per month had a 50% lower risk of colorectal cancer [OR > 4 times vs. ≤ 1 time per month = 0.5 (95% CI = 0.2-1.1)] compared with people consuming fish ≤ 1 time per month. Our findings for an inverse

association between marine n-3 PUFAs and colorectal cancer among those with combined *GSTT1* and *GSTM1* positive genotypes were consistent with their results, since fish consumption is the source of marine n-3 PUFAs.

We observed that marine n-3 PUFAs and the ratio of marine n-3 to n-6 PUFAs intake was positively associated with colorectal cancer risk among those with one or more *GSTT1* and *GSTM1* null genotypes. This finding is consistent with our previous finding for a positive association with marine n-3 PUFAs for colorectal cancer among people possessing the *PARP* codon 762 Ala allele (i.e., carriers of a PARP protein with reduced enzymatic activity).[90] PARP is a key enzyme involved in repairing lipid hydroperoxide-induced oxidative DNA base modifications and single-strand breaks. Together, our findings in the present study for interaction between marine n-3 PUFAs and GST genotype, and our previous finding for interaction with marine n-3 PUFAs and *PARP* genotype suggest that higher intake of marine n-3 PUFAs increases risk for colorectal cancer among individuals with reduced ability to eliminate lipid peroxidation products or repairing lipid hydroperoxide-induced oxidative DNA damage. In summary, these findings support the role of lipid peroxidation in explaining the observed associations between marine n-3 PUFAs and colorectal cancer risk.

Strengths of our study include the use of an FFQ and a food composition database that were developed for the population, and the validation of FFQ showed a good correlation between FFQ- and 24-hr recall fat intake levels.[106] The Singapore nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases.[111] Therefore, cancer case ascertainment was complete. There are some limitations. First, the small number of cases in this nested case-control study while assessing modification of fatty acid-colon/rectal cancer association by GST genotypes resulted in rather imprecise estimates. Second,

recall bias may exist, since the dietary and covariate information relies on self-reported data. This nondifferential misclassification of exposure could result in bias toward or away from the null.

Our study shows that *GSTM1* and *GSTT1* genotypes modify the association between dietary marine n-3 PUFAs and colorectal cancer risk. It suggests that unmeasured genetic susceptibility could be a possible explanation of the overall null association between marine n-3 PUFAs and colorectal cancer among epidemiologic studies, and indirectly support the role of lipid peroxidation products in the colorectal carcinogenesis. However, more research is necessary to confirm the interaction between GST genotypes and PUFAs on colorectal cancer risk. First, given that people having different DNA repair ability determined by DNA repair gene, it needs further studied if the interaction between GST genotypes and PUFAs is different according to DNA repair gene, such as *PARP* genotype. Second, more biological research is needed to investigate the key role of lipid peroxidation products in the development of colorectal cancer, and the relationship between serum level of lipid peroxidation products and GST expression. In conclusion, our study shows that *GSTT1* and *GSTM1* genotypes modify the association between marine n-3 PUFAs and colorectal cancer. This effect modification could be due to the role of GST enzymes in eliminating lipid peroxidation products.

5.0 PUBLIC HEALTH SIGNIFICANCE

The effect of marine n-3 PUFAs on colorectal cancer has been investigated by many studies. Although the potential beneficial effect was found by *in vivo* and *in vitro* experiments, epidemiological studies did not support this association. Our results supported the role of GST genotypes in marine n-3 PUFAs-colorectal cancer association. This finding indirectly suggested the importance of lipid peroxidation process in the effect of marine n-3 PUFAs on colorectal cancer. This thesis provided evidence that GST genotypes contribute to understanding the association between marine n-3 PUFAs and colorectal cancer risk.

APPENDIX A: TABLES

Table 1. Characteristics and results of epidemiologic studies investigating the association between dietary n-3 PUFAs and colorectal cancer risk[a]

Reference	Study name	Population	Age at baseline	Country	Study design	Mean Follow-up period	Exposure[b]	Range	Exposure assessment	Outcome (n)	RR (95% CI)	*P* for trend
Bostick (1994)[44]	Iowa Women's Health Study	F	55-60	US	cohort	4 years	Total n-3 PUFAs (g/d)	>0.18 vs. <0.03	FFQ	Colon cancer (212)	0.70 (0.45, 1.09)	0.26
Terry (2001)[81]	The Swedish Mammography Screening Cohort	F	40-74	Sweden	cohort	9.6 years	Dietary EPA (g/d)	0.09 vs. 0.03	FFQ	Colorectal cancer (460)	0.96 (0.72, 1.28)	0.91
		F					Dietary DHA	0.18 vs. 0.08			0.90 (0.67, 1.20)	0.49
Murff (2009)[69]	Shanghai Women's Health Study	F	40-70	China	cohort	11 years	Total n-3 PUFA (g/d)	1.61 vs. 0.64	FFQ	Colorectal cancer (396)	1.41 (0.77, 2.57)	0.37
Pietinen (1999)[49]	Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study	Male smokers	50-69	Finland	cohort	8 years	Marine n-3 PUFAs (g/d)	0.7 vs. 0.2	FFQ	Colorectal cancer (185)	1.2 (0.8, 1.9)	0.84
Hall (2008)[41]	Physicians' Health Study	M	43-63	US	cohort	22 years	Marine n-3 PUFAs intake (g/d)	Q4 vs. Q1 (0.474-0.048)[c]	FFQ	Colorectal cancer (500)	**0.76 (0.59, 0.98)**	**0.02**
Butler (2009)[71]	Singapore Chinese Health Study	M & F	45-74	Singapore	cohort	9.8 years	Marine n-3 PUFAs (g/1000 kcal)	0.29 vs. 0.09	FFQ	Colorectal cancer (961)	**1.22 (1.02, 1.45)**	**0.03**
Daniel (2009)[48]	Cancer Prevenion Study-II Nutrition Cohort	M	68-70	US	cohort	6 years	Marine n-3 PUFAs intake (g/d)	≥0.25 vs. <0.10	FFQ	Colorectal cancer (452)	1.00 (0.75, 1.33)	0.9
		F						≥0.24 vs. <0.10		Colorectal cancer (417)	0.94 (0.72, 1.24)	0.83

Table 1 (continued)

Reference	Study name	Population	Age at baseline	Country	Study design	Mean Follow-up period	Exposure[b]	Range	Exposure assessment	Outcome (n)	RR (95% CI)	*P* for trend
Sasazuki (2010)[72]	Japan Public Health Center-Based prospective study	M	40-69	Japan	cohort	9.3 years	Marine n-3 PUFAs (g/d)	2.18 vs. 0.49	FFQ	colorectal cancer (521)	0.96 (0.57, 1.61)	0.82
		F						1.92 vs. 0.42		colorectal cancer (253)	0.60 (0.31, 1.14)	**0.04**
Kantor (2014)[79]	VITamins And Lifestyle cohort	M & F	50-76	US	cohort	6-8 years	Dietary EPA+DHA (g/d)	≥0.29 vs. <0.08	FFQ	Colorectal cancer (488)	0.92 (0.68, 1.24)	0.61
Song (2014)[68]	Nurses' Health Study	F	38-63	US	cohort	25 years	Marine n-3 PUFAs (g/day)	≥0.30 vs. <0.15	FFQ	Colorectal cancer (1469)	1.03 (0.89, 1.20)	0.68
	Health Professionals Follow-up Study	M	40-75	US	cohort	25 years	Marine n-3 PUFAs (g/d)	≥0.41 vs. <0.16	FFQ	Colorectal cancer (987)	1.05 (0.85, 1.30)	0.82
Nkondjock (2003)[112]	n/a	M & F	35-79	Canada	case-control	n/a	Total n-3 PUFAs (g/d)	>2.92 vs. <1.46	FFQ	Colorectal cancer (402)	0.73 (0.51, 1.05)	0.017
Kraja (2015)[77]	The Rotterdam Study	M	55-	The Netherlands	cohort	14.6 years	Total n-3 PUFAs (g/d)	1.5 vs. 0.7	FFQ	Colorectal cancer (222)	**1.44 (1.02, 2.04)**	**0.04**
Kim (2010)[80]	North Carolina Colon Cancer Study II	M & F	40-80	US	case-control	n/a	Total n-3 PUFAs (g/d)	≥2.31 vs. <1.27	FFQ	Distal large bowel cancer (716)	0.96 (0.61, 1.51)	0.86

[a] Studies were identified using the snowball strategy in an effort to include all published studies as of 02/01/2016.
[b] If both of total and marine n-3 PUFAs were presented, marine n-3 PUFAs was selected.
[c] For the values which were not provided by the publication, the range was found in other publications using the same data.[113]

Table 2. Association of baseline characteristics with colorectal cancer

Baseline characteristics	Cases [n (%)]	Noncases [n (%)]	OR[a] (95% CI)	*P* value[b]
Age (years), n				0.17
45-54	104 (22.2)	540 (46.3)	1.00 (reference)	
55-64	191 (40.7)	393 (33.7)	1.45 (0.90, 2.34)	
≥65	174 (37.1)	234 (20.1)	1.40 (0.63, 3.12)	
Sex, n				<0.01
Men	272 (58.0)	506 (43.4)	1.00 (reference)	
Women	197 (42.0)	661 (56.6)	0.60 (0.48, 0.75)	
BMI (kg/m^2), n				0.11
<20	77 (16.4)	186 (15.9)	1.00 (reference)	
20-23.9	243 (51.8)	651 (55.8)	0.82 (0.60, 1.13)	
24-27.9	118 (25.2)	268 (23.0)	1.04 (0.73, 1.49)	
≥28	31 (6.6)	62 (5.3)	1.32 (0.78, 2.25)	
Education level, n				0.19
No formal education	129 (27.5)	313 (26.8)	1.00 (reference)	
Primary level	232 (49.5)	502 (43.0)	1.24 (0.92, 1.66)	
Secondary level or higher	108 (23.0)	352 (30.2)	1.00 (0.71, 1.42)	
Family history, n				<0.01
No	447 (95.3)	1139 (97.6)	1.00 (reference)	
Yes	22 (4.7)	28 (2.4)	2.32 (1.27, 4.23)	
Diabetes at baseline, n				0.12
No	403 (85.9)	1058 (90.7)	1.00 (reference)	
Yes	66 (14.1)	109 (9.3)	1.31 (0.93, 1.84)	
Smoking, n				<0.01
Never	271 (57.8)	845 (72.4)	1.00 (reference)	
<13 cigarette/day or started smoking at age 15 or older	158 (33.7)	292 (25.0)	1.12 (0.85, 1.49)	
≥13 cigarette/day and started smoking before age 15	40 (8.5)	30 (2.6)	3.09 (1.82, 5.25)	

Table 2 (continued)

Baseline characteristics	Cases [n (%)]	Noncases [n (%)]	OR[a] (95% CI)	*P* value[b]
Alcohol, n				0.02
Never	361 (77.0)	957 (82.0)	1.00 (reference)	
<7 drinks per week	70 (14.9)	164 (14.1)	1.06 (0.77, 1.48)	
≥7 drinks per week	38 (8.1)	46 (3.9)	2.01 (1.25, 3.24)	
Weekly vigorous work or strenuous sports, n				0.96
No	424 (90.4)	1021 (87.5)	1.00 (reference)	
Yes	45 (9.6)	146 (12.5)	0.99 (0.68, 1.45)	

Abbreviations: Body mass index (BMI), Confidence interval (CI), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, and dialect group.

[b] From Wald Test

Table 3. Distribution of baseline characteristics according to quartile (Q) intake of marine n-3 PUFAs among noncases (n=1,167)

Baseline characteristics	Marine n-3 PUFAs, g/1000kcal				*p* value[b]
	Q1[a]	Q2	Q3	Q4	
Mean age in year (SD)	57.1 (8.0)	56.1 (8.4)	56.5 (7.8)	56.2 (8.0)	0.407
Sex, % men	48.6	44.4	44.3	36.3	0.026
Mean BMI in kg/m^2 (SD)	22.4 (3.0)	22.7 (3.1)	22.9 (3.0)	23.5 (3.2)	<0.001
Education level, % no formal education	25.7	24.9	24.8	31.9	0.144
Diabetes at baseline, % yes	7.9	9.4	8.7	11.3	0.534
Smoking index, % never smoker	70.7	72.1	74.5	72.3	0.245
Alcohol, % nondrinker	79.3	79.5	81.5	87.7	0.030
Weekly vigorous work or strenuous sports, % yes	15.7	14.1	12.8	7.5	0.019
Mean daily intake (SD)					
Total energy, kcal	1557.6 (515.6)	1620.3 (561.0)	1577.5 (519.7)	1466.3 (530.1)	<0.001
Vegetables, g	96.3 (55.9)	111.3 (58.4)	115.7 (60.4)	119.1 (70.0)	<0.001
Fruits, g	198.8 (157.5)	223.4 (166.0)	214.4 (169.0)	195.2 (169.4)	0.080
Red meat, g	24.4 (23.4)	32.7 (28.4)	32.8 (22.4)	32.5 (28.2)	<0.001
Fish, g	26.0 (13.1)	47.7 (17.5)	61.9 (20.8)	83.7 (29.6)	<0.001
Folate, µg/1000kcal	96.4(32.4)	101.0 (30.7)	103.6 (32.2)	104.2 (32.2)	0.010
Calcium, mg/1000kcal	262.4 (134.7)	277.3 (135.5)	279.9 (126.8)	283.3 (119.3)	0.003
Fiber, g/1000kcal	8.1 (2.7)	8.5 (2.8)	8.4 (2.7)	8.4 (2.6)	0.290
Total fat, g/1000kcal	22.6 (6.2)	25.0 (5.5)	25.8 (4.8)	27.6 (5.5)	<0.001
Saturated fat, g/1000kcal	8.2 (2.8)	8.9 (2.6)	8.8 (2.3)	9.5 (2.5)	<0.001
Monounsaturated fat, g/1000kcal	7.7 (2.2)	8.5 (2.0)	8.7 (1.8)	9.3 (2.1)	<0.001
Total PUFAs, g/1000kcal	4.5 (1.7)	5.0 (1.7)	5.6 (2.0)	5.8 (2.0)	<0.001
Total n-6 PUFAs, g/1000kcal	4.1 (1.6)	4.5 (1.6)	5.0 (1.9)	5.1 (1.9)	<0.001
Total n-3 PUFAs, g/1000kcal	0.40 (0.12)	0.49 (0.15)	0.56 (0.18)	0.65 (0.16)	<0.001
Marine n-3 PUFAs, g/1000kcal	0.08 (0.03)	0.15 (0.02)	0.21 (0.02)	0.31 (0.06)	<0.001

Abbreviations: Body mass index (BMI), Polyunsaturated fatty acids (PUFAs), Standard deviation (SD).

[a] Quartiles of marine n-3 PUFAs are based on the distribution of the entire cohort.

[b] From χ^2 test for categorical variables and Kruskal-Wallis Test for continuous variables.

Table 4. Odds ratios and 95% confidence intervals for *GST* genotype and colorectal cancer risk

	Case/noncase	OR[a] (95% CI)	OR[b] (95% CI)
GSTT1			
Null	182/476	1.00 (reference)	1.00 (reference)
Positive	287/691	1.09 (0.87, 1.37)	1.08 (0.86, 1.36)
GSTM1			
Null	230/526	1.00 (reference)	1.00 (reference)
Positive	239/641	0.89 (0.71, 1.12)	0.88 (0.70, 1.11)
GSTP1			
AB/BB	135/396	1.00 (reference)	1.00 (reference)
AA	334/771	1.22 (0.96, 1.56)	1.23 (0.96, 1.58)
# of null genotypes in *GSTT1* and *GSTM1*			
0	150/387	1.00 (reference)	1.00 (reference)
1	226/558	1.00 (0.78, 1.29)	0.99 (0.77, 1.28)
2	93/222	1.03 (0.75, 1.42)	1.06 (0.77, 1.47)
# of null or low activity genotypes in *GSTT1, GSTM1,GSTP1*			
0	104/263	1.00 (reference)	1.00 (reference)
1	205/483	1.06 (0.79, 1.41)	1.03 (0.76, 1.38)
2	138/348	0.97 (0.71, 1.33)	0.97 (0.71, 1.34)
3	22/73	0.78 (0.45, 1.35)	0.78 (0.45, 1.36)

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase (*GST*), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Glutathione *S*-transferase pi 1 (*GSTP1*), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, and dialect group.

[b] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table 5. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal, colon and rectal cancer

	Median value	Colorectal cancer		Colon cancer		Rectal cancer	
		Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)
Total PUFAs, g/day							
Q1	5.57	116/265	1.00 (reference)	63/265	1.00 (reference)	53/265	1.00 (reference)
Q2	7.58	124/296	1.10 (0.80, 1.52)	72/296	1.08 (0.73, 1.60)	52/296	1.06 (0.68, 1.65)
Q3	9.14	128/286	1.19 (0.86, 1.66)	80/286	1.25 (0.85, 1.86)	48/286	1.06 (0.67, 1.68)
Q4	12.56	101/320	0.86 (0.61, 1.20)	56/320	0.80 (0.53, 1.22)	45/320	0.91 (0.57, 1.45)
P for trend			0.47		0.46		0.71
n-6 PUFAs, g/day							
Q1	4.88	120/269	1.00 (reference)	64/269	1.00 (reference)	56/269	1.00 (reference)
Q2	6.71	112/289	0.96 (0.69, 1.33)	69/289	1.00 (0.67, 1.49)	43/289	0.82 (0.52, 1.30)
Q3	8.12	133/288	1.22 (0.88, 1.69)	78/288	1.23 (0.83, 1.82)	55/288	1.17 (0.75, 1.83)
Q4	11.30	104/321	0.85 (0.61, 1.18)	60/321	0.83 (0.55, 1.26)	44/321	0.84 (0.53, 1.33)
P for trend			0.64		0.61		0.78
Total n-3 PUFAs, g/day							
Q1	0.62	116/273	1.00 (reference)	71/273	1.00 (reference)	45/273	1.00 (reference)
Q2	0.79	115/294	1.02 (0.73, 1.41)	65/294	0.87 (0.59, 1.30)	50/294	1.29 (0.81, 2.06)
Q3	0.91	127/289	1.16 (0.84, 1.61)	68/289	0.96 (0.65, 1.42)	59/289	1.55 (0.98, 2.46)
Q4	1.14	111/311	0.96 (0.69, 1.33)	67/311	0.88 (0.59, 1.30)	44/311	1.11 (0.69, 1.79)
P for trend			0.96		0.63		0.53
Marine n-3 PUFAs, g/day							
Q1	0.16	107/276	1.00 (reference)	65/276	1.00 (reference)	42/276	1.00 (reference)
Q2	0.26	109/305	0.90 (0.65, 1.26)	68/305	0.88 (0.60, 1.31)	41/305	0.86 (0.53, 1.39)
Q3	0.35	124/285	1.12 (0.81, 1.55)	70/285	1.00 (0.67, 1.48)	54/285	1.28 (0.81, 2.02)
Q4	0.48	129/301	1.09 (0.79, 1.50)	68/301	0.92 (0.62, 1.36)	61/301	1.37 (0.87, 2.15)
P for trend			0.36		0.84		0.06

Table 5 (continued)

	Median value	Colorectal cancer		Colon cancer		Rectal cancer	
		Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)
Marine n-3/n-6 PUFAs							
Q1	0.021	91/305	1.00 (reference)	59/305	1.00 (reference)	32/305	1.00 (reference)
Q2	0.034	118/322	1.28 (0.92, 1.78)	64/322	1.04 (0.70, 1.55)	54/322	1.72 (1.06, 2.80)
Q3	0.047	136/273	1.58 (1.14, 2.20)	82/273	1.43 (0.97, 2.11)	54/273	1.77 (1.08, 2.88)
Q4	0.073	124/267	1.40 (1.00, 2.00)	66/267	1.18 (0.78, 1.77)	58/267	1.88 (1.15, 3.06)
P for trend			0.03		0.20		0.02

Abbreviations: Confidence interval (CI), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table 6. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal cancer by *GSTT1* and *GSTM1* genotype

	***GSTT1* null**		***GSTT1* positive**		***GSTM1* null**		***GSTM1* positive**	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
Total PUFAs								
Q1	46/100	1.00 (reference)	70/165	1.00 (reference)	51/126	1.00 (reference)	65/139	1.00 (reference)
Q2	54/120	1.10 (0.66, 1.84)	70/176	1.10 (0.72, 1.67)	58/125	1.40 (0.86, 2.30)	66/171	0.94 (0.61, 1.44)
Q3	43/130	0.85 (0.49, 1.46)	85/156	1.47 (0.97, 2.23)	67/128	1.65 (1.01, 2.71)	61/158	0.93 (0.59, 1.45)
Q4	39/126	0.82 (0.47, 1.43)	62/194	0.89 (0.58, 1.37)	54/147	1.23 (0.74, 2.04)	47/137	0.65 (0.41, 1.04)
P for trend		0.32		0.95		0.37		0.09
P for interaction				0.26				0.08
N-6 PUFAs								
Q1	46/102	1.00 (reference)	74/167	1.00 (reference)	54/128	1.00 (reference)	66/141	1.00 (reference)
Q2	58/115	0.97 (0.57, 1.63)	64/174	0.95 (0.62, 1.44)	49/122	1.09 (0.66, 1.80)	63/167	0.88 (0.57, 1.36)
Q3	48/126	1.01 (0.59, 1.73)	85/162	1.36 (0.90, 2.06)	69/127	1.71 (1.05, 2.78)	64/161	0.95 (0.61, 1.48)
Q4	40/133	0.79 (0.46, 1.38)	64/188	0.89 (0.58, 1.37)	58/149	1.21 (0.74, 1.99)	46/172	0.63 (0.40, 1.01)
P for trend		0.46		0.94		0.22		0.09
P for interaction				0.32				<0.05
N-3 PUFAs								
Q1	40/111	1.00 (reference)	76/162	1.00 (reference)	52/123	1.00 (reference)	64/150	1.00 (reference)
Q2	41/121	1.18 (0.68, 2.06)	74/173	0.95 (0.63, 1.44)	55/136	1.10 (0.68, 1.79)	60/158	0.95 (0.61, 1.49)
Q3	58/120	1.68 (0.99, 2.87)	69/169	0.92 (0.60, 1.39)	62/127	1.35 (0.83, 2.18)	65/162	1.03 (0.66, 1.60)
Q4	43/124	1.27 (0.73, 2.22)	68/187	0.83 (0.55, 1.25)	61/140	1.29 (0.79, 2.09)	50/171	0.72 (0.46, 1.15)
P for trend		0.22		0.36		0.23		0.23
P for interaction				0.18				0.09

Table 6 (continued)

	GSTT1 null		*GSTT1* positive		*GSTM1* null		*GSTM1* positive	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
Marine n-3 PUFAs								
Q1	35/108	1.00 (reference)	72/168	1.00 (reference)	51/139	1.00 (reference)	56/137	1.00 (reference)
Q2	40/122	0.84 (0.48, 1.47)	69/183	0.94 (0.62, 1.41)	51/145	0.92 (0.57, 1.49)	58/160	0.89 (0.56, 1.41)
Q3	49/113	1.38 (0.80, 2.39)	75/172	1.01 (0.67, 1.52)	58/111	1.54 (0.95, 2.50)	66/174	0.87 (0.56, 1.35)
Q4	58/133	1.28 (0.75, 2.18)	71/168	0.98 (0.65, 1.48)	70/131	1.54 (0.97, 2.46)	59/170	0.78 (0.50, 1.23)
P for trend		0.14		1.00		0.02		0.30
P for interaction				0.19				0.02
Marine n-3/n-6 PUFAs								
Q1	28/116	1.00 (reference)	63/189	1.00 (reference)	49/144	1.00 (reference)	42/161	1.00 (reference)
Q2	40/145	1.15 (0.65, 2.06)	78/177	1.41 (0.94, 2.13)	49/149	1.06 (0.65, 1.72)	69/173	1.53 (0.96, 2.42)
Q3	61/111	2.06 (1.19, 3.59)	75/162	1.37 (0.90, 2.08)	66/114	1.69 (1.05, 2.73)	70/159	1.53 (0.96, 2.44)
Q4	53/104	1.91 (1.08, 3.36)	71/163	1.15 (0.75, 1.77)	66/119	1.46 (0.90, 2.38)	58/148	1.29 (0.80, 2.09)
P for trend		<0.01		0.56		0.04		0.36
P for interaction				0.02				0.39

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table 7. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal cancer by number of null genotypes in *GSTT1* and *GSTM1*

	≥1		0	
	Case/noncase	**OR[a] (95% CI)**	**Case/noncase**	**OR[a] (95% CI)**
Marine n-3 PUFAs				
Q1	63/196	1.00 (reference)	44/80	1.00 (reference)
Q2	73/204	1.03 (0.68, 1.55)	36/101	0.70 (0.40, 1.23)
Q3	85/175	1.59 (1.06, 2.39)	39/110	0.62 (0.36, 1.07)
Q4	98/205	1.49 (1.00, 2.23)	31/96	0.57 (0.32, 1.01)
P for trend		0.01		<0.05
P for interaction				<0.01
Marine n-3/n-6 PUFAs				
Q1	62/209	1.00 (reference)	29/96	1.00 (reference)
Q2	66/217	1.08 (0.71, 1.65)	52/105	1.67 (0.96, 2.93)
Q3	97/180	1.73 (1.16, 2.59)	39/93	1.26 (0.70, 2.28)
Q4	94/174	1.64 (1.09, 2.37)	30/93	0.94 (0.50, 1.77)
P for trend		<0.01		0.58
P for interaction				0.01

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table 8. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of localized and advanced colorectal cancer by number of null genotypes in *GSTT1* and *GSTM1*

	≥1		0	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
LOCALIZED				
Marine n-3 PUFAs				
Q1	20/205	1.00 (reference)	24/81	1.00 (reference)
Q2	34/208	1.50 (0.81, 2.78)	19/104	0.59 (0.29, 1.20)
Q3	38/180	2.26 (1.23, 4.15)	16/114	0.39 (0.19, 0.82)
Q4	44/214	2.08 (1.15, 3.78)	14/99	0.40 (0.19, 0.86)
P for trend		<0.01		<0.01
P for interaction				<0.01
Marine n-3/n-6 PUFAs				
Q1	28/215	1.00 (reference)	17/98	1.00 (reference)
Q2	22/223	0.81 (0.44, 1.51)	28/107	1.44 (0.72, 2.87)
Q3	41/186	1.60 (0.92, 2.78)	19/97	0.89 (0.42, 1.88)
Q4	45/183	1.73 (0.99, 3.00)	9/96	0.43 (0.17, 1.07)
P for trend		0.01		0.04
P for interaction				<0.01
ADVANCED				
Marine n-3 PUFAs				
Q1	34/205	1.00 (reference)	19/83	1.00 (reference)
Q2	35/208	0.96 (0.56, 1.65)	14/105	0.74 (0.34, 1.61)
Q3	42/180	1.53 (0.91, 2.58)	19/114	0.77 (0.37, 1.58)
Q4	45/214	1.33 (0.79, 2.22)	14/99	0.64 (0.30, 1.39)
P for trend		0.12		0.30
P for interaction				0.07

Table 8 (continued)

(ADVANCED)	≥1		0	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
Marine n-3/n-6 PUFAs				
Q1	28/215	1.00 (reference)	10/98	1.00 (reference)
Q2	38/223	1.45 (0.84, 2.50)	22/107	2.21 (0.97, 5.02)
Q3	50/186	2.05 (1.21, 3.48)	16/97	1.70 (0.71, 4.06)
Q4	40/183	1.51 (0.87, 2.63)	18/96	1.81 (0.76, 4.28)
P for trend		0.07		0.37
P for interaction				0.61

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Odds ratio (OR), Polyunsaturated fatty acids (PUFAs).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

APPENDIX B: FIGURES

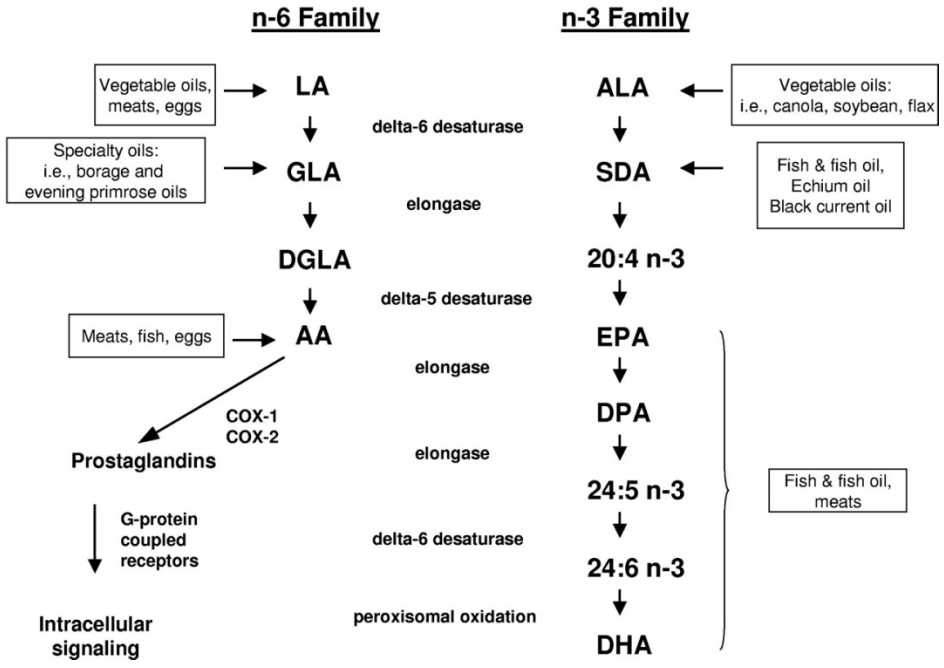

Figure 1[67] Metabolism of (n-6) and (n-3) families of PUFA

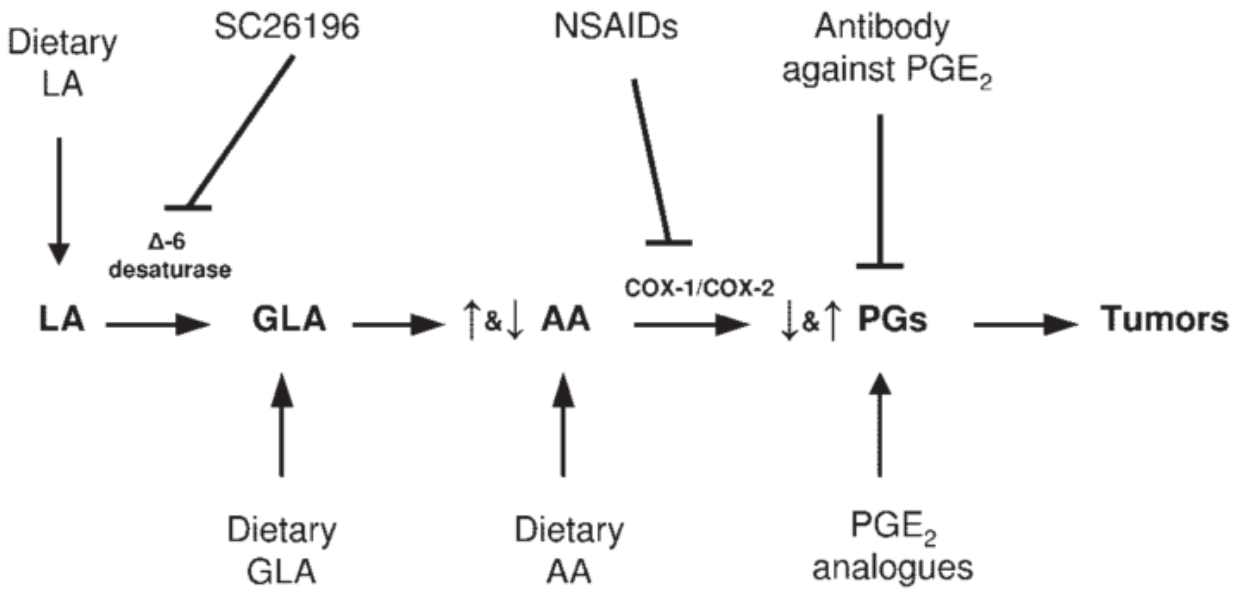

Figure 2[67] The AA cascade, from LA to PGs

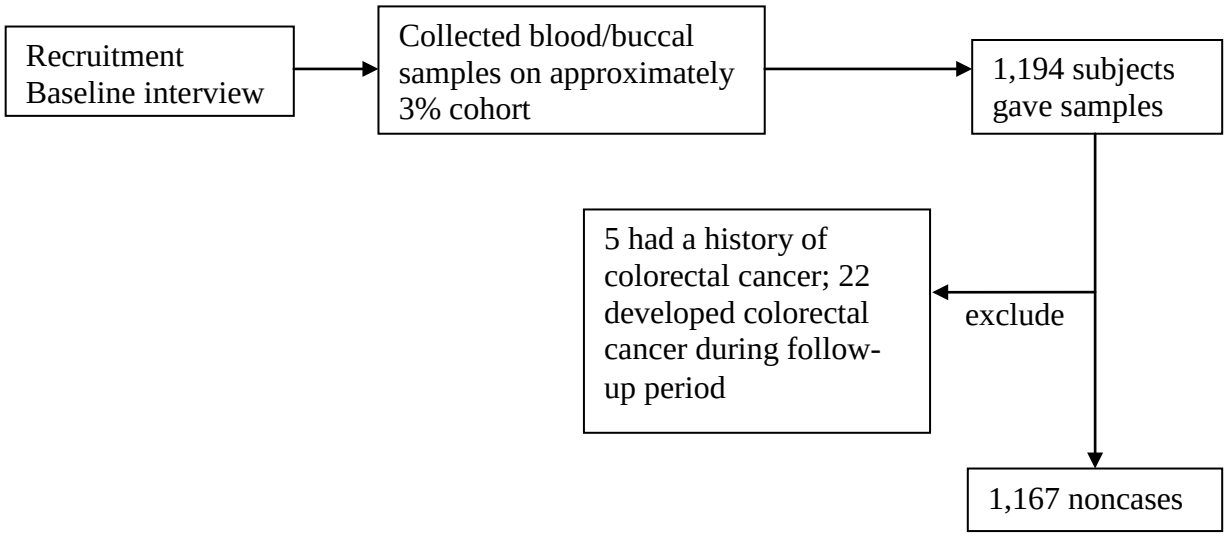

Figure 3 The selection process of noncases from the entire cohort

APPENDIX C: SUPPLEMENTARY TABLES

Table S1. Odds ratios and 95% confidence intervals for GST genotype and colon and rectal cancer risk

	Colon Cancer		Rectal Cancer	
	Case/noncase	**OR[a] (95% CI)**	**Case/noncase**	**OR[a] (95% CI)**
GSTT1				
Null	100/476	1.00 (reference)	82/476	1.00 (reference)
Positive	171/691	1.20 (0.90, 1.59)	116/691	0.94 (0.68, 1.30)
GSTM1				
Null	138/526	1.00 (reference)	92/526	1.00 (reference)
Positive	133/641	0.81 (0.61, 1.07)	106/641	0.96 (0.70, 1.33)
GSTP1				
AB/BB	81/396	1.00 (reference)	54/396	1.00 (reference)
AA	190/771	1.16 (0.86, 1.57)	144/771	1.35 (0.95, 1.92)
# of null genotypes in *GSTT1* and *GSTM1*				
0	87/387	1.00 (reference)	63/387	1.00 (reference)
1	130/558	0.99 (0.72, 1.35)	96/558	1.03 (0.72, 1.48)
2	54/222	1.05 (0.71, 1.56)	39/222	1.11 (0.70, 1.75)
# of null or low activity genotypes in *GSTT1*, *GSTM1*,*GSTP1*				
0	60/263	1.00 (reference)	44/263	1.00 (reference)
1	119/483	1.03 (0.72, 1.47)	86/483	1.02 (0.67, 1.55)
2	76/348	0.92 (0.63, 1.36)	62/348	1.07 (0.69, 1.67)
3	16/73	0.99 (0.53, 1.86)	6/73	0.49 (0.20, 1.25)

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase (*GST*), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Glutathione *S*-transferase pi 1 (*GSTP1*), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table S2. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of localized and advanced colorectal cancer

	Localized		Advanced	
	Case/noncase	**OR[a] (95% CI)**	**Case/noncase**	**OR[a] (95% CI)**
Total PUFAs				
Q1	51/274	1.00 (reference)	56/274	1.00 (reference)
Q2	56/306	1.12 (0.72, 1.73)	58/306	1.07 (0.70, 1.62)
Q3	59/298	1.22 (0.78, 1.89)	57/298	1.10 (0.72, 1.69)
Q4	43/327	0.80 (0.50, 1.27)	51/327	0.92 (0.59, 1.42)
P for trend		0.44		0.74
N-6 PUFAs				
Q1	53/277	1.00 (reference)	59/277	1.00 (reference)
Q2	48/301	0.89 (0.57, 1.40)	52/301	0.88 (0.58, 1.35)
Q3	62/299	1.27 (0.82, 1.95)	60/299	1.11 (0.73, 1.69)
Q4	46/328	0.81 (0.51, 1.29)	51/328	0.85 (0.55, 1.32)
P for trend		0.72		0.72
Total n-3 PUFAs				
Q1	51/281	1.00 (reference)	57/281	1.00 (reference)
Q2	55/303	1.14 (0.73, 1.76)	51/303	0.96 (0.63, 1.49)
Q3	62/297	1.27 (0.83, 1.96)	57/297	1.15 (0.75, 1.76)
Q4	41/324	0.76 (0.48, 1.21)	57/324	1.03 (0.68, 1.57)
P for trend		0.36		0.72
Marine n-3 PUFAs				
Q1	44/286	1.00 (reference)	53/286	1.00 (reference)
Q2	53/312	1.03 (0.66, 1.62)	49/312	0.86 (0.56, 1.34)
Q3	54/294	1.11 (0.71, 1.74)	61/294	1.18 (0.78, 1.79)
Q4	58/313	1.11 (0.71, 1.74)	59/313	1.04 (0.68, 1.58)
P for trend		0.59		0.54

Table S2 (continued)

	Localized		Advanced	
	Case/noncase	**OR[a] (95% CI)**	**Case/noncase**	**OR[a] (95% CI)**
Marine n-3/n-6 PUFAs				
Q1	45/313	1.00 (reference)	38/313	1.00 (reference)
Q2	50/330	1.07 (0.68, 1.68)	60/330	1.63 (1.04, 2.55)
Q3	60/283	1.32 (0.85, 2.05)	66/283	1.91 (1.23, 2.99)
Q4	54/279	1.16 (0.74, 1.83)	58/279	1.60 (1.01, 2.53)
P for trend		0.36		0.04

Abbreviations: Confidence interval (CI), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table S3. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal, colon and rectal cancer among men

	Median value	Colorectal cancer		Colon cancer		Rectal cancer	
		Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)
Total PUFAs, g/day							
Q1	5.36	93/158	1.00 (reference)	51/158	1.00 (reference)	42/158	1.00 (reference)
Q2	7.62	64/134	0.83 (0.55, 1.26)	30/134	0.66 (0.39, 1.11)	34/134	1.01 (0.59, 1.73)
Q3	9.18	58/102	0.88 (0.57, 1.38)	30/102	0.75 (0.43, 1.30)	28/102	1.03 (0.58, 1.85)
Q4	13.00	57/112	0.81 (0.52, 1.26)	28/112	0.64 (0.37, 1.12)	29/112	0.97 (0.55, 1.73)
P for trend			0.39		0.14		0.95
N-6 PUFAs, g/day							
Q1	4.70	95/157	1.00 (reference)	50/157	1.00 (reference)	45/157	1.00 (reference)
Q2	6.72	57/136	0.69 (0.45, 1.06)	31/136	0.65 (0.39, 1.11)	26/136	0.67 (0.38, 1.17)
Q3	8.12	63/99	0.96 (0.62, 1.49)	29/99	0.75 (0.43, 1.30)	34/99	1.19 (0.68, 2.07)
Q4	11.66	57/114	0.76 (0.49, 1.19)	29/114	0.65 (0.37, 1.13)	28/114	0.83 (0.47, 1.48)
P for trend			0.41		0.16		0.93
N-3 PUFAs, g/day							
Q1	0.59	85/159	1.00 (reference)	49/159	1.00 (reference)	36/159	1.00 (reference)
Q2	0.79	63/130	0.90 (0.58, 1.37)	33/130	0.78 (0.46, 1.33)	30/130	1.10 (0.61, 1.96)
Q3	0.91	64/99	1.05 (0.67, 1.63)	26/99	0.72 (0.40, 1.27)	38/99	1.53 (0.87, 2.71)
Q4	1.18	60/118	0.87 (0.57, 1.35)	31/118	0.74 (0.43, 1.26)	29/118	1.06 (0.59, 1.90)
P for trend			0.70		0.23		0.57
Marine n-3 PUFAs, g/day							
Q1	0.14	75/140	1.00 (reference)	44/140	1.00 (reference)	31/140	1.00 (reference)
Q2	0.26	53/123	0.62 (0.40, 0.98)	26/123	0.49 (0.28, 0.88)	27/123	0.79 (0.43, 1.44)
Q3	0.35	63/118	0.80 (0.52, 1.25)	30/118	0.65 (0.37, 1.14)	33/118	1.06 (0.59, 1.89)
Q4	0.50	81/125	0.96 (0.63, 1.47)	39/125	0.75 (0.44, 1.27)	42/125	1.26 (0.72, 2.19)
P for trend			0.86		0.45		0.26
Marine n-3/n-6 PUFAs							
Q1	0.02	59/142	1.00 (reference)	37/142	1.00 (reference)	22/142	1.00 (reference)
Q2	0.03	61/119	1.19 (0.76, 1.88)	28/119	0.88 (0.50, 1.56)	33/119	1.73 (0.93, 3.20)
Q3	0.05	67/112	1.24 (0.79, 1.95)	29/112	0.87 (0.49, 1.55)	38/112	1.85 (1.01, 3.39)
Q4	0.08	85/133	1.32 (0.85, 2.05)	45/133	1.21 (0.70, 2.06)	40/133	1.66 (0.91, 3.04)
P for trend			0.22		0.51		0.12

Abbreviations: Confidence interval (CI), Odds ratio (OR).
[a] Unconditional logistic regression models were adjusted for age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table S4. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal, colon and rectal cancer among women

	Median value	Colorectal cancer		Colon cancer		Rectal cancer	
		Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)	Case/ noncase	OR[a] (95% CI)
Total PUFAs, g/day							
Q1	5.89	23/107	1.00 (reference)	12/107	1.00 (reference)	11/107	1.00 (reference)
Q2	7.55	60/162	1.88 (1.07, 3.31)	42/162	2.46 (1.21, 4.98)	18/162	1.26 (0.55, 2.88)
Q3	9.13	70/184	1.96 (1.13, 3.41)	50/184	2.74 (1.37, 5.48)	20/184	1.14 (0.50, 2.59)
Q4	12.38	44/208	1.12 (0.63, 2.02)	28/208	1.37 (0.65, 2.88)	16/208	0.92 (0.39, 2.16)
P for trend			0.89		0.80		0.70
N-6 PUFAs, g/day							
Q1	5.26	25/112	1.00 (reference)	14/112	1.00 (reference)	11/112	1.00 (reference)
Q2	6.69	55/153	1.71 (0.98, 2.98)	38/153	2.03 (1.03, 3.99)	17/153	1.33 (0.58, 3.05)
Q3	8.12	70/189	1.87 (1.09, 3.21)	49/189	2.36 (1.22, 4.57)	21/189	1.24 (0.55, 2.82)
Q4	11.18	47/207	1.13 (0.64, 2.00)	31/207	1.33 (0.66, 2.67)	16/207	0.96 (0.41, 2.25)
P for trend			0.92		0.65		0.79
N-3 PUFAs, g/day							
Q1	0.65	31/114	1.00 (reference)	22/114	1.00 (reference)	9/114	1.00 (reference)
Q2	0.80	52/164	1.17 (0.69, 1.99)	32/164	1.01 (0.55, 1.89)	20/164	1.50 (0.63, 3.60)
Q3	0.92	63/190	1.40 (0.84, 2.35)	42/190	1.29 (0.72, 2.34)	21/190	1.79 (0.76, 4.20)
Q4	1.13	51/193	1.14 (0.67, 1.95)	36/193	1.13 (0.61, 2.08)	15/193	1.27 (0.51, 3.13)
P for trend			0.56		0.53		0.64
Marine n-3 PUFAs, g/day							
Q1	0.17	32/136	1.00 (reference)	21/136	1.00 (reference)	11/136	1.00 (reference)
Q2	0.27	56/182	1.45 (0.86, 2.42)	42/182	1.57 (0.87, 2.85)	14/182	1.14 (0.48, 2.69)
Q3	0.35	61/167	1.76 (1.06, 2.94)	40/167	1.69 (0.93, 3.08)	21/167	1.79 (0.80, 3.99)
Q4	0.48	48/176	1.30 (0.77, 2.20)	29/176	1.19 (0.63, 2.24)	19/176	1.53 (0.68, 3.45)
P for trend			0.30		0.67		0.19
Marine n-3/n-6 PUFAs							
Q1	0.02	32/163	1.00 (reference)	22/163	1.00 (reference)	10/163	1.00 (reference)
Q2	0.03	57/203	1.38 (0.83, 2.29)	36/203	1.29 (0.71, 2.33)	21/203	1.49 (0.67, 3.35)
Q3	0.05	69/161	2.08 (1.26, 3.43)	53/161	2.28 (1.28, 4.04)	16/161	1.45 (0.62, 3.39)
Q4	0.07	39/134	1.48 (0.86, 2.58)	21/134	1.16 (0.59, 2.27)	18/134	2.12 (0.91, 4.94)
P for trend			<0.05		0.18		0.10

Abbreviations: Confidence interval (CI), Odds ratio (OR).
[a] Unconditional logistic regression models were adjusted for age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table S5. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colon and rectal cancer by *GSTT1* and *GSTM1* genotype

	***GSTT1* null**		***GSTT1* positive**		***GSTM1* null**		***GSTM1* positive**	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
COLON CANCER								
Total PUFAs								
Q1	25/100	1.00 (reference)	38/165	1.00 (reference)	25/126	1.00 (reference)	38/139	1.00 (reference)
Q2	30/120	1.03 (0.54, 1.93)	42/176	1.10 (0.66, 1.84)	33/125	1.41 (0.76, 2.61)	39/171	0.93 (0.55, 1.57)
Q3	27/130	0.85 (0.44, 1.66)	53/156	1.55 (0.94, 2.55)	48/128	2.13 (1.18, 3.86)	32/158	0.79 (0.46, 1.37)
Q4	18/126	0.63 (0.31, 1.31)	38/194	0.92 (0.54, 1.56)	32/147	1.32 (0.70, 2.49)	24/173	0.55 (0.30, 0.99)
P for trend		0.18		0.89		0.21		0.04
P for interaction				0.14				0.02
N-6 PUFAs								
Q1	25/102	1.00 (reference)	39/167	1.00 (reference)	25/128	1.00 (reference)	39/141	1.00 (reference)
Q2	28/115	0.93 (0.49, 1.77)	41/174	1.02 (0.61, 1.69)	32/122	1.31 (0.71, 2.43)	37/167	0.85 (0.50, 1.43)
Q3	27/126	0.89 (0.46, 1.72)	51/162	1.44 (0.88, 2.36)	45/127	2.10 (1.16, 3.81)	33/161	0.79 (0.46, 1.37)
Q4	20/133	0.66 (0.32, 1.33)	40/188	0.97 (0.58, 1.62)	36/149	1.43 (0.77, 2.65)	24/172	0.54 (0.30, 0.96)
P for trend		0.25		0.75		0.13		0.04
P for interaction				0.17				0.01
N-3 PUFAs								
Q1	21/111	1.00 (reference)	50/162	1.00 (reference)	30/123	1.00 (reference)	41/150	1.00 (reference)
Q2	25/121	1.25 (0.63, 2.49)	40/173	0.74 (0.45, 1.21)	34/136	1.07 (0.60, 1.94)	31/158	0.71 (0.41, 1.24)
Q3	30/120	1.62 (0.82, 3.19)	38/169	0.73 (0.45, 1.21)	36/127	1.26 (0.70, 2.25)	32/162	0.76 (0.44, 1.30)
Q4	24/124	1.29 (0.63, 2.62)	43/187	0.75 (0.46, 1.22)	38/140	1.27 (0.71, 2.27)	29/171	0.61 (0.35, 1.06)
P for trend		0.38		0.27		0.35		0.11
P for interaction				0.20				0.09

Table S5 (continued)

(COLON CANCER)	*GSTT1* null		*GSTT1* positive		*GSTM1* null		*GSTM1* positive	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
Marine n-3 PUFAs								
Q1	17/108	1.00 (reference)	48/168	1.00 (reference)	29/139	1.00 (reference)	36/137	1.00 (reference)
Q2	25/122	1.05 (0.51, 2.14)	43/183	0.84 (0.52, 1.36)	38/145	1.17 (0.66, 2.05)	30/160	0.67 (0.38, 1.17)
Q3	31/113	1.90 (0.95, 3.80)	39/172	0.76 (0.46, 1.24)	34/111	1.55 (0.86, 2.78)	36/174	0.69 (0.40, 1.19)
Q4	27/133	1.23 (0.61, 2.50)	41/168	0.86 (0.53, 1.40)	37/131	1.39 (0.78, 2.47)	31/170	0.62 (0.35, 1.08)
P for trend		0.30		0.47		0.18		0.12
P for interaction				0.17				0.06
Marine n-3/n-6 PUFAs								
Q1	14/116	1.00 (reference)	45/189	1.00 (reference)	32/144	1.00 (reference)	27/161	1.00 (reference)
Q2	22/145	1.19 (0.56, 2.54)	42/177	1.07 (0.66, 1.74)	29/149	0.95 (0.53, 1.71)	35/173	1.17 (0.67, 2.07)
Q3	37/111	2.43 (1.20, 4.95)	45/162	1.15 (0.70, 1.87)	41/114	1.59 (0.91, 2.77)	41/159	1.34 (0.77, 2.34)
Q4	27/104	1.98 (0.94, 4.14)	39/163	0.96 (0.58, 1.60)	36/119	1.27 (0.71, 2.28)	30/148	1.05 (0.58, 1.89)
P for trend		0.01		0.99		0.18		0.76
P for interaction				0.01				0.46
RECTAL CANCER								
Total PUFAs								
Q1	21/100	1.00 (reference)	32/165	1.00 (reference)	26/126	1.00 (reference)	27/139	1.00 (reference)
Q2	24/120	1.08 (0.54, 2.15)	28/176	1.04 (0.58, 1.87)	25/125	1.27 (0.66, 2.45)	27/171	0.88 (0.48, 1.62)
Q3	16/130	0.75 (0.35, 1.61)	32/156	1.32 (0.74, 2.38)	19/128	0.97 (0.48, 1.96)	29/158	1.13 (0.60, 2.10)
Q4	21/126	1.03 (0.49, 2.14)	24/194	0.82 (0.44, 1.52)	22/147	1.06 (0.54, 2.09)	23/173	0.78 (0.41, 1.50)
P for trend		0.83		0.76		0.95		0.65
P for interaction				0.83				0.80

Table S5 (continued)

(RECTAL CANCER)	*GSTT1* null		*GSTT1* positive		*GSTM1* null		*GSTM1* positive	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
N-6 PUFAs								
Q1	21/102	1.00 (reference)	35/167	1.00 (reference)	29/128	1.00 (reference)	27/141	1.00 (reference)
Q2	20/115	0.91 (0.45, 1.86)	23/174	0.79 (0.43, 1.44)	17/122	0.75 (0.38, 1.50)	26/167	0.86 (0.46, 1.59)
Q3	21/126	1.09 (0.52, 2.26)	34/162	1.23 (0.70, 2.19)	24/127	1.20 (0.62, 2.34)	31/161	1.17 (0.63, 2.16)
Q4	20/133	0.94 (0.45, 1.96)	24/188	0.78 (0.42, 1.42)	22/149	0.92 (0.47, 1.80)	22/172	0.76 (0.39, 1.46)
P for trend		0.98		0.75		0.91		0.64
P for interaction				0.91				0.66
N-3 PUFAs								
Q1	19/111	1.00 (reference)	26/162	1.00 (reference)	22/123	1.00 (reference)	23/150	1.00 (reference)
Q2	16/121	1.02 (0.47, 2.20)	34/173	1.51 (0.83, 2.74)	21/136	1.14 (0.57, 2.30)	29/158	1.41 (0.74, 2.70)
Q3	28/120	1.71 (0.84, 3.46)	31/169	1.36 (0.74, 2.52)	26/127	1.48 (0.76, 2.92)	33/162	1.57 (0.84, 2.97)
Q4	19/124	1.25 (0.59, 2.67)	25/187	1.02 (0.54, 1.90)	23/140	1.31 (0.66, 2.62)	21/171	0.90 (0.46, 1.77)
P for trend		0.30		0.91		0.33		0.83
P for interaction				0.44				0.38
Marine n-3 PUFAs								
Q1	18/108	1.00 (reference)	24/168	1.00 (reference)	22/139	1.00 (reference)	20/137	1.00 (reference)
Q2	15/122	0.57 (0.26, 1.25)	26/183	1.10 (0.59, 2.06)	13/145	0.50 (0.24, 1.08)	28/160	1.28 (0.66, 2.47)
Q3	18/113	0.89 (0.41, 1.90)	37/172	1.55 (0.86, 2.80)	24/111	1.42 (0.73, 2.76)	30/174	1.15 (0.60, 2.19)
Q4	31/133	1.34 (0.67, 2.66)	30/168	1.30 (0.71, 2.40)	33/131	1.71 (0.91, 3.22)	28/170	1.06 (0.55, 2.04)
P for trend		0.18		0.24		0.01		1.00
P for interaction				0.68				0.07

Table S5 (continued)

(RECTAL CANCER)	***GSTT1* null**		***GSTT1* positive**		***GSTM1* null**		***GSTM1* positive**	
	Case/ noncase	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**	**Case/ noncase**	**OR[a] (95% CI)**
Marine n-3/n-6 PUFAs								
Q1	14/116	1.00 (reference)	18/189	1.00 (reference)	17/144	1.00 (reference)	15/161	1.00 (reference)
Q2	18/145	1.12 (0.51, 2.44)	37/177	2.33 (1.23, 4.39)	20/149	1.29 (0.63, 2.63)	34/173	2.16 (1.09, 4.25)
Q3	24/111	1.63 (0.77, 3.47)	30/162	1.88 (0.97, 3.62)	25/114	1.76 (0.88, 3.55)	29/159	1.78 (0.89, 3.57)
Q4	26/104	1.93 (0.91, 4.08)	32/163	1.79 (0.93, 3.45)	30/119	1.81 (0.91, 3.60)	28/148	1.84 (0.91, 3.71)
P for trend		<0.05		0.20		0.06		0.21
P for interaction				0.30				0.61

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase theta 1 (*GSTT1*), Glutathione *S*-transferase mu 1 (*GSTM1*), Odds ratio (OR).

[a] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

Table S6. Odds ratios and 95% confidence intervals for dietary polyunsaturated fatty acid (PUFA) intake and risk of colorectal, colon and rectal cancer by *GSTP1* genotype

	***GSTP1* Low activity[a]**		***GSTP1* High activity[a]**	
	Case/ noncase	**OR[b] (95% CI)**	**Case/ noncase**	**OR[b] (95% CI)**
COLORECTAL CANCER				
Total PUFAs				
Q1	34/93	1.00 (reference)	82/172	1.00 (reference)
Q2	44/97	1.46 (0.82, 2.60)	80/199	0.96 (0.64, 1.42)
Q3	35/100	1.03 (0.56, 1.87)	93/186	1.26 (0.85, 1.87)
Q4	22/106	0.64 (0.33, 1.23)	79/214	0.92 (0.61, 1.38)
P for trend		0.11		0.99
P for interaction				0.15
N-6 PUFAs				
Q1	34/96	1.00 (reference)	86/173	1.00 (reference)
Q2	42/92	1.45 (0.82, 2.59)	70/197	0.78 (0.52, 1.16)
Q3	35/103	1.05 (0.58, 1.92)	98/185	1.29 (0.87, 1.90)
Q4	24/105	0.72 (0.38, 1.38)	80/216	0.86 (0.58, 1.28)
P for trend		0.23		0.95
P for interaction				0.26
N-3 PUFAs				
Q1	41/92	1.00 (reference)	75/181	1.00 (reference)
Q2	35/102	0.83 (0.47, 1.48)	80/192	1.12 (0.75, 1.67)
Q3	31/92	0.84 (0.46, 1.51)	96/197	1.34 (0.91, 1.99)
Q4	28/110	0.58 (0.32, 1.05)	83/201	1.19 (0.80, 1.79)
P for trend		0.09		0.28
P for interaction				0.05

Table S6 (continued)

	***GSTP1* Low activity**[a]		***GSTP1* High activity**[a]	
(COLORECTAL CANCER)	**Case/ noncase**	**OR**[b] **(95% CI)**	**Case/ noncase**	**OR**[b] **(95% CI)**
Marine n-3 PUFAs				
Q1	33/91	1.00 (reference)	74/185	1.00 (reference)
Q2	34/96	0.80 (0.44, 1.46)	75/209	0.93 (0.63, 1.39)
Q3	37/98	0.89 (0.49, 1.60)	87/187	1.23 (0.83, 1.82)
Q4	31/111	0.59 (0.32, 1.08)	98/190	1.39 (0.95, 2.04)
P for trend		0.13		0.04
P for interaction				0.03
Marine n-3/n-6 PUFAs				
Q1	29/107	1.00 (reference)	62/198	1.00 (reference)
Q2	34/95	1.23 (0.67, 2.25)	84/227	1.29 (0.87, 1.93)
Q3	38/94	1.33 (0.73, 2.41)	98/179	1.73 (1.16, 2.58)
Q4	34/100	0.93 (0.50, 1.70)	90/167	1.68 (1.12, 2.54)
P for trend		0.86		<0.01
P for interaction				0.14
COLON CANCER				
Total PUFAs				
Q1	20/93	1.00 (reference)	43/172	1.00 (reference)
Q2	28/97	1.50 (0.75, 3.01)	44/199	0.95 (0.58, 1.54)
Q3	23/100	1.06 (0.52, 2.17)	57/186	1.40 (0.87, 2.26)
Q4	10/106	0.45 (0.19, 1.08)	46/214	0.95 (0.58, 1.56)
P for trend		0.07		0.77
P for interaction				0.09

Table S6 (continued)

(COLON CANCER)	***GSTP1* Low activity[a]**		***GSTP1* High activity[a]**	
	Case/ noncase	**OR[b] (95% CI)**	**Case/ noncase**	**OR[b] (95% CI)**
N-6 PUFAs				
Q1	20/96	1.00 (reference)	44/173	1.00 (reference)
Q2	27/92	1.44 (0.72, 2.88)	42/197	0.86 (0.52, 1.40)
Q3	21/103	0.95 (0.46, 1.97)	57/185	1.40 (0.87, 2.24)
Q4	13/105	0.59 (0.26, 1.34)	47/216	0.92 (0.56, 1.51)
P for trend		0.14		0.76
P for interaction				0.18
N-3 PUFAs				
Q1	30/92	1.00 (reference)	41/181	1.00 (reference)
Q2	18/102	0.54 (0.27, 1.08)	47/192	1.15 (0.70, 1.88)
Q3	17/92	0.60 (0.29, 1.22)	51/197	1.25 (0.77, 2.04)
Q4	16/110	0.43 (0.21, 0.87)	51/201	1.26 (0.78, 2.06)
P for trend		0.03		0.32
P for interaction				0.03
Marine n-3 PUFAs				
Q1	22/91	1.00 (reference)	43/185	1.00 (reference)
Q2	22/96	0.66 (0.32, 1.35)	46/209	0.97 (0.60, 1.57)
Q3	21/98	0.76 (0.37, 1.54)	49/187	1.14 (0.71, 1.84)
Q4	16/111	0.44 (0.21, 0.94)	52/190	1.23 (0.77, 1.98)
P for trend		0.06		0.29
P for interaction				<0.05

Table S6 (continued)

	***GSTP1* Low activity**[a]		***GSTP1* High activity**[a]	
(COLON CANCER)	**Case/ noncase**	**OR**[b] **(95% CI)**	**Case/ noncase**	**OR**[b] **(95% CI)**
Marine n-3/n-6 PUFAs				
Q1	18/107	1.00 (reference)	41/198	1.00 (reference)
Q2	20/95	1.07 (0.51, 2.24)	44/227	1.02 (0.63, 1.66)
Q3	24/94	1.35 (0.66, 2.78)	58/179	1.50 (0.94, 2.40)
Q4	19/100	0.90 (0.42, 1.90)	47/167	1.35 (0.83, 2.21)
P for trend		0.93		0.09
P for interaction				0.37
RECTAL CANCER				
Total PUFAs				
Q1	14/93	1.00 (reference)	39/172	1.00 (reference)
Q2	16/97	1.28 (0.55, 2.96)	36/199	0.93 (0.55, 1.57)
Q3	12/100	0.89 (0.36, 2.20)	36/186	1.04 (0.60, 1.80)
Q4	12/106	0.85 (0.35, 2.10)	33/214	0.86 (0.49, 1.49)
P for trend		0.57		0.70
P for interaction				0.75
N-6 PUFAs				
Q1	14/96	1.00 (reference)	42/173	1.00 (reference)
Q2	15/92	1.27 (0.54, 2.98)	28/197	0.66 (0.38, 1.14)
Q3	14/103	1.11 (0.46, 2.67)	41/185	1.12 (0.66, 1.90)
Q4	11/105	0.86 (0.34, 2.15)	33/216	0.77 (0.45, 1.33)
P for trend		0.70		0.73
P for interaction				0.80

Table S6 (continued)

(RECTAL CANCER)	*GSTP1* Low activity[a]		*GSTP1* High activity[a]	
	Case/ noncase	**OR[b] (95% CI)**	**Case/ noncase**	**OR[b] (95% CI)**
N-3 PUFAs				
Q1	11/92	1.00 (reference)	34/181	1.00 (reference)
Q2	17/102	1.71 (0.71, 4.14)	33/192	1.11 (0.63, 1.95)
Q3	14/92	1.62 (0.65, 4.03)	45/197	1.52 (0.89, 2.61)
Q4	12/110	0.90 (0.35, 2.31)	32/201	1.12 (0.63, 1.98)
P for trend		0.72		0.46
P for interaction				0.58
Marine n-3 PUFAs				
Q1	11/91	1.00 (reference)	31/185	1.00 (reference)
Q2	12/96	0.92 (0.37, 2.32)	29/209	0.85 (0.47, 1.51)
Q3	16/98	1.24 (0.51, 2.98)	38/187	1.29 (0.75, 2.23)
Q4	15/111	0.84 (0.34, 2.06)	46/190	1.65 (0.97, 2.80)
P for trend		0.84		0.02
P for interaction				0.23
Marine n-3/n-6 PUFAs				
Q1	11/107	1.00 (reference)	21/198	1.00 (reference)
Q2	14/95	1.47 (0.61, 3.55)	40/227	1.89 (1.05, 3.41)
Q3	14/94	1.25 (0.51, 3.04)	40/179	2.08 (1.15, 3.77)
Q4	15/100	0.98 (0.40, 2.38)	43/167	2.52 (1.39, 4.58)
P for trend		0.84		<0.01
P for interaction				0.12

Abbreviations: Confidence interval (CI), Glutathione *S*-transferase pi 1 (*GSTP1*), Odds ratio (OR).

[a] *GSTP1* Low activity was defined as *GSTP1 AB/BB*; *GSTP1* High activity was defined as *GSTP1 AA.*

[b] Unconditional logistic regression models were adjusted for sex, age at interview, interview year, dialect group, body mass index, education, family history, diabetes at baseline, smoking, drinking, and physical activity.

BIBLIOGRAPHY

1. Ferlay J SI, Ervik M, et al. Cancer incidence and mortality worldwide: IARC CancerBase no. 11 (online). GLOBOCAN 2012 v1.0. Lyon, France: International Agency for Research on Cancer, 2013.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA: a cancer journal for clinicians.* 2015;65(2):87-108.
3. Registry SC. Trends in cancer incidence in Singapore 2010-2014. Singapore, Singapore: National Registry of Diseases Office; 2015.
4. Soo KC. Role of comprehensive cancer centres during economic and disease transition: National Cancer Centre, Singapore--a case study. *The Lancet. Oncology.* 2008;9(8):796-802.
5. Wong MT, Eu KW. Rise of colorectal cancer in Singapore: an epidemiological review. *ANZ journal of surgery.* 2007;77(6):446-449.
6. Taylor DP, Burt RW, Williams MS, Haug PJ, Cannon-Albright LA. Population-based family history-specific risks for colorectal cancer: a constellation approach. *Gastroenterology.* 2010;138(3):877-885.
7. Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. *The American journal of gastroenterology.* 2001;96(10):2992-3003.
8. Liang PS, Chen TY, Giovannucci E. Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. *International journal of cancer. Journal international du cancer.* 2009;124(10):2406-2415.
9. Tsong WH, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. *British journal of cancer.* 2007;96(5):821-827.
10. Fedirko V, Tramacere I, Bagnardi V, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2011;22(9):1958-1972.
11. Harriss DJ, Atkinson G, George K, et al. Lifestyle factors and colorectal cancer risk (1): systematic review and meta-analysis of associations with body mass index. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2009;11(6):547-563.
12. Guraya SY. Association of type 2 diabetes mellitus and the risk of colorectal cancer: A meta-analysis and systematic review. *World journal of gastroenterology.* 2015;21(19):6026-6031.
13. Giovannucci E. Insulin and colon cancer. *Cancer causes & control : CCC.* 1995;6(2):164-179.
14. Liu JJ, Druta M, Shibata D, et al. Metabolic syndrome and colorectal cancer: is hyperinsulinemia/insulin receptor-mediated angiogenesis a critical process? *Journal of geriatric oncology.* 2014;5(1):40-48.

15. Odegaard AO, Koh WP, Yu MC, Yuan JM. Body mass index and risk of colorectal cancer in Chinese Singaporeans: the Singapore Chinese Health Study. *Cancer.* 2011;117(16):3841-3849.
16. Luo W, Cao Y, Liao C, Gao F. Diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2012;14(11):1307-1312.
17. Limburg PJ, Anderson KE, Johnson TW, et al. Diabetes mellitus and subsite-specific colorectal cancer risks in the Iowa Women's Health Study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2005;14(1):133-137.
18. Larsson SC, Giovannucci E, Wolk A. Diabetes and colorectal cancer incidence in the cohort of Swedish men. *Diabetes care.* 2005;28(7):1805-1807.
19. He J, Stram DO, Kolonel LN, Henderson BE, Le Marchand L, Haiman CA. The association of diabetes with colorectal cancer risk: the Multiethnic Cohort. *British journal of cancer.* 2010;103(1):120-126.
20. Seow A, Yuan JM, Koh WP, Lee HP, Yu MC. Diabetes mellitus and risk of colorectal cancer in the Singapore Chinese Health Study. *Journal of the National Cancer Institute.* 2006;98(2):135-138.
21. Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. *Gastroenterology.* 2004;127(4):1044-1050.
22. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes care.* 2009;32(9):1620-1625.
23. Mansouri D, McMillan DC, Crighton EM, Horgan PG. Comment on Luo et al.: diabetes mellitus and the incidence and mortality of colorectal cancer: a meta-analysis of 24 cohort studies. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2013;15(8):1045.
24. Sehdev A, Shih YC, Vekhter B, Bissonnette MB, Olopade OI, Polite BN. Metformin for primary colorectal cancer prevention in patients with diabetes: a case-control study in a US population. *Cancer.* 2015;121(7):1071-1078.
25. Research WCRFAIfC. Continuous Update Project: Keeping the science current. Colorectal cancer 2011 Report: Food, nutrition, physical activity, and the prevention of colorectal cancer.
26. Samad AK, Taylor RS, Marshall T, Chapman MA. A meta-analysis of the association of physical activity with reduced risk of colorectal cancer. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2005;7(3):204-213.
27. Liu L, Shi Y, Li T, et al. Leisure time physical activity and cancer risk: evaluation of the WHO's recommendation based on 126 high-quality epidemiological studies. *British journal of sports medicine.* 2015.
28. Boyle T, Heyworth J, Bull F, McKerracher S, Platell C, Fritschi L. Timing and intensity of recreational physical activity and the risk of subsite-specific colorectal cancer. *Cancer causes & control : CCC.* 2011;22(12):1647-1658.

29. Odegaard AO, Koh WP, Yuan JM. Combined lifestyle factors and risk of incident colorectal cancer in a Chinese population. *Cancer prevention research (Philadelphia, Pa.).* 2013;6(4):360-367.
30. Din FV, Theodoratou E, Farrington SM, et al. Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. *Gut.* 2010;59(12):1670-1679.
31. Bosetti C, Rosato V, Gallus S, Cuzick J, La Vecchia C. Aspirin and cancer risk: a quantitative review to 2011. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2012;23(6):1403-1415.
32. Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet (London, England).* 2010;376(9754):1741-1750.
33. Friis S, Riis AH, Erichsen R, Baron JA, Sorensen HT. Low-Dose Aspirin or Nonsteroidal Anti-inflammatory Drug Use and Colorectal Cancer Risk: A Population-Based, Case-Control Study. *Annals of internal medicine.* 2015;163(5):347-355.
34. Stolfi C, De Simone V, Pallone F, Monteleone G. Mechanisms of action of non-steroidal anti-inflammatory drugs (NSAIDs) and mesalazine in the chemoprevention of colorectal cancer. *International journal of molecular sciences.* 2013;14(9):17972-17985.
35. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology.* 2015;148(6):1244-1260.e1216.
36. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project: Keeping the science current. Colorectal cancer 2011 Report: Food, nutrition, physical activity, and the prevention of colorectal cancer.
37. Bouvard V, Loomis D, Guyton KZ, et al. Carcinogenicity of consumption of red and processed meat. *The Lancet. Oncology.* 2015;16(16):1599-1600.
38. Chan DS, Lau R, Aune D, et al. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PloS one.* 2011;6(6):e20456.
39. Aune D, Chan DS, Lau R, et al. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ (Clinical research ed.).* 2011;343:d6617.
40. Yu XF, Zou J, Dong J. Fish consumption and risk of gastrointestinal cancers: a meta-analysis of cohort studies. *World journal of gastroenterology.* 2014;20(41):15398-15412.
41. Hall MN, Chavarro JE, Lee IM, Willett WC, Ma J. A 22-year prospective study of fish, n-3 fatty acid intake, and colorectal cancer risk in men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2008;17(5):1136-1143.
42. Norat T, Bingham S, Ferrari P, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *Journal of the National Cancer Institute.* 2005;97(12):906-916.
43. Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. *Nutrition and cancer.* 1997;28(3):276-281.
44. Bostick RM, Potter JD, Kushi LH, et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). *Cancer causes & control : CCC.* 1994;5(1):38-52.

45. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *The New England journal of medicine.* 1990;323(24):1664-1672.
46. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer research.* 1994;54(9):2390-2397.
47. Hsing AW, McLaughlin JK, Chow WH, et al. Risk factors for colorectal cancer in a prospective study among U.S. white men. *International journal of cancer. Journal international du cancer.* 1998;77(4):549-553.
48. Daniel CR, McCullough ML, Patel RC, et al. Dietary intake of omega-6 and omega-3 fatty acids and risk of colorectal cancer in a prospective cohort of U.S. men and women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2009;18(2):516-525.
49. Pietinen P, Malila N, Virtanen M, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. *Cancer causes & control : CCC.* 1999;10(5):387-396.
50. Tiemersma EW, Kampman E, Bueno de Mesquita HB, et al. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. *Cancer causes & control : CCC.* 2002;13(4):383-393.
51. Engeset D, Andersen V, Hjartaker A, Lund E. Consumption of fish and risk of colon cancer in the Norwegian Women and Cancer (NOWAC) study. *The British journal of nutrition.* 2007;98(3):576-582.
52. Luchtenborg M, Weijenberg MP, de Goeij AF, et al. Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands). *Cancer causes & control : CCC.* 2005;16(9):1041-1054.
53. Larsson SC, Rafter J, Holmberg L, Bergkvist L, Wolk A. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. *International journal of cancer. Journal international du cancer.* 2005;113(5):829-834.
54. Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. *International journal of cancer. Journal international du cancer.* 1999;80(6):852-856.
55. Sanjoaquin MA, Appleby PN, Thorogood M, Mann JI, Key TJ. Nutrition, lifestyle and colorectal cancer incidence: a prospective investigation of 10998 vegetarians and non-vegetarians in the United Kingdom. *British journal of cancer.* 2004;90(1):118-121.
56. English DR, MacInnis RJ, Hodge AM, Hopper JL, Haydon AM, Giles GG. Red meat, chicken, and fish consumption and risk of colorectal cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2004;13(9):1509-1514.
57. Sugawara Y, Kuriyama S, Kakizaki M, et al. Fish consumption and the risk of colorectal cancer: the Ohsaki Cohort Study. *British journal of cancer.* 2009;101(5):849-854.
58. Kojima M, Wakai K, Tamakoshi K, et al. Diet and colorectal cancer mortality: results from the Japan Collaborative Cohort Study. *Nutrition and cancer.* 2004;50(1):23-32.

59. Lee SA, Shu XO, Yang G, Li H, Gao YT, Zheng W. Animal origin foods and colorectal cancer risk: a report from the Shanghai Women's Health Study. *Nutrition and cancer.* 2009;61(2):194-205.
60. Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food science & nutrition.* 2014;2(5):443-463.
61. Yang K, Li H, Dong J, Dong Y, Wang CZ. Expression profile of polyunsaturated fatty acids in colorectal cancer. *World journal of gastroenterology.* 2015;21(8):2405-2412.
62. DHA EPA Omega-3 Institute. http://www.dhaomega3.org/Overview/Dietary-Sources-of-Omega-3-Fatty-Acids.
63. Davidson MH. Omega-3 fatty acids: new insights into the pharmacology and biology of docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid. *Current opinion in lipidology.* 2013;24(6):467-474.
64. Center MI. http://lpi.oregonstate.edu/mic/other-nutrients/essential-fatty-acids.
65. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochimica et biophysica acta.* 1994;1213(3):277-288.
66. United States Department of Agriculture Agricultural Research Service. Farmed Salmon Raises Blood Levels of Omega-3s.;2016(2/17).
67. Whelan J, McEntee MF. Dietary (n-6) PUFA and intestinal tumorigenesis. *The Journal of nutrition.* 2004;134(12 Suppl):3421s-3426s.
68. Song M, Chan AT, Fuchs CS, et al. Dietary intake of fish, omega-3 and omega-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women. *International journal of cancer. Journal international du cancer.* 2014;135(10):2413-2423.
69. Murff HJ, Shu XO, Li H, et al. A prospective study of dietary polyunsaturated fatty acids and colorectal cancer risk in Chinese women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2009;18(8):2283-2291.
70. Lin J, Zhang SM, Cook NR, Lee IM, Buring JE. Dietary fat and fatty acids and risk of colorectal cancer in women. *American journal of epidemiology.* 2004;160(10):1011-1022.
71. Butler LM, Wang R, Koh WP, Stern MC, Yuan JM, Yu MC. Marine n-3 and saturated fatty acids in relation to risk of colorectal cancer in Singapore Chinese: a prospective study. *International journal of cancer. Journal international du cancer.* 2009;124(3):678-686.
72. Sasazuki S, Inoue M, Iwasaki M, et al. Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer by subsite: Japan Public Health Center-based prospective study. *International journal of cancer. Journal international du cancer.* 2011;129(7):1718-1729.
73. Cai F, Dupertuis YM, Pichard C. Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments. *Current opinion in clinical nutrition and metabolic care.* 2012;15(2):99-106.
74. Cockbain AJ, Toogood GJ, Hull MA. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut.* 2012;61(1):135-149.

75. Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis : an international journal on programmed cell death.* 2000;5(5):415-418.
76. Geelen A, Schouten JM, Kamphuis C, et al. Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. *American journal of epidemiology.* 2007;166(10):1116-1125.
77. Kraja B, Muka T, Ruiter R, et al. Dietary Fiber Intake Modifies the Positive Association between n-3 PUFA Intake and Colorectal Cancer Risk in a Caucasian Population. *The Journal of nutrition.* 2015;145(8):1709-1716.
78. Shen XJ, Zhou JD, Dong JY, Ding WQ, Wu JC. Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. *The British journal of nutrition.* 2012;108(9):1550-1556.
79. Kantor ED, Lampe JW, Peters U, Vaughan TL, White E. Long-chain omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer. *Nutrition and cancer.* 2014;66(4):716-727.
80. Kim S, Sandler DP, Galanko J, Martin C, Sandler RS. Intake of polyunsaturated fatty acids and distal large bowel cancer risk in whites and African Americans. *American journal of epidemiology.* 2010;171(9):969-979.
81. Terry P, Bergkvist L, Holmberg L, Wolk A. No association between fat and fatty acids intake and risk of colorectal cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2001;10(8):913-914.
82. Reddy BS, Burill C, Rigotty J. Effect of diets high in omega-3 and omega-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. *Cancer research.* 1991;51(2):487-491.
83. Tuncer S, Banerjee S. Eicosanoid pathway in colorectal cancer: Recent updates. *World journal of gastroenterology.* 2015;21(41):11748-11766.
84. Song JH, Fujimoto K, Miyazawa T. Polyunsaturated (n-3) fatty acids susceptible to peroxidation are increased in plasma and tissue lipids of rats fed docosahexaenoic acid-containing oils. *The Journal of nutrition.* 2000;130(12):3028-3033.
85. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free radical biology & medicine.* 1991;11(1):81-128.
86. Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity.* 2014;2014:360438.
87. Wang M, Dhingra K, Hittelman WN, Liehr JG, de Andrade M, Li D. Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 1996;5(9):705-710.
88. Bastide NM, Chenni F, Audebert M, et al. A central role for heme iron in colon carcinogenesis associated with red meat intake. *Cancer research.* 2015;75(5):870-879.
89. Pettazzoni P, Pizzimenti S, Toaldo C, et al. Induction of cell cycle arrest and DNA damage by the HDAC inhibitor panobinostat (LBH589) and the lipid peroxidation end

product 4-hydroxynonenal in prostate cancer cells. *Free radical biology & medicine.* 2011;50(2):313-322.
90. Stern MC, Butler LM, Corral R, et al. Polyunsaturated fatty acids, DNA repair single nucleotide polymorphisms and colorectal cancer in the Singapore Chinese Health Study. *Journal of nutrigenetics and nutrigenomics.* 2009;2(6):273-279.
91. Sprenger R, Schlagenhaufer R, Kerb R, et al. Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. *Pharmacogenetics.* 2000;10(6):557-565.
92. Koh WP, Nelson HH, Yuan JM, et al. Glutathione S-transferase (GST) gene polymorphisms, cigarette smoking and colorectal cancer risk among Chinese in Singapore. *Carcinogenesis.* 2011;32(10):1507-1511.
93. Ginsberg G, Smolenski S, Hattis D, Guyton KZ, Johns DO, Sonawane B. Genetic Polymorphism in Glutathione Transferases (GST): Population distribution of GSTM1, T1, and P1 conjugating activity. *Journal of toxicology and environmental health. Part B, Critical reviews.* 2009;12(5-6):389-439.
94. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *The Journal of biological chemistry.* 1997;272(15):10004-10012.
95. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology.* 2000;61(3):154-166.
96. Yoshida K, Osawa K, Kasahara M, et al. Association of CYP1A1, CYP1A2, GSTM1 and NAT2 gene polymorphisms with colorectal cancer and smoking. *Asian Pacific journal of cancer prevention : APJCP.* 2007;8(3):438-444.
97. Fan CH, Jin MJ, Zhang Y, et al. [Association between genetic polymorphisms of metabolic enzymes and susceptibility of colorectal cancer]. *Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine].* 2006;40(1):13-17.
98. Lee E, Huang Y, Zhao B, Seow-Choen F, Balakrishnan A, Chan SH. Genetic polymorphism of conjugating enzymes and cancer risk: GSTM1, GSTT1, NAT1 and NAT2. *The Journal of toxicological sciences.* 1998;23 Suppl 2:140-142.
99. Seow A, Yuan JM, Sun CL, Van Den Berg D, Lee HP, Yu MC. Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. *Carcinogenesis.* 2002;23(12):2055-2061.
100. Zhong S, Yang JH, Liu K, Jiao BH, Chang Z. Null genotype of glutathione S-transferase Tl contributes to colorectal cancer risk in the Asian population: a meta-analysis. *Journal of gastroenterology and hepatology.* 2012;27(2):231-237.
101. Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free radical research.* 1999;31(4):273-300.
102. Fukuda A, Nakamura Y, Ohigashi H, Osawa T, Uchida K. Cellular response to the redox active lipid peroxidation products: induction of glutathione S-transferase P by 4-hydroxy-2-nonenal. *Biochemical and biophysical research communications.* 1997;236(2):505-509.
103. Yuan JM, Stram DO, Arakawa K, Lee HP, Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer*

Research, cosponsored by the American Society of Preventive Oncology. 2003;12(9):890-898.
104. Koh WP, Yuan JM, Sun CL, et al. Angiotensin I-converting enzyme (ACE) gene polymorphism and breast cancer risk among Chinese women in Singapore. *Cancer research.* 2003;63(3):573-578.
105. Registry SC. Trends in cancer incidence in Singapore 2006–2010. Singapore, Singapore: National Registry of Diseases Office; 2012.
106. Hankin JH, Stram DO, Arakawa K, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. *Nutrition and cancer.* 2001;39(2):187-195.
107. Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic acids research.* 1993;21(16):3761-3766.
108. Willett W. *Nutritional epidemiology.* Vol 40;40.;. New York: Oxford University Press; 2013.
109. Larsson K, Harrysson H, Havenaar R, Alminger M, Undeland I. Formation of malondialdehyde (MDA), 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE) in fish and fish oil during dynamic gastrointestinal in vitro digestion. *Food & function.* 2016;7(2):1176-1187.
110. Leuratti C, Watson MA, Deag EJ, et al. Detection of malondialdehyde DNA adducts in human colorectal mucosa: relationship with diet and the presence of adenomas. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2002;11(3):267-273.
111. Forman, D., Bray, F., Brewster, D.H., et al. Cancer Incidence in Five Continents. IARC, Lyon. 2014.
112. Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Assessment of risk associated with specific fatty acids and colorectal cancer among French-Canadians in Montreal: a case-control study. *International journal of epidemiology.* 2003;32(2):200-209.
113. Wilk JB, Tsai MY, Hanson NQ, Gaziano JM, Djousse L. Plasma and dietary omega-3 fatty acids, fish intake, and heart failure risk in the Physicians' Health Study. *The American journal of clinical nutrition.* 2012;96(4):882-888.