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Zinc and heme iron intakes and risk of colorectal cancer: a population-based prospective cohort study in Japan[1–3]

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ABSTRACT

Background: Food sources and intakes of zinc and heme iron may differ between Western and Asian populations. However, all of the studies on the association between zinc and heme iron intakes and colorectal cancer have been conducted in Western populations.

Objective: We investigated the association between zinc and heme iron intakes and colorectal cancer risk in a Japanese general population.

Design: We conducted a large, population-based prospective study in 39,721 men and 45,376 women aged 45–74 y. Heme iron and zinc intakes were measured by using a validated food-frequency questionnaire in either 1995 or 1998.

Results: During as many as 808,053 person-years of follow-up until the end of 2006, 1284 colorectal cancer cases were identified. In multivariate-adjusted models, zinc and heme iron intakes were not associated with colorectal cancer in either men or women. In comparison with the lowest quartile, the HRs (95% CIs) for developing colorectal cancer in the fourth quartile of zinc and heme iron intakes were 0.77 (0.58, 1.03; *P*-trend = 0.2) and 1.06 (0.79, 1.42; *P*-trend = 0.6), respectively, for men and 1.05 (0.77, 1.44; *P*-trend = 0.4) and 0.88 (0.61, 1.29; *P*-trend = 0.4), respectively, for women.

Conclusion: Our results in a Japanese population with lower intakes and different major food sources of zinc and heme iron in comparison with those of Western populations suggest that zinc and heme iron intakes are not associated with colorectal cancer. *Am J Clin Nutr* 2012;96:864–73.

INTRODUCTION

A recent joint report by the World Cancer Research Fund and the American Institute for Cancer Research concluded that there was "convincing" evidence to support a positive association between colorectal cancer (CRC) and intakes of both red and processed meats (1). Red meat is rich in zinc and heme iron. Zinc is an antioxidant and is involved in various cellular functions, including DNA repair and apoptosis (2), whereas heme iron is a prooxidant and may contribute to colorectal carcinogenesis by promoting free radical production and lipid peroxidation (3–5).

Only 3 prospective studies (6–8) and one case-control study (9) have examined an association of dietary zinc intake with CRC risk, whereas several studies have reported an association between heme iron intake and CRC risk (6–8, 10–13). However, all of these studies were conducted in Western populations, and we know of no data reported for prospective cohort studies in Asian general populations. Food sources of zinc and heme iron might differ between Western and Asian populations, because Asian populations tend to consume more fish (14) and poultry (15) and less red meat (16) than do Western populations. Therefore, similar studies in Asian populations are important to confirm the generalizability of these associations.

In addition, zinc and heme iron concentrations may be modified by alcohol consumption. For example, serum zinc concentrations in alcohol-drinking patients are reportedly lower than those in nondrinkers (17), and this difference may be due to an ethanol-induced increase in urinary zinc excretion (18). In addition, alcohol consumption is known to disrupt iron homeostasis (19–22). Some previous studies have reported that associations of CRC with zinc or heme iron intake are more pronounced among alcohol drinkers (6, 7, 10).

Vitamins B-6 and B-12, which are plentiful in foods rich in zinc and heme iron, such as meat and fish, may also affect the association between zinc and heme iron intakes and CRC risk. We previously reported that vitamins B-6 and B-12 are associated with decreased and increased risk of CRC, respectively (23). Discrepancies between the results of previous studies may be due to confounding or effect modification of vitamin B-6 or vitamin B-12, which was not taken into account previously. Therefore, more studies that consider vitamins B-6 and B-12 as confounding factors are needed.

Here, we investigated the association between zinc and heme iron intakes and CRC risk in a population-based, prospective cohort study in Japan. Our hypothesis was that a higher intake of

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zinc would decrease CRC risk, whereas higher heme iron intake would increase CRC risk. We also considered the effects of vitamin B-6 and B-12 intakes on CRC risk and tested whether any associations depended on alcohol consumption.

SUBJECTS AND METHODS

Study population

The Japan Public Health Center–based Prospective Study was initiated in 1990 for cohort I and in 1993 for cohort II. Participants were all registered Japanese inhabitants of 11 public health center areas and were aged 40–69 y (cohort I: 40–59 y; cohort II: 40–69 y) at the beginning of each cohort's baseline survey. Details of the study design have been described previously (24). The institutional review board of the National Cancer Center, Tokyo, Japan, approved the study. The participants in the present study were subjects in the Japan Public Health Center study who responded to a 5-y follow-up questionnaire during the period from 1995 to 1999 at ages 45–74 y. The present study used the 5-y follow-up survey as a baseline because this survey included a detailed self-administered food-frequency questionnaire (FFQ). The participants from 2 public health center areas (Tokyo and Osaka) were excluded from the present analysis because the selection criteria of participants differed from those in other public health center areas, which left 116,896 participants as the study population. After the exclusion of non-Japanese participants (n = 51), late reports of emigration occurring before the starting point (n = 168), ineligibility due to incorrect birth date (n = 4), and duplicate enrollments (n = 4), we established a population-based cohort of 116,669 participants. After the exclusion of 1626 participants who had died, moved out of the study area, or were lost to follow-up before the starting point, 115,043 eligible participants remained. From these, 91,245 responded to the questionnaire, yielding a response rate of 79.3%. We excluded participants who had been diagnosed with or reported as having CRC before the starting point (n = 605) or who reported extreme total energy intakes (upper 2.5% or lower 2.5%; n = 5543). The final analysis included 85,097 participants (39,721 men and 45,376 women).

Questionnaire

We asked participants to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, and diet. We designed the FFQ to estimate dietary intake from 138 food items and validated it for the estimation of various nutrients and food groups (25). The participants were asked to estimate how often they consumed the individual food items (frequency of intake) and to estimate representative relative portion sizes compared with standard portions during the previous year (26). The FFQ contained questions on frequency (never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, once per day, 2–3 times/d, 4–6 times/d, or ≥7 times/d) and portion sizes relative to a standard portion [small (50% smaller), medium (same as standard), and large (50% larger)]. Daily food intake was calculated by multiplying frequency by standard portion and relative size for each food item in the FFQ. Daily intakes of zinc and iron were calculated by using the Fifth Revised and Enlarged Edition of the Standard Tables of Food Composition in Japan (27). Heme iron intake was calculated by multiplying type-specific percentages of heme iron by total iron content (mg/g) to yield heme iron contents for the reported intake of 16 meat items (7 food groups) and 19 fish and shellfish items (one food group); the percentages of heme iron used for the various types of meat, fish, and shellfish were as follows: 69% for beef; 39% for pork, ham, bacon, and luncheon meat; 26% for chicken, fish, and shellfish (10, 12); and 21% for liver (12). We did not collect information on the use of iron and zinc supplements, because zinc and iron supplementation is reported to be low in Japan (<0.5% in both men and women, and 0.2% in men and 2.4% in women, respectively) (28). Intakes of food and nutrients were log-transformed and adjusted for total energy intake by means of the residual model (29).

The validity of the energy-adjusted zinc or iron intake assessed from the 5-y FFQ was evaluated in a subsample with consecutive 14- or 28-d dietary records. Spearman's correlation coefficients between the energy-adjusted intakes of zinc and iron from the questionnaire and from dietary records were 0.50 and 0.44 (cohort I) and 0.44 and 0.54 (cohort II), respectively, for men and 0.35 and 0.38 (cohort I) and 0.40 and 0.55 (cohort II), respectively, for women (30). We also calculated Spearman's correlation coefficients between the energy-adjusted heme iron intakes from the questionnaire and from dietary records in cohorts I and II: 0.26 and 0.28 for men and 0.11 and 0.27 for women, respectively (A Hara, unpublished data, 2012).

Follow-up and identification of CRC cases

We followed participants from the 5-y follow-up survey until 31 December 2006. We identified changes in residence status (including survival) annually through the residential registry in each area or, for those who had moved out of the area, through the municipal office of the area to which they had moved. Mortality data for persons in the residential registry are forwarded to the Ministry of Health, Labor, and Welfare and are coded for inclusion in the national vital statistics database. Residency registration and death registration are required by the Basic Residential Register Law and the Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period in the present study, 9425 (11.1%) participants died, 3695 (4.4%) moved out of the study area, and 308 (0.4%) were lost to follow-up.

We identified incidence data for CRC by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries. We coded CRC cases according to the *International Classification of Diseases for Oncology, third edition* (31) (C18–C20). We conducted analyses of site-specific cancers: C18 for colon cancer (C18.0–C18.5 for proximal colon cancer and C18.6–C18.7 for distal colon cancer) and C19 and C20 for rectal cancer. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 2.7%.

Statistical analysis

We calculated person-years of follow-up for each participant from the starting point to the date of CRC diagnosis, date of emigration from the study area, date of death, or end of the follow-up (31 December 2006), whichever came first. We censored

participants lost to follow-up at the last confirmed date of presence in the study area.

We calculated HRs and 95% CIs for developing CRC for the categories of energy-adjusted intakes of zinc and heme iron in quartiles for men and women separately, with the lowest consumption category as the reference. We used Cox proportional hazards models with adjustment for potential confounding variables as follows: age (y); public health center area; BMI (in kg/m^2; <18.4, 18.5–19.9, 20–22.4, 22.5–24.9, 25–29.9, or ≥30); smoking status (never, past, or current); alcohol consumption (for men—none; drinker: <150, 150–299, 300–449, or ≥450 g ethanol/wk; for women—none; drinker: <150 or ≥150 g ethanol/wk); quartile of physical activity in metabolic equivalent task-hours/d; history of type 2 diabetes (yes or no); screening examinations for CRC (fecal occult blood test, barium enema, or colonoscopy); menopausal status (premenopausal or natural or induced postmenopausal) and use of exogenous female hormones (never or ever) in women; and quartiles of energy-adjusted intakes of calcium, magnesium, vitamin B-6, vitamin B-12, folic acid, vitamin D, n−3 PUFAs, and fiber.

We calculated *P* values for the analyses of linear trends by assigning ordinal values for categories of zinc and heme iron intakes and entering the values as continuous terms in the regression model. We also statistically evaluated the interactions between sex and zinc and heme iron intakes and between alcohol consumption and zinc and heme iron intakes with regard to the risk of CRC based on the likelihood ratio test with 1 df. The interaction was assessed by a product term consisting of a dichotomous variable for alcohol drinking and an ordinal variable for heme or zinc. We then created an interaction term by multiplying the dichotomous value for alcohol consumption by ordinal values for zinc or heme iron intake. All *P* values are 2-sided, and significance was determined at the $P < 0.05$ level. We performed all statistical analyses with SAS software, version 9.1 (SAS Institute Inc).

RESULTS

During 808,053 person-years of follow-up, we identified 1284 new CRC cases (786 for men, 498 for women).

The major sources of zinc in our population were grains (37%), red meat (15%), pulses (9.5%), dairy products (9.2%), and fish (9.1%). Dietary heme iron was derived mainly from various types of fish and shellfish (49%), beef (20%), and pork, ham, bacon, and luncheon meat (19%).

The characteristics of participants according to zinc and heme iron intakes are shown in **Tables 1** and **2** for men and women, respectively. Mean (±SE) zinc and heme iron intakes were 8.5 ± 0.007 and 0.50 ± 0.001 mg/d, respectively, in men and 7.9 ± 0.004 and 0.44 ± 0.001 mg/d, respectively, in women. Men and women with a high intake of zinc were less likely to be drinkers (alcohol consumption ≥1 g ethanol/wk), were more likely to have a history of type 2 diabetes, and generally consumed more of most of the foods and nutrients listed in Tables 1 and 2, compared with those with a low intake of zinc. Men with a higher zinc intake were less likely to be ever smokers, and women with a higher zinc intake were more likely to be postmenopausal. Both men and women whose heme iron intakes were higher were more likely to consume zinc, vitamin D, vitamin B-6, vitamin B-12, n−3 PUFAs, fish, and red meat and less likely to consume calcium and fiber compared with those whose heme iron intakes were lower. In men, individuals with a higher heme iron intake were less likely to be drinkers than those with a lower heme iron intake. The relations between magnesium, folate, and vegetable intakes and heme iron intake for men differed from the relations for women.

Associations between zinc and heme iron intakes and CRC risk in men and women are shown separately (**Tables 3** and **4**, respectively). In an age- and area-adjusted model, the quartile category of zinc intake was associated with decreased risk of colorectal, colon, and rectal cancer in men, whereas heme iron intake was not associated with CRC risk. However, in multivariate-adjusted models, zinc intake was not significantly associated with the risk of CRC among men; the HRs (95% CIs) for the highest quartile compared with the lowest quartile of zinc intake were 0.77 (0.58, 1.03) for colorectal, 0.76 (0.54, 1.07) for colon, and 0.80 (0.49, 1.32) for rectal cancer. Heme iron intake was not associated with CRC risk, whereas we found significantly higher HRs for vitamin B-12 intake and lower HRs for vitamin B-6 intake in the higher-intake categories in the same multivariate-adjusted model; the HRs (95% CIs) for the highest quartile compared with the lowest quartile were 1.52 (1.05, 2.20; *P*-trend = 0.01) and 0.68 (0.49, 0.94; *P*-trend = 0.009), respectively. Similar results were observed when we evaluated the risk of either proximal or distal colon cancer (data not shown). In women, there was no significant association between zinc and heme iron intakes and CRC risk in the age- and area-adjusted model or in the multivariate-adjusted model. Results were essentially unchanged when analyses were restricted to postmenopausal women (data not shown). There was no statistical interaction between sex and zinc or heme iron intakes with regard to the risk of CRC (all *P* values for interaction were >0.1).

The results of stratified analysis by alcohol intake among men are shown in **Table 5**. We found no significant interactions (all *P* values for interaction were >0.07), although a significant inverse association between zinc intake and CRC risk was observed only among drinkers; HRs (95% CIs) for the highest quartile compared with the lowest quartile of zinc intake were 0.63 (0.47, 0.85; *P*-trend = 0.001) for colorectal, 0.62 (0.43, 0.89; *P*-trend = 0.01) for colon, and 0.67 (0.39, 1.13; *P*-trend = 0.04) for rectal cancer. However, among drinkers, the dose-response was not clear; the HR for men who consumed more alcohol (≥450 g/wk) was similar to those who consumed less alcohol (<150 g/wk) in the highest quartile of zinc intake. No significant association was observed between heme iron intake and CRC risk in the analysis stratified by alcohol intake. These effects of alcohol intake could not be examined in women, because the number of women who consumed ≥150 g alcohol/wk was insufficient. The results among all nondrinkers in women were similar to the results among all women; the HRs (95% CI) for the highest quartile compared with the lowest quartile were 1.10 (0.77, 1.57; *P*-trend = 0.3) for zinc intake and 0.88 (0.58, 1.33; *P*-trend = 0.4) for heme iron intake.

DISCUSSION

To our knowledge, our study is the first large-scale prospective cohort study to evaluate the effect of heme iron and zinc intakes on CRC risk in Asia, where the dietary sources of zinc and heme iron differ from those in Western countries. Zinc and heme iron

TABLE 1
Characteristics of the study participants at the 5-y follow-up survey according to quartiles of energy-adjusted intakes of heme iron and zinc among men in the JPHC study (n = 39,721)[1]

Variable	Q1	Q2	Q3	Q4	P value[2]
Zinc intake					
No. of participants	9930	9930	9931	9930	
Heme iron intake (mg/d)[3]	0.37 ± 0.002[4]	0.45 ± 0.002	0.50 ± 0.002	0.66 ± 0.004	<0.0001
Zinc intake (mg/d)[3]	6.8 ± 0.008	8.2 ± 0.002	8.9 ± 0.002	10.1 ± 0.008	<0.0001
Age (y)	55.4 ± 0.08	56.3 ± 0.08	57.2 ± 0.08	57.8 ± 0.08	<0.0001
BMI (kg/m^2)	23.6 ± 0.03	23.5 ± 0.03	23.6 ± 0.03	23.6 ± 0.03	0.7
Ever smoker (%)	66.8	61.7	57.1	54.5	<0.0001
Alcohol drinker (%)[5]	93.8	79.1	64.8	51.8	<0.0001
METs (MET-h/d)	33.1 ± 0.07	33.0 ± 0.07	33.0 ± 0.07	32.4 ± 0.07	<0.0001
History of type 2 diabetes (%)	6.0	5.8	7.0	10.0	<0.0001
CRC screening, yes (%)[6]	27.9	33.9	34.5	32.8	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	2187 ± 6.2	2133 ± 6.1	2119 ± 6.5	2230 ± 7.2	<0.0001
Calcium (mg/d)	365 ± 1.4	453 ± 1.6	516 ± 1.9	633 ± 2.8	<0.0001
Vitamin D (μg/d)	7.6 ± 0.05	9.5 ± 0.06	10.4 ± 0.06	11.4 ± 0.07	<0.0001
Magnesium (mg/d)	245 ± 0.5	276 ± 0.5	291 ± 0.5	313 ± 0.7	<0.0001
Vitamin B-6 (mg/d)	1.4 ± 0.004	1.5 ± 0.003	1.6 ± 0.003	1.7 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	7.0 ± 0.04	8.4 ± 0.04	9.2 ± 0.04	10.8 ± 0.05	<0.0001
Folate (μg/d)	302 ± 1.1	356 ± 1.2	390 ± 1.3	437 ± 1.6	<0.0001
Fiber (g/d)	9.3 ± 0.04	11.6 ± 0.04	12.6 ± 0.04	13.3 ± 0.05	<0.0001
n−3 PUFAs (g/d)	2.4 ± 0.01	3.0 ± 0.01	3.3 ± 0.01	3.6 ± 0.01	<0.0001
Vegetables (g/d)	150 ± 1.0	188 ± 1.1	211 ± 1.3	228 ± 1.5	<0.0001
Fish (g/d)	73.2 ± 0.5	88.5 ± 0.5	93.7 ± 0.5	99.0 ± 0.6	<0.0001
Red meat (g/d)	36.3 ± 0.3	45.0 ± 0.3	52.6 ± 0.3	70.4 ± 0.5	<0.0001
Heme iron intake					
No. of participants	9930	9930	9931	9930	
Heme iron intake (mg/d)[3]	0.22 ± 0.0008	0.39 ± 0.0004	0.53 ± 0.0005	0.84 ± 0.003	<0.0001
Zinc intake (mg/d)[3]	7.9 ± 0.01	8.2 ± 0.01	8.6 ± 0.01	9.3 ± 0.01	<0.0001
Age (y)	56.5 ± 0.08	56.7 ± 0.08	56.7 ± 0.08	56.7 ± 0.08	0.2
BMI (kg/m^2)	23.4 ± 0.03	23.4 ± 0.03	23.7 ± 0.03	23.8 ± 0.03	<0.0001
Ever smoker (%)	61.5	60.0	59.3	59.2	0.02
Alcohol drinker (%)[5]	77.6	72.8	71.1	68.0	<0.0001
METs (MET-h/d)	33.5 ± 0.07	32.8 ± 0.07	32.6 ± 0.07	32.7 ± 0.07	<0.0001
History of type 2 diabetes (%)	6.0	7.0	8.3	7.5	<0.0001
CRC screening, yes (%)[6]	33.6	33.9	32.4	29.3	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	2286 ± 6.0	2058 ± 6.0	2053 ± 6.3	2273 ± 7.4	<0.0001
Calcium (mg/d)	516 ± 2.8	499 ± 2.1	488 ± 2.0	465 ± 1.9	<0.0001
Vitamin D (μg/d)	6.7 ± 0.04	9.0 ± 0.05	10.6 ± 0.06	12.6 ± 0.08	<0.0001
Magnesium (mg/d)	274 ± 0.7	282 ± 0.6	284 ± 0.6	284 ± 0.6	<0.0001
Vitamin B-6 (mg/d)	1.4 ± 0.003	1.5 ± 0.003	1.6 ± 0.003	1.7 ± 0.004	<0.0001
Vitamin B-12 (μg/d)	5.6 ± 0.02	7.8 ± 0.03	9.5 ± 0.04	12.4 ± 0.06	<0.0001
Folate (μg/d)	349 ± 1.5	367 ± 1.4	377 ± 1.4	391 ± 1.4	<0.0001
Fiber (g/d)	11.8 ± 0.05	11.9 ± 0.05	11.8 ± 0.04	11.2 ± 0.04	<0.0001
n−3 PUFAs (g/d)	2.3 ± 0.008	2.9 ± 0.008	3.3 ± 0.009	3.9 ± 0.014	<0.0001
Vegetables (g/d)	184 ± 1.5	196 ± 1.3	198 ± 1.2	198 ± 1.1	<0.0001
Fish (g/d)	53.6 ± 0.3	79.5 ± 0.4	97.9 ± 0.5	123.2 ± 0.7	<0.0001
Red meat (g/d)	23.3 ± 0.2	39.5 ± 0.2	55.7 ± 0.3	85.8 ± 0.5	<0.0001

[1] CRC, colorectal cancer; JPHC, Japan Public Health Center–based; MET, metabolic equivalent task; Q, quartile.
[2] Derived by using ANOVA or the chi-square test.
[3] All mean total intakes of food and nutrition were energy adjusted.
[4] Mean ± SE (all such values).
[5] Alcohol consumption ≥1 g ethanol/wk.
[6] CRC screening included fecal occult blood test, barium enema, or colonoscopy.

intakes were not significantly associated with CRC risk. We found an inverse association between zinc intake and CRC risk among drinkers in men, although there were no significant interactions.

Food sources of zinc and heme iron vary among ethnic groups and cultures. In the Western diet, the major food sources of dietary zinc are red meat, poultry, dairy foods, whole grains, and fortified cereals (7, 8), whereas in our study's Japanese population, the main sources were grains, red meat, pulses, dairy products, and fish. Fish was also the main food source of heme iron in our study. To date, only 3 cohort studies (6–8) and one case-control study (9) have examined the association between

TABLE 2
Characteristics of the study participants at the 5-y follow-up survey according to quartiles of energy-adjusted intakes of heme iron and zinc among women in the JPHC study (n = 45,376)[1]

Variable	Q1	Q2	Q3	Q4	P value[2]
Zinc intake					
No. of participants	11,344	11,344	11,344	11,344	
Heme iron intake (mg/d)[3]	0.34 ± 0.002[4]	0.40 ± 0.002	0.45 ± 0.002	0.58 ± 0.003	<0.0001
Zinc intake (mg/d)[3]	6.9 ± 0.005	7.7 ± 0.001	8.1 ± 0.001	9.0 ± 0.006	<0.0001
Age (y)	55.9 ± 0.07	57.2 ± 0.07	57.3 ± 0.07	57.9 ± 0.07	<0.0001
BMI (kg/m^2)	23.4 ± 0.03	23.6 ± 0.03	23.6 ± 0.03	23.7 ± 0.03	<0.0001
Ever smoker (%)	8.0	4.4	3.8	4.7	<0.0001
Alcohol drinker (%)[5]	23.0	15.7	14.4	13.0	<0.0001
METs (MET-h/d)	32.1 ± 0.06	32.0 ± 0.06	32.0 ± 0.06	31.6 ± 0.06	<0.0001
History of type 2 diabetes (%)	2.3	3.2	3.8	5.5	<0.0001
CRC screening, yes (%)[6]	27.1	32.3	34.4	33.0	<0.0001
Postmenopausal status (%)	68.3	72.8	73.8	74.3	<0.0001
Ever hormone use (%)	12.4	12.4	12.9	14.1	<0.0001
Dietary intake[3]					
Total energy (kcal/d)	1873 ± 5.3	1812 ± 5.0	1822 ± 5.2	1908 ± 5.9	<0.0001
Calcium (mg/d)	432 ± 1.3	497 ± 1.5	557 ± 1.7	653 ± 2.5	<0.0001
Vitamin D (μg/d)	8.2 ± 0.06	9.4 ± 0.05	10.0 ± 0.05	10.6 ± 0.0	<0.0001
Magnesium (mg/d)	254 ± 0.4	269 ± 0.4	282 ± 0.4	298 ± 0.6	<0.0001
Vitamin B-6 (mg/d)	1.3 ± 0.003	1.4 ± 0.002	1.5 ± 0.002	1.5 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	6.8 ± 0.03	7.9 ± 0.03	8.6 ± 0.04	10.0 ± 0.05	<0.0001
Folate (μg/d)	358 ± 1.2	388 ± 1.2	413 ± 1.3	442 ± 1.5	<0.0001
Fiber (g/d)	12.8 ± 0.04	13.2 ± 0.04	13.6 ± 0.04	13.6 ± 0.05	<0.0001
n−3 PUFAs (g/d)	2.8 ± 0.009	3.1 ± 0.009	3.2 ± 0.008	3.4 ± 0.010	<0.0001
Vegetables (g/d)	202 ± 1.1	223 ± 1.1	236 ± 1.2	240 ± 1.4	<0.0001
Fish (g/d)	75.7 ± 0.5	83.9 ± 0.4	86.7 ± 0.4	89.5 ± 0.5	<0.0001
Red meat (g/d)	34.4 ± 0.2	41.4 ± 0.3	46.0 ± 0.3	58.5 ± 0.4	<0.0001
Heme iron intake					
No. of participants	11,344	11,344	11,344	11,344	
Heme iron intake (mg/d)[3]	0.21 ± 0.0007	0.35 ± 0.0003	0.47 ± 0.0004	0.74 ± 0.002	<0.0001
Zinc intake (mg/d)[3]	7.6 ± 0.009	7.7 ± 0.007	7.9 ± 0.007	8.4 ± 0.008	<0.0001
Age (y)	57.1 ± 0.07	57.3 ± 0.07	57.0 ± 0.07	57.0 ± 0.07	0.003
BMI (kg/m^2)	23.4 ± 0.03	23.5 ± 0.03	23.6 ± 0.03	23.9 ± 0.03	<0.0001
Ever smoker (%)	4.6	4.5	5.4	6.4	<0.0001
Alcohol drinker (%)[5]	16.2	16.3	17.1	16.6	0.2
METs (MET-h/d)	32.2 ± 0.06	31.9 ± 0.06	31.8 ± 0.06	31.8 ± 0.06	<0.0001
History of type 2 diabetes (%)	3.3	3.7	4.0	3.8	0.04
CRC screening, yes (%)[6]	33.7	32.8	31.4	29.0	<0.0001
Postmenopausal status (%)	73.1	72.9	71.7	71.6	0.06
Ever hormone use (%)	12.7	12.3	13.5	13.2	0.001
Dietary intake[3]					
Total energy (kcal/d)	1969 ± 5.0	1734 ± 4.7	1756 ± 5.1	1957 ± 6.1	<0.0001
Calcium (mg/d)	591 ± 2.3	542 ± 1.8	522 ± 1.8	484 ± 1.7	<0.0001
Vitamin D (μg/d)	7.0 ± 0.04	9.0 ± 0.04	10.4 ± 0.05	11.8 ± 0.07	<0.0001
Magnesium (mg/d)	282 ± 0.5	278 ± 0.5	274 ± 0.5	269 ± 0.5	<0.0001
Vitamin B-6 (mg/d)	1.3 ± 0.003	1.4 ± 0.002	1.5 ± 0.002	1.6 ± 0.003	<0.0001
Vitamin B-12 (μg/d)	5.5 ± 0.02	7.4 ± 0.02	9.0 ± 0.03	11.3 ± 0.05	<0.0001
Folate (μg/d)	404 ± 1.4	402 ± 1.3	397 ± 1.3	398 ± 1.3	0.0004
Fiber (g/d)	14.5 ± 0.05	13.7 ± 0.04	13.0 ± 0.04	11.9 ± 0.04	<0.0001
n−3 PUFAs (g/d)	2.5 ± 0.007	3.0 ± 0.007	3.3 ± 0.008	3.7 ± 0.011	<0.0001
Vegetables (g/d)	238 ± 1.4	230 ± 1.2	223 ± 1.1	211 ± 1.1	<0.0001
Fish (g/d)	54.8 ± 0.3	76.5 ± 0.3	92.7 ± 0.4	111.7 ± 0.6	<0.0001
Red meat (g/d)	21.4 ± 0.1	35.1 ± 0.2	49.1 ± 0.2	74.7 ± 0.4	<0.0001

[1] CRC, colorectal cancer; JPHC, Japan Public Health Center–based; MET, metabolic equivalent task; Q, quartile.
[2] Derived by using ANOVA or the chi-square test.
[3] All mean total intakes of food and nutrition were energy adjusted.
[4] Mean ± SE (all such values).
[5] Alcohol consumption ≥1 g ethanol/wk.
[6] CRC screening included fecal occult blood test, barium enema, or colonoscopy.

zinc intake and CRC risk, and all of these studies involved Western populations. The results indicated a decreased risk of CRC of zinc intake in 2 studies from the United States (6, 8) and one from Australia (9) but not in a study from Sweden (7). In the present study, we observed no significant association between zinc intake and CRC risk in the general population of Japan,

TABLE 3
HRs and 95% CIs for colorectal cancer risk according to quartiles of intakes of zinc and heme iron among men[1]

	Q1	Q2	Q3	Q4	*P*-trend
Zinc intake					
Median (mg/d)	7.05	8.19	8.9	9.83	
Person-years	91,139	93,391	93,237	92,355	
Colorectal cancer					
No. of cases	230	182	185	189	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.71 (0.58, 0.86)	0.68 (0.56, 0.83)	0.70 (0.57, 0.85)	0.0004
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.79 (0.63, 0.98)	0.80 (0.63, 1.03)	0.77 (0.58, 1.03)	0.2
Colon cancer					
No. of cases	157	116	130	124	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.66 (0.52, 0.84)	0.71 (0.56, 0.90)	0.68 (0.54, 0.87)	0.005
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.74 (0.57, 0.97)	0.84 (0.62, 1.13)	0.76 (0.54, 1.07)	0.3
Rectal cancer					
No. of cases	73	66	55	65	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.81 (0.58, 1.13)	0.63 (0.44, 0.90)	0.73 (0.52, 1.02)	0.03
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.87 (0.60, 1.28)	0.72 (0.46, 1.12)	0.80 (0.49, 1.32)	0.3
Heme iron intake					
Median (mg/d)	0.24	0.39	0.53	0.77	
Person-years	94,132	92,107	91,799	92,084	
Colorectal cancer					
No. of cases	214	174	194	204	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.85 (0.69, 1.03)	0.99 (0.81, 1.21)	1.06 (0.87, 1.29)	0.3
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.87 (0.69, 1.08)	1.00 (0.78, 1.28)	1.06 (0.79, 1.42)	0.6
Colon cancer					
No. of cases	149	111	132	135	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.61, 1.00)	0.98 (0.78, 1.25)	1.02 (0.80, 1.29)	0.6
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.77 (0.59, 1.01)	0.97 (0.72, 1.30)	1.02 (0.71, 1.46)	0.7
Rectal cancer					
No. of cases	65	63	62	69	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.99 (0.70, 1.40)	1.01 (0.71, 1.44)	1.15 (0.82, 1.63)	0.4
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	1.11 (0.75, 1.63)	1.09 (0.70, 1.71)	1.17 (0.69, 1.98)	0.6

[1] Cox proportional hazards models were used. Q, quartile.
[2] Adjusted for age, area, BMI, smoking status, ethanol intake, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Zinc and heme iron intakes were simultaneously included in the model.

where the major food sources of zinc differ from those in Western countries and where zinc intake is lower than that in Western countries; the intake ranges of the third quintiles in Western studies were 9.7–10.3 mg/d (7) to 11.5–14.8 mg/d (6).

Six cohort studies reported an association between heme iron intake and CRC risk, but the association remains controversial (6–8, 10–12). A recent meta-analysis of 5 of these studies suggests a modest positive association between heme iron intake and colon cancer risk (highest quintile compared with lowest quintile; HR: 1.18; 95% CI: 1.06, 1.32) (13). The controversy may be due to confounding or effect modification of vitamin B-6 or vitamin B-12, which was not taken into account previously. These vitamins are present in some of the same foods as heme iron, such as meat and fish (27) (the correlation coefficients between vitamins B-6 and B-12 and heme iron in this study were 0.40 and 0.58 in men and 0.31 and 0.54 in women, respectively). In addition, we previously reported that higher vitamin B-6 intake is associated with a decreased risk of CRC, whereas high vitamin B-12 intake tends to increase CRC risk (*P*-trend = 0.05) in men (23). Vitamins B-6 and B-12 are coenzymes in one-carbon metabolism, which is critical for the synthesis and methylation of DNA (32, 33). Low dietary intake of these nutrients may result in colon carcinogenesis via the induction of aberrations in DNA methylation and synthesis (34, 35). However, high concentrations of vitamin B-12 may also induce hypermethylation in this pathway. Associations of vitamin B-12 with DNA methylation have been observed in rats (36, 37) and humans (38, 39), although the idea that DNA methylation is a cause of CRC remains speculative. In the present study, we found significantly higher CRC risk associated with high vitamin B-12 intake, lower CRC risk associated with high vitamin B-6 intake, and no association of CRC risk with heme iron intake in the multivariate model. These findings suggest that heme iron intake may only partly explain the apparent increased risk of CRC and that other factors present in foods along with heme iron, such as vitamin B-12, might be involved in colorectal carcinogenesis. In addition, differences in other constituents of the major food sources of heme iron between Western and Japanese populations might have produced the different associations with CRC risk observed in previous studies and the present study. Major sources of heme iron in Western populations are red and processed meats. Other constituents of red and processed meats, such as nitrate and heterocyclic amines, reportedly increase CRC risk (11). In contrast, fish and shellfish were the major sources of heme iron in the Japanese population. These foods also include nutrients such as n−3 PUFAs and vitamin D, which protect against CRC (40, 41).

TABLE 4
HRs and 95% CIs for colorectal cancer risk according to quartiles of intakes of zinc and heme iron among women[1]

	Q1	Q2	Q3	Q4	*P*-trend
Zinc intake					
Median (mg/d)	7.04	7.67	8.14	8.83	
Person-years	107,466	109,520	110,258	110,687	
Colorectal cancer					
No. of cases	107	110	143	138	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.93 (0.71, 1.22)	1.19 (0.92, 1.53)	1.10 (0.85, 1.43)	0.2
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.92 (0.70, 1.22)	1.16 (0.88, 1.54)	1.05 (0.77, 1.44)	0.4
Colon cancer					
No. of cases	71	76	105	99	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.96 (0.69, 1.33)	1.30 (0.96, 1.77)	1.18 (0.87, 1.61)	0.1
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.97 (0.69, 1.36)	1.33 (0.95, 1.86)	1.19 (0.81, 1.74)	0.2
Rectal cancer					
No. of cases	36	34	38	39	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.88 (0.55, 1.40)	0.95 (0.60, 1.51)	0.95 (0.60, 1.51)	0.9
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.83 (0.51, 1.35)	0.85 (0.51, 1.41)	0.81 (0.46, 1.43)	0.5
Heme iron intake					
Median (mg/d)	0.23	0.35	0.47	0.67	
Person-years	110,299	108,752	109,056	109,823	
Colorectal cancer					
No. of cases	128	127	120	123	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	1.02 (0.80, 1.30)	1.01 (0.78, 1.29)	1.05 (0.81, 1.35)	0.8
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.96 (0.73, 1.26)	0.89 (0.65, 1.22)	0.88 (0.61, 1.29)	0.4
Colon cancer					
No. of cases	88	95	80	88	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	1.11 (0.83, 1.48)	0.98 (0.72, 1.33)	1.10 (0.82, 1.48)	0.7
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	1.05 (0.76, 1.44)	0.88 (0.60, 1.28)	0.94 (0.60, 1.46)	0.6
Rectal cancer					
No. of cases	40	32	40	35	
Age- and area-adjusted HR (95% CI)	1.00 (reference)	0.83 (0.52, 1.32)	1.05 (0.68, 1.64)	0.93 (0.58, 1.48)	0.99
Multivariate-adjusted HR (95% CI)[2]	1.00 (reference)	0.77 (0.47, 1.29)	0.93 (0.53, 1.63)	0.78 (0.39, 1.58)	0.6

[1] Cox proportional hazards models were used. Q, quartile.
[2] Adjusted for age, area, BMI, smoking status, ethanol intake, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, menopausal status, use of exogenous female hormones, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Zinc and heme iron intakes were simultaneously included in the model.

In the present study, we observed a significant inverse association between high zinc intake and CRC risk among drinkers in men. However, there was no significant interaction and the dose-response was not clear, which suggests that the inverse association between zinc intake and CRC risk among drinkers in men might be a random finding. Alternatively, the study might be underpowered to detect the significant interaction between alcohol consumption and zinc intakes with regard to CRC risk. Further research in a large population is needed to investigate the interaction with alcohol consumption.

The strength of this study was its prospective design, which enabled us to avoid exposure recall bias. Participants were selected from the general population, the sample size was large, the response rate for the surveys was acceptable for studies of settings such as this, and the loss to follow-up was negligible. In addition, the cancer registry was of sufficient quality to reduce misclassification of the outcomes.

Several limitations of the study warrant mention. First, we assessed zinc and iron intakes by using an FFQ, and heme iron content values were calculated on the basis of type-specific percentages of total iron content. In addition, in our FFQ, we included only 2 types of shellfish that were rich in zinc. Therefore, there may have been some misclassification of zinc and heme iron intakes. Especially for heme iron intake in women, the lower validity of the FFQ (r = 0.11–0.27 for women) may have resulted in the misclassification of individual intakes. The lack of association between heme iron intake and CRC risk among women in the present study may have been due partly to the poor validity of the FFQ for heme iron intake. However, no previous study has reported the validation of heme iron intake from an FFQ. Two studies showed the validation of iron intake (8) or major food sources of dietary heme iron, such as red meat and processed meat (8, 10), but none of the other studies showed the validation of heme iron intake (6, 7, 9, 11, 12). Therefore, we could not compare our validation of heme iron intake to validations in previous studies. Second, we did not collect information on zinc and iron supplement use. However, a survey of supplement use in Japan from 2000 to 2002 showed that the prevalence of zinc and iron supplementation is low (<0.5% in both men and women and 0.2% in men and 2.4% in women, respectively) (28), and thus we considered intake from supplements to be negligible. Third, although we measured and adjusted for possible confounding variables to the extent possible, the possibility of unmeasured confounding variables cannot be totally disregarded. Also, some of the significant findings may have been due to chance.

In conclusion, in this large-scale, population-based prospective cohort study in middle-aged Japanese men and women, whose

TABLE 5
HRs and 95% CIs for colorectal cancer risk among men according to quartiles of zinc and heme iron intakes and alcohol consumption[1]

	Q1		Q2		Q3		Q4			
	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	No. of cases	HR (95% CI)[2]	*P*-trend	*P*-interaction
Zinc intake										
Colorectal cancer										
Nondrinker	14	1.00 (ref)	30	0.69 (0.36, 1.33)	66	0.86 (0.46, 1.61)	80	0.75 (0.39, 1.46)	0.8	0.1
Drinker[3] (overall)	216	1.00 (ref)	150	0.74 (0.59, 0.92)	113	0.62 (0.48, 0.81)	101	0.63 (0.47, 0.85)	0.001	
<150 g/wk	12	1.00 (ref)	29	0.71 (0.35, 1.43)	46	0.66 (0.33, 1.33)	47	0.58 (0.27, 1.23)	0.2	
150–299 g/wk	33	1.00 (ref)	49	0.83 (0.52, 1.32)	33	0.75 (0.44, 1.29)	32	1.08 (0.59, 1.96)	0.9	
300–449 g/wk	51	1.00 (ref)	45	0.91 (0.59, 1.41)	24	0.77 (0.44, 1.36)	17	0.87 (0.44, 1.70)	0.7	
≥450 g/wk	120	1.00 (ref)	27	0.74 (0.47, 1.19)	10	0.67 (0.33, 1.38)	5	0.55 (0.21, 1.47)	0.1	
Colon cancer										
Nondrinker	7	1.00 (ref)	22	1.05 (0.44, 2.51)	48	1.32 (0.57, 3.07)	53	1.08 (0.44, 2.63)	0.9	0.07
Drinker[3] (overall)	150	1.00 (ref)	92	0.65 (0.49, 0.86)	79	0.63 (0.46, 0.86)	68	0.62 (0.43, 0.89)	0.01	
<150 g/wk	10	1.00 (ref)	19	0.57 (0.26, 1.26)	29	0.48 (0.22, 1.07)	29	0.41 (0.17, 0.99)	0.08	
150–299 g/wk	20	1.00 (ref)	27	0.69 (0.38, 1.26)	28	0.94 (0.49, 1.80)	21	1.00 (0.47, 2.10)	0.7	
300–449 g/wk	37	1.00 (ref)	30	0.87 (0.52, 1.48)	15	0.66 (0.33, 1.33)	15	1.11 (0.52, 2.38)	0.9	
≥450 g/wk	83	1.00 (ref)	16	0.57 (0.31, 1.02)	7	0.61 (0.26, 1.44)	3	0.45 (0.13, 1.56)	0.08	
Rectal cancer										
Nondrinker	7	1.00 (ref)	8	0.30 (0.10, 0.88)	18	0.36 (0.13, 0.96)	27	0.37 (0.13, 1.06)	0.4	1.0
Drinker[3] (overall)	66	1.00 (ref)	58	0.93 (0.63, 1.37)	34	0.61 (0.38, 0.98)	33	0.67 (0.39, 1.13)	0.04	
<150 g/wk	2	1.00 (ref)	10	1.45 (0.31, 6.87)	17	1.54 (0.33, 7.28)	18	1.42 (0.28, 7.23)	0.9	
150–299 g/wk	13	1.00 (ref)	22	1.04 (0.50, 2.17)	5	0.36 (0.12, 1.10)	11	1.32 (0.48, 3.67)	0.8	
300–449 g/wk	14	1.00 (ref)	15	0.99 (0.44, 2.24)	9	1.04 (0.37, 2.87)	2	0.31 (0.06, 1.62)	0.3	
≥450 g/wk	37	1.00 (ref)	11	1.27 (0.58, 2.79)	3	0.78 (0.21, 2.95)	2	0.89 (0.18, 4.54)	0.9	
Heme iron intake										
Colorectal cancer										
Nondrinker	45	1.00 (ref)	42	0.88 (0.55, 1.40)	46	0.91 (0.54, 1.53)	57	1.10 (0.60, 2.04)	0.7	0.7
Drinker[3] (overall)	166	1.00 (ref)	127	0.83 (0.64, 1.07)	145	1.02 (0.77, 1.36)	142	1.04 (0.74, 1.47)	0.6	
<150 g/wk	25	1.00 (ref)	27	0.87 (0.48, 1.57)	44	1.30 (0.70, 2.41)	38	1.13 (0.53, 2.38)	0.5	
150–299 g/wk	33	1.00 (ref)	39	1.04 (0.62, 1.74)	39	1.04 (0.57, 1.87)	36	0.92 (0.44, 1.91)	0.8	
300–449 g/wk	47	1.00 (ref)	23	0.56 (0.33, 0.98)	31	0.91 (0.50, 1.66)	36	1.11 (0.54, 2.30)	0.6	
≥450 g/wk	61	1.00 (ref)	38	0.94 (0.60, 1.49)	31	1.01 (0.59, 1.72)	32	1.13 (0.60, 2.13)	0.8	
Colon cancer										
Nondrinker	33	1.00 (ref)	28	0.87 (0.50, 1.52)	32	1.03 (0.56, 1.91)	37	1.30 (0.63, 2.72)	0.5	0.4
Drinker[3] (overall)	114	1.00 (ref)	80	0.72 (0.53, 0.99)	98	0.97 (0.69, 1.37)	97	1.01 (0.66, 1.53)	0.7	
<150 g/wk	19	1.00 (ref)	16	0.67 (0.33, 1.39)	28	1.14 (0.54, 2.40)	24	1.01 (0.41, 2.51)	0.7	
150–299 g/wk	21	1.00 (ref)	24	0.92 (0.48, 1.76)	27	0.98 (0.47, 2.03)	24	0.80 (0.32, 1.95)	0.7	
300–449 g/wk	33	1.00 (ref)	15	0.49 (0.25, 0.96)	21	0.84 (0.41, 1.73)	28	1.09 (0.46, 2.59)	0.8	
≥450 g/wk	41	1.00 (ref)	25	0.88 (0.51, 1.53)	22	1.08 (0.57, 2.04)	21	1.30 (0.60, 2.82)	0.5	
Rectal cancer										
Nondrinker	12	1.00 (ref)	14	0.91 (0.37, 2.21)	14	0.71 (0.26, 1.95)	20	0.78 (0.25, 2.44)	0.7	0.5
Drinker[3] (overall)	52	1.00 (ref)	47	1.08 (0.70, 1.68)	47	1.13 (0.68, 1.88)	45	1.13 (0.61, 2.08)	0.7	
<150 g/wk	6	1.00 (ref)	11	1.51 (0.51, 4.48)	16	1.90 (0.59, 6.10)	14	1.60 (0.41, 6.25)	0.6	
150–299 g/wk	12	1.00 (ref)	15	1.30 (0.56, 3.05)	12	1.13 (0.40, 3.18)	12	1.20 (0.34, 4.21)	0.9	
300–449 g/wk	14	1.00 (ref)	8	0.86 (0.32, 2.34)	10	1.23 (0.40, 3.74)	8	1.13 (0.27, 4.68)	0.7	
≥450 g/wk	20	1.00 (ref)	13	1.05 (0.48, 2.31)	9	0.82 (0.31, 2.17)	11	0.81 (0.26, 2.55)	0.7	

[1] Cox proportional hazards models were used. Q, quartile; ref, reference.
[2] Adjusted for age, public health center area, BMI, smoking status, metabolic equivalent tasks, history of type 2 diabetes, screening for colorectal cancer, and intakes of energy-adjusted magnesium, vitamin B-6, vitamin B-12, folate, calcium, vitamin D, n−3 PUFAs, and fiber. Heme iron and zinc intakes were simultaneously included in the model.
[3] Alcohol consumption ≥1 g ethanol/wk.

sources of zinc and heme iron intakes differed from those of Western populations and whose intakes of zinc and heme iron were moderate by Western standards, we found no substantial association between dietary zinc and heme iron intakes and CRC.

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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Pathway Analyses Identify *TGFBR2* as Potential Breast Cancer Susceptibility Gene: Results from a Consortium Study among Asians

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Abstract

Background: The TGF-β signaling pathway plays a significant role in the carcinogenic process of breast cancer.

Methods: We systematically evaluated associations of common variants in TGF-β signaling pathway genes with breast cancer risk using a multistage, case–control study among Asian women.

Results: In the first stage, 341 single-nucleotide polymorphisms with minor allele frequencies ≥ 0.05 across 11 genes were evaluated among 2,926 cases and 2,380 controls recruited as a part of the Shanghai Breast Cancer Genetics Study (SBCGS). In the second stage, 20 SNPs with promising associations were evaluated among an additional 1,890 cases and 2,000 controls from the SBCGS. One variant, *TGFBR2 rs1078985*, had highly consistent and significant associations with breast cancer risk among participants in both study stages, as well as promising results from *in silico* analysis. Additional genotyping was carried out among 2,475 cases and 2,343 controls from the SBCGS, as well as among 5,077 cases and 5,384 controls from six studies in the Asian Breast Cancer Consortium (stage III). Pooled analysis of all data indicated that minor allele homozygotes (*GG*) of *TGFBR2 rs1078985* had a 24% reduced risk of breast cancer compared with major allele carriers (*AG* or *AA*; OR, 0.76; 95% CI, 0.65–0.89; $P = 8.42 \times 10^{-4}$).

Conclusion: These findings support a role for common genetic variation in TGF-β signaling pathway genes, specifically in *TGFBR2*, in breast cancer susceptibility.

Impact: These findings may provide new insights into the etiology of breast cancer as well as future potential therapeutic targets. *Cancer Epidemiol Biomarkers Prev; 21(7); 1176–84. ©2012 AACR.*

Introduction

The TGF-β signaling pathway is composed of several multifunctional cytokines and receptors that are involved in regulating various essential cellular processes including growth, differentiation, apoptosis, angiogenesis, and homeostasis (1, 2). This pathway also plays an important role in the development and progression of multiple human diseases, such as cancer, asthma, autoimmune, and cardiovascular diseases (2–6). In the context of cancer, the TGF-β signaling pathway has both tumor-suppressing and tumor-promoting functions depending upon the cellular context. In the early stages of cancer, TGF-β signaling

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can inhibit tumor growth, whereas in later stage cancers, tumor invasiveness, and metastasis are promoted by TGF-β signaling (1, 3).

Signal transduction of the TGF-β ligands [TGF-β1, TGF-β2, and TGF-β3] is mediated through interactions with their receptors. The ligands first bind to TGFBR2, a transmembrane serine/threonine protein kinase receptor; this interaction is sometimes mediated by TGFBR3. This complex then binds to and activates TGF-β receptor 1 (TGFBR1), which in turn phosphorylates SMAD family member 2 (SMAD2) and SMAD3. Phosphorylated SMAD2 and SMAD3, in association with SMAD4, form a complex which accumulates in the nucleus and acts as a transcription factor to regulate target genes. SMAD7 can block the activation of SMAD2 and SMAD3, while the SMAD anchor for receptor activation (SARA, also known as ZFYVE9) stabilizes the SMAD4–TGFBR1 interaction (7, 8). Genetic variation in constituents of the TGF-β signaling pathway may result in altered protein function, increased or decreased target gene transcription, and therefore, the development and progression of breast cancer.

Although there is considerable biologic plausibility for the involvement of the TGF-β signaling pathway in the development of breast cancer, limited information is available about the impact of genetic variation on breast cancer risk. Most studies of genetic variation in TGF-β signaling pathway genes and breast cancer risk have focused on a few single-nucleotide polymorphisms (SNP), and findings have been inconsistent. The most extensively studied variant in the TGF-β pathway is a SNP located in exon 1 of the *TGFB1* gene (*T29C*, also known as *rs1982073*, which merged into *rs1800470*; refs. 7, 9–13). Although this SNP was shown to be associated with increased TGFB1 protein secretion, its association with breast cancer risk has been inconsistent (7, 9–12). Our recent field synopsis included data from 32 studies for this variant; no significant association with breast cancer risk was found using allelic, dominant, or recessive models (10). Notably, results from a large study that evaluated 354 genetic variants in 17 TGF-β pathway genes for associations with breast cancer risk among women of European ancestry found that only this SNP (*rs1982073*, which merged into *rs1800470*) retained statistical significance after correction for multiple comparisons in analyses of progesterone receptor negative (PR−) breast cancer (14). Three other *TGFB1* variants (*rs1800468*, *rs1800469*, and *rs1800471*) and one *TGFBR1* variant (*rs11466445*) have also been previously evaluated; meta-analyses for these SNPs have not found significant associations with breast cancer risk (10, 15, 16).

This study was undertaken to comprehensively evaluate the associations of genetic variants in the TGF-β signaling pathway with breast cancer risk among Asian women. In the discovery stage, 341 genetic variants in 11 TGF-β pathway genes [*TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, TGF-β receptor 2 (*TGFBR2*), TGF-β receptor 3 (*TGFBR3*), *SMAD2*, *SMAD3*, *SMAD4*, *SMAD7*, and *SARA*] were evaluated among 2,926 cases and 2,380 controls from studies of Chinese women in Shanghai. Promising SNPs were then evaluated for replication of associations with breast cancer risk among an additional 1,890 cases and 2,000 controls from Shanghai. Finally, one SNP was further genotyped among 7,552 cases and 7,727 controls, comprised of 7 additional independent studies conducted among Chinese and Japanese women, as part of the Asian Breast Cancer Consortium.

Materials and Methods

Study population

The Shanghai Breast Cancer Genetics Study (SBCGS) includes data from 4 population-based studies conducted among Chinese women in urban Shanghai: the Shanghai Breast Cancer Study (SBCS; refs. 17, 18), the Shanghai Breast Cancer Survival Study (SBCSS; ref. 19), the Shanghai Endometrial Cancer Study (SECS; ref. 20), and the Shanghai Women's Health Study (SWHS; ref. 21). Details of these studies have been described previously (17). Briefly, the SBCS is a 2-stage (SBCS-I and SBCS-II), population-based, case–control study. SBCS-I recruitment occurred between August 1996 and March 1998; SBCS-II recruitment occurred between April 2002 and February 2005. The SBCSS included newly diagnosed breast cancer cases ascertained via the population-based Shanghai Cancer Registry between April 2002 and December 2006. The SECS is a population-based, case–control study of endometrial cancer conducted between January 1997 and December 2003 using a protocol similar to the SBCS; only community controls from the SECS were included in the SBCGS. The SWHS is a population-based cohort study of women from urban communities in Shanghai who were recruited between 1996 and 2000. In this analysis, stage I (SBCGS I) included 2,926 cases and 2,380 controls from the SBCS, SBCSS, and SWHS; stage II (SBCGS II) included 1,890 cases and 2,000 controls from the SBCS, SBCSS, SWHS, and SECS. Stage III included 2,475 cases from the SBCSS and 2,343 controls from the SWHS and SECS (SBCGS III), as well as data from 6 collaboration studies, including 2,095 women from Taiwan (22, 23); 1,050 women from Hong Kong (24); 3,580 women from Nanjing, China (25, 26); 1,657 women from Guangzhou, China; 1,284 women from Nagoya, Japan (27); and 795 women from Nagano, Japan (28), participating in the Asian Breast Cancer Consortium. Appropriate approval was granted from all relevant Institutional Review Boards and all included participants provided informed consent.

Genotyping and quality control

Over the past few years, we have genotyped TGF-β signaling pathway genes in several projects. To maximize our coverage of genetic variation for these genes, in the discovery stage, we included all genotyping data generated in these projects for this analysis. First, 88 haplotype

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tagging SNPs (htSNPs) in 11 genes were genotyped among 2,083 participants using a targeted genotyping system (Affymetrix Inc.). Second, 412 SNPs in 11 TGF-β pathway genes were genotyped as part of the Affymetrix Genome-Wide SNP Array 6.0 (Affymetrix Inc.) for 5,242 participants. Third, one SNP (*rs1800469*) was genotyped by TaqMan (Applied Biosystems) among 1,978 participants. Fourth, 3 SNPs (*rs1800469*, *rs1800470*, and *rs1800471*) were genotyped by RFLP among 2,277 participants. Finally, 2 SNPs (*rs1461085* and *rs2026811*) were genotyped with the Sequenom iPLEX MassARRAY platform (Sequenom) among 1,978 participants. Twenty-seven SNPs were genotyped by more than one method, so that the total number of SNPs genotyped was 479. Analysis for stage I was conducted for 341 SNPs with a minimum minor allele frequency (MAF) of 5% among genotyped controls. Twenty promising SNPs were selected for additional stage II genotyping by the Sequenom iPLEX MassARRAY platform (stage II). One replicated SNP (*rs1078985*) was further evaluated among participants of SBCGS III and 6 Asian collaboration studies (stage III). Genotyping of these women was also carried out with the Sequenom iPLEX MassARRAY. For all genotyping methods, blinded duplicate samples and quality controls (QC) were included as described previously (17, 18). All included SNPs had call rates and concordance rates of at least 95% among duplicates within each platform, as well as across genotyping platforms. Laboratory personnel were blinded to the case–control and QC status of all samples.

Statistical analysis

All statistical analyses, except where noted, were conducted with SAS version 9.2 (SAS Institute Inc.) and Stata 11.0 (Stata Corporation). Characteristics of demographic data between cases and controls were compared with the χ^2 or *t* test for categorical or continuous variables, respectively. Hardy–Weinberg equilibrium among controls was evaluated using χ^2 tests. ORs and corresponding CIs were determined by logistic regression models that included adjustment for age. Additive, dominant, and recessive models of effect were used for all SNPs. Interactions were evaluated using likelihood ratio tests for nested models; case-only analyses were used to evaluate associations between SNPs and tumor characteristics. Heterogeneity between stages I and II results was evaluated using the Cochran's Q statistic; significant heterogeneity was determined by $P \leq 0.10$ (29). Pooled analysis was conducted with data from the SBCGS and the Asian Breast Cancer Consortium for the association between *TGFBR2 rs1078985* and breast cancer risk. Linkage disequilibrium (LD) was assessed with SNAP (30). The Bonferroni adjustment was used to address the issue of false positive findings arising from multiple comparisons. Quanto was used for power calculations (31). All statistical tests were 2-tailed, and $P \leq 0.05$ was interpreted as statistically significant unless otherwise indicated.

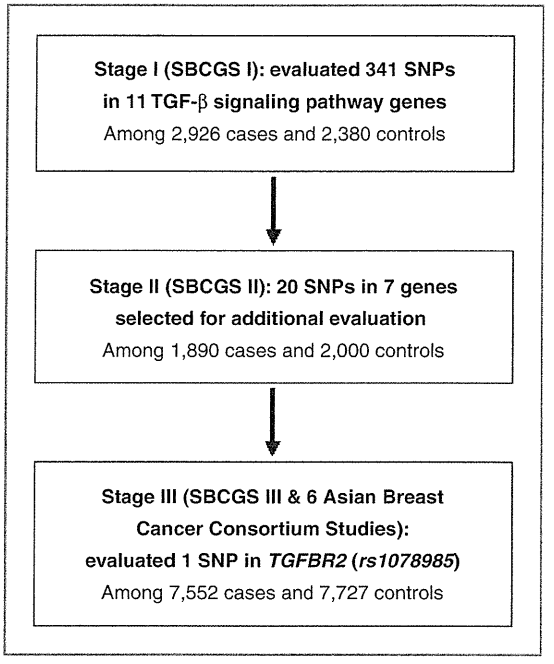

Figure 1. Study design.

Results

A 3-stage study design was used (Fig. 1). In total, 12,368 breast cancer cases and 12,107 controls were included in this analysis (Table 1). The 3 study stages included 2,926 cases and 2,380 controls from the SBCGS (stage I), 1,890 cases and 2,000 controls from the SBCGS (stage II), and 7,552 cases and 7,727 controls (stage III) from the SBCGS and the Asian Breast Cancer Consortium. Overall, cases were slightly older and more likely to be postmenopausal than controls. Information on breast cancer stage was available for the majority of the cases (91.8%) from the Shanghai studies; only 193 (3.0%) were *in situ* breast cancer (data not shown). Information about included SNPs and genetic coverage of 11 TGF-β signaling pathway genes is shown in Table 2. In stage I, a total of 479 SNPs were genotyped across the *TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGFBR2*, *TGFBR3*, *SMAD2*, *SMAD3*, *SMAD4*, *SMAD7*, and *SARA* genes; of these, 341 had MAFs $\geq$ 5% among controls in our study population (Supplementary Table S1). Our coverage of the polymorphisms in these 11 genes (MAFs $\geq$ 5%) was estimated to be approximately 83.6% using an $r^2 = 0.8$ and a pairwise tagging approach.

Associations with breast cancer risk for the 341 TGF-β signaling pathway SNPs with MAFs $\geq$ 5% yielded significant *P* values from additive, dominant, or recessive models for 43 SNPs (Supplementary Table S2). When possible, consistency of associations between SBCS-I and SBCS-II study populations was assessed; 5 SNPs (*rs6696224*, *rs10493858*, *rs12132114*, *rs11165293*, and

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Table 1. Selected characteristics of participants in the Asian Breast Cancer Consortium by study stage

Study stage	Ethnicity	Cases	Controls	Mean age[a]	% Postmenopausal[a]	ER+[b]
Stage I						
SBCGS I	Chinese	2,926	2,380	**51.7/50.2**	42.9/41.4	1,581 (54.3%)
Stage II						
SBCGS II	Chinese	1,890	2,000	52.8/53.3	**48.8/55.0**	924 (59.9%)
Stage III						
SBCGS III	Chinese	2,475	2,343	**53.8/55.0**	50.4/52.5	1,542 (62.3%)
Taiwan	Chinese	1,049	1,046	**51.6/47.5**	**52.6/39.6**	634 (66.1%)
Hong Kong	Chinese	429	621	45.8/45.6	50.3/41.8	157 (70.4%)
Nanjing	Chinese	1,757	1,823	50.6/50.2	50.9/47.0	651 (54.9%)
Guangzhou	Chinese	804	853	49.0/49.2	**39.8/50.7**	156 (73.6%)
Nagoya	Japanese	640	644	51.4/51.1	48.4/48.5	353 (73.2%)
Nagano	Japanese	398	397	53.8/54.1	**54.8/65.7**	294 (74.4%)
Total		12,368	12,107	**51.8/51.3**	**47.8/47.0**	

[a]Cases/controls; bold values denote significant difference at $P \leq 0.01$.
[b]Number and percentage of estrogen receptor positive (ER^+) breast cancer cases among those with data available.

rs12562433) in 4 loci had inconsistent associations between the 2 SBCS study populations and were not further evaluated. Using an $r^2 \geq 0.3$, the remaining variants were found to represent 20 independent loci. One SNP from each of these loci was selected for additional genotyping in stage II. Design failed for one variant (*rs12403389*), so it was replaced with another SNP in high LD (*rs10874915*), despite not having a statistically significant association itself. Genotyping failed for one variant (*rs745103*), and so it could not be further analyzed.

Eight SNPs were found to have significant associations with breast cancer risk in the combined analysis of stages I and II data (Table 3); results from the 2 study stages for all 8 were not significantly heterogeneous ($P > 0.10$; data not shown). One SNP (*TGFBR2 rs1078985*) had significant associations with breast cancer risk in both study stages, as well as highly consistent risk estimates. When results from the 2 stages were combined, both heterozygotes (OR, 0.84; 95% CI, 0.765–0.93) and homozygotes (OR, 0.73; 95% CI: 0.55–0.97) had significantly lower risks of breast cancer

Table 2. Gene and SNP information for included TGF-β signaling pathway genes among women in Shanghai

TGFβ pathway genes	Genomic location	Gene span, kb	SNPS in HapMap[a]	SNPs genotyped		Genetic variation coverage (%)[b]
				All	MAF ≥ 5%	
Ligands						
TGFB1	19q13.1	23.2	16	14	13	93.8
TGFB2	1q41	95.1	106	44	29	81.1
TGFB3	14q24	23.1	19	13	6	68.4
Receptors						
TGFBR1	9q22	49.1	49	24	16	91.8
TGFBR2	3p22	87.6	118	102	80	97.5
TGFBR3	1p33-p32	203.7	298	112	86	81.5
Cofactors						
SMAD2	18q21	98.1	87	45	37	100.0
SMAD3	15q21-22	129.3	170	76	51	77.6
SMAD4	18q21.1	49.5	25	10	8	92.0
SMAD7	18q21.1	30.9	27	17	6	48.1
SARA (ZFYVE9)	1p32.3	204.3	50	22	9	88.0

[a]SNPs with a MAF ≥ 5% in HapMap (v2 R24), Han Chinese (CHB) data, ± 10 kb for each gene.
[b]Coverage of HapMap CHB SNPs by our genotyped SNPs for $r^2 = 0.8$, using a pairwise tagging approach in Tagger.

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Table 3. Associations with breast cancer risk for selected TGF-β signaling pathway variants, the SBCGS

Information[a]	N (cases/controls)	Breast cancer risk, additive model[b] AB OR (95% CI)	BB OR (95% CI)	P	Dominant model[c] AB/BB OR (95% CI)	P	Recessive model[d] BB OR (95% CI)	P
***TGFBR2 rs1078985* (A/G), 15.9%, intron 3**								
SBCGS I	2,909/2,316	0.87 (0.76–0.98)	0.79 (0.55–1.12)	**0.0117**	0.86 (0.76–0.97)	**0.0132**	0.82 (0.57–1.17)	0.2656
SBCGS II	1,543/1,746	0.81 (0.69–0.95)	0.64 (0.39–1.04)	**0.0024**	0.79 (0.68–0.93)	**0.0038**	0.67 (0.42–1.09)	0.1064
Combined	4,452/4,062	0.84 (0.76–0.93)	0.73 (0.55–0.97)	**1.15×10^{-4}**	0.84 (0.76–0.92)	**1.88×10^{-4}**	0.77 (0.58–1.02)	0.0605
***TGFB2 rs2799086* (C/T), 25.0%, intron 2**								
SBCGS I	2,764/2,177	1.04 (0.92–1.17)	1.34 (1.05–1.71)	0.0589	1.07 (0.96–1.20)	0.2247	1.32 (1.04–1.68)	**0.0228**
SBCGS II	1,613/1,800	1.06 (0.92–1.23)	1.18 (0.90–1.55)	0.1821	1.08 (0.94–1.24)	0.2583	1.16 (0.89–1.51)	0.2883
Combined	4,377/3,977	1.05 (0.96–1.15)	1.27 (1.06–1.52)	**0.0191**	1.08 (0.99–1.18)	0.0907	1.25 (1.04–1.49)	**0.0147**
***TGFB2 rs17047740* (C/T), 9.9%, intron 2**								
SBCGS I	2,771/2,176	1.04 (0.90–1.20)	2.22 (1.19–4.12)	0.1088	1.08 (0.94–1.24)	0.2772	2.20 (1.18–4.08)	**0.0126**
SBCGS II	1,601/1,784	1.12 (0.94–1.34)	1.08 (0.54–2.14)	0.2107	1.12 (0.95–1.33)	0.1892	1.05 (0.53–2.09)	0.8801
Combined	4,372/3,960	1.08 (0.97–1.20)	1.58 (1.01–2.47)	**0.0418**	1.10 (0.99–1.23)	0.0883	1.56 (1.00–2.44)	0.0511
***TGFBR1 rs2026811* (C/A), 46.9%, intron 1**								
SBCGS I	1,616/1,585	0.82 (0.70–0.96)	0.88 (0.72–1.07)	0.1340	0.84 (0.72–0.97)	**0.0221**	1.00 (0.84–1.18)	0.9834
SBCGS II	908/888	0.89 (0.71–1.10)	0.91 (0.70–1.18)	0.4278	0.89 (0.73–1.10)	0.2830	0.98 (0.78–1.23)	0.8552
Combined	2,524/2,473	0.84 (0.74–0.96)	0.89 (0.76–1.04)	0.0956	0.86 (0.76–0.97)	**0.0125**	0.99 (0.87–1.14)	0.9274
***TGFBR1 rs10733710* (G/A), 18.7%, intron 6**								
SBCGS I	947/889	1.28 (1.05–1.55)	1.42 (0.69–2.93)	**0.0111**	1.28 (1.06–1.55)	**0.0108**	1.31 (0.64–2.68)	0.4682
SBCGS II	1,609/1,799	1.10 (0.95–1.27)	1.04 (0.74–1.47)	0.2952	1.09 (0.95–1.25)	0.2262	1.01 (0.72–1.42)	0.9466
Combined	2,556/2,688	1.16 (1.03–1.30)	1.11 (0.82–1.52)	**0.0226**	1.15 (1.03–1.29)	**0.0132**	1.06 (0.78–1.44)	0.7092
***TGFBR2 rs304822* (C/T), 38.9%, 3′ flanking region**								
SBCGS I	2,853/2,255	0.84 (0.74–0.95)	0.92 (0.78–1.10)	0.0791	0.86 (0.76–0.96)	**0.0084**	1.02 (0.87–1.20)	0.8101
SBCGS II	1,598/1,780	0.91 (0.78–1.05)	0.88 (0.72–1.08)	0.1589	0.90 (0.78–1.03)	0.1382	0.93 (0.77–1.13)	0.4687
Combined	4,451/4,035	0.87 (0.79–0.95)	0.91 (0.80–1.04)	**0.0272**	0.88 (0.80–0.96)	**0.0034**	0.98 (0.87–1.11)	0.7868
***TGFBR3 rs284185* (T/A), 11.0%, intron 4**								
SBCGS I	2,763/2,167	1.00 (0.87–1.15)	1.71 (1.04–2.83)	0.3103	1.04 (0.90–1.19)	0.6176	1.71 (1.04–2.83)	**0.0352**
SBCGS II	1,609/1,791	0.86 (0.72–1.02)	1.72 (0.96–3.07)	0.5621	0.90 (0.76–1.07)	0.2207	1.77 (0.99–3.16)	0.0532
Combined	4,372/3,958	0.94 (0.84–1.05)	1.72 (1.17–2.51)	0.6682	0.98 (0.88–1.09)	0.7060	1.74 (1.19–2.54)	**0.0042**
***SMAD3 rs7178117* (G/C), 10.0%, intron 1**								
SBCGS I	2,771/2,177	1.14 (0.99–1.32)	1.57 (0.94–2.64)	**0.0190**	1.17 (1.01–1.34)	**0.0340**	1.53 (0.91–2.58)	0.1055
SBCGS II	1,600/1,781	1.11 (0.93–1.32)	0.82 (0.43–1.58)	0.4599	1.09 (0.92–1.29)	0.3293	0.81 (0.42–1.55)	0.5203
Combined	4,371/3,958	1.13 (1.01–1.27)	1.22 (0.82–1.82)	**0.0185**	1.14 (1.02–1.27)	**0.0195**	1.19 (0.81–1.77)	0.3761

NOTE: Estimates and *P* values in bold denote significance at $P \leq 0.05$.

[a]Information includes alleles (major or reference allele/minor allele) as determined by allele frequency among all genotyped controls, MAF among all genotyped controls, and region of the gene where the SNP is located.

[b]Breast cancer risk for heterozygotes (AB) and minor allele homozygotes (BB), compared with major allele homozygotes (AA), in models adjusted for age and genotyping stage when appropriate; P_{trend}.

[c]Breast cancer risk for minor allele carriers (AB/BB) compared with major allele homozygotes (AA), in models adjusted for age and genotyping stage when appropriate; *P* value for dominant association.

[d]Breast cancer risk for minor allele homozygotes (BB) compared with major allele carriers (AA/AB), in models adjusted for age and genotyping stage when appropriate; *P* value for recessive association.

than major allele homozygotes (*AA*). Furthermore, both additive and dominant effect models were highly significant ($P < 1.9 \times 10^{-4}$). This surpassed a Bonferroni corrected significance threshold for the number of variants evaluated in stage II (P, $0.05/19 = 2.63 \times 10^{-3}$). In addition, nominally significant associations with breast cancer risk were also found for *TGFB2 rs2799086*, *TGFB2 rs17047740*, *TGFBR1 rs2026811*, *TGFBR1 rs10733710*, *TGFBR2 rs304822*, *TGFBR3 rs284185*, and *SMAD3 rs7178117* in the combined analyze, although none of these SNPs had significant associations in stage II. Regression models shown in Table 3 include adjustment for age and genotyping stage when appropriate; additional adjustment for education, age at menarche, age at menopause, age at first live birth, menopausal status, a first-degree relative with breast cancer, use of hormone replacement therapy, previous history of fibroadenoma, physical activity, body mass index, and waist-to-hip ratio

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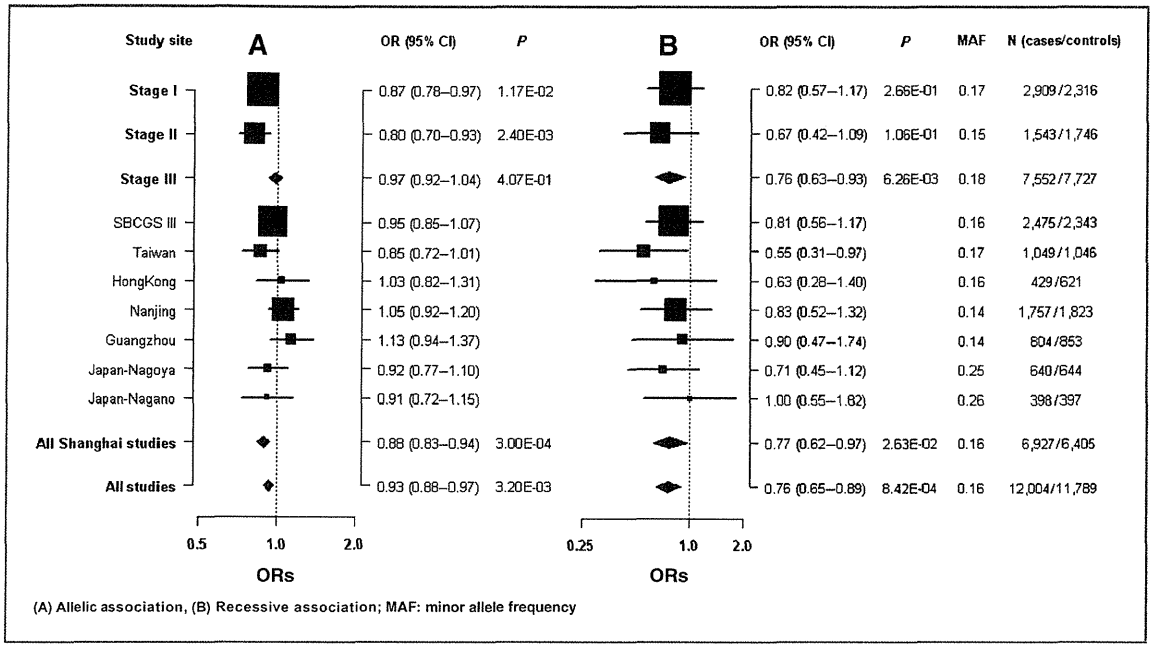

Figure 2 Forest plots for associations of breast cancer risk with *TGFBR2 rs1078985* by study site, the Asian Breast Cancer Consortium.

did not materially alter these findings (data not shown). Results for the remaining 11 SNPs evaluated in stage 2 are shown in Supplementary Table S3; results from analyses of the 2 stages combined were either nonsignificant or else had significant heterogeneity of associations with breast cancer risk.

TGFBR2 rs1078985 was then evaluated among an additional 4,818 subjects from the SBCGS III, as well as among 10,461 participants of 6 collaboration studies (Fig. 2). Pooled analysis of all data indicated a highly significant recessive effect (OR, 0.76; 95% CI, 0.65–0.89, $P = 8.42 \times 10^{-4}$). This was driven by the results of stage III, in which a 24% reduced risk of breast cancer (OR, 0.76; 95% CI, 0.63–0.93; P value $= 6.26 \times 10^{-3}$) was observed for minor allele homozygotes compared with major allele carriers. To evaluate a potential dual rule of the *TGFBR2 rs1078985* SNP with breast cancer risk, further analysis was conducted using data from the SBCGS by tumor stage (Table 4). Strong additive trends were seen among women with early and midstage disease, while the association with breast cancer risk was attenuated among women with advanced stage cancer (TNM stages III and IV). Heterogeneity tests, however, were not statistically significant ($P > 0.10$).

Discussion

In this multistage study, we comprehensively evaluated genetic variation of 11 genes in the TGF-β signaling pathway with breast cancer risk among Asian women. One SNP (*rs1078985*) in intron 3 of the *TGFBR2* gene, showed a consistent association in all 3 stages. Pooled analysis revealed a significantly reduced risk of breast cancer in a recessive genetic model (OR, 0.76; 95% CI, 0.65–0.89; $P = 8.42 \times 10^{-4}$). This novel finding provides support for a role of TGF-β signaling pathway in the etiology of breast cancer. Although the association of *rs1078985* with breast cancer risk was identified in our study initially under the additive model (stages I and II), after evaluating data from 7 additional studies, a recessive model seemed to best explain the association. This may be due to the reduced power for detecting recessive associations in stages I and II. Using Quanto, we found that the power to find an association for an SNP with an MAF of 16% was less than 42% for a recessive model in the analysis of stages I and II combined. After including data from the SBCGS III and the 6 collaboration studies, however, we had >87% power to detect such an association.

Results from *in silico* analysis were supportive of an association between *TGFBR2 rs1078985* and breast cancer risk. Using TFSEARCH (32), a web-based program that searches for transcription factor binding sites, an Nkx-2.5 binding site was found to be present when the major *A* allele was present, but not when the minor *G* allele was. Nkx-2.5 is a transcriptional regulator of iodide transport in thyroid and mammary cells; a role in cancer has been implicated, as Nkx-2.5 has been shown to be expressed in breast cancer cell lines, as well as in mammary glands during lactation (33). The ENCODE transcription factor chromatin immunoprecipitation (ChIP) track of the UCSC Genome Browser (Build 36 assembly, hg18; ref. 34), was also evaluated; this track shows regions where

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Table 4. Association of *TGFBR2 rs1078985* and breast cancer risk by tumor stage, the SBCGS

	Stage 0 or I		Stage II		Stage III or IV		Total[a]		
	N_{cases}	OR (95% CI)	N_{cases}	OR (95% CI)	N_{cases}	OR (95% CI)	N_{cases}	OR (95% CI)	P^d
TGFBR2 rs1078985									
AA	1,661	1.00 (reference)	2,502	1.00 (reference)	495	1.00 (reference)	5,079	1.00 (reference)	0.7738
AG	568	**0.91 (0.82–1.02)**	823	**0.87 (0.79–0.96)**	181	0.98 (0.82–1.17)	1,704	**0.89 (0.82–0.96)**	
GG	45	**0.72 (0.52–1.01)**	72	**0.76 (0.58–1.01)**	15	0.81 (0.47–1.38)	144	**0.75 (0.60–0.94)**	
P^b		**0.0198**		**0.0011**		0.5294		**0.0003**	
AA/AG	2,229	1.00 (reference)	3,325	1.00 (reference)	676	1.00 (reference)	6,783	1.00 (reference)	0.6869
GG	45	0.74 (0.53–1.03)	72	0.79 (0.60–1.05)	15	0.81 (0.48–1.39)	144	**0.77 (0.62–0.97)**	
P^c		0.0769		0.1026		0.4502		**0.0263**	

NOTE: Bold values denote significance at $P \leq 0.05$.

[a]Includes 565 women without information on tumor stage.

[b]P_{trend}.

[c]*P* value for recessive association.

[d]*P* value for heterogeneity test.

transcription factors have been shown to bind by ChIP with specific antibodies followed by DNA sequencing (35). SNP *rs1078985* was found to be within one experimentally verified transcription factor binding region (NF-κB) and adjacent to 7 additional regions, the strongest signal of which was found for PU.1. NF-κB regulates genes involved in the immune and inflammatory responses, as well as genes important for cell proliferation, apoptosis, angiogenesis, invasion, and therefore carcinogenesis (36, 37). Overexpression of NF-κB1 and NF-κB2 has been shown in breast cancer cell lines and breast carcinomas (36), and many cancer cells show aberrant or constitutive NF-κB activation, which mediates resistance to chemotherapy and radiotherapy (36, 37). PU.1 is an erythroblast transformation specific–domain transcription factor that binds purine-rich sequences and can regulate alternative splicing of target genes; it has been postulated that PU.1 can reduce the transcriptional activity of the p53 tumor suppressor family, thereby altering cell-cycle regulation and apoptosis (38). Together, these data provide considerable biologic plausibility for a role for this *TGFBR2* SNP in breast cancer etiology.

Four SNPs in *TGFB1* (*rs1800468*, *rs1800469*, *rs1800470*, and *rs1800471*), and 1 SNP in *TGFBR1* (*rs11466445*) have been reported to be associated with breast cancer risk in previous studies (7, 9–13, 15, 16). Among them, *rs1800470* (also known as T29C or *rs1982073*) has been the most frequently investigated (7, 9–13). Similar to other previously reported SNPs, the association of *rs1800470* with breast cancer risk has not been robustly replicated (10). In our study, none of these SNPs were significantly associated with breast cancer risk. Instead, in addition to the one replicated association (*rs1078985*), 7 additional SNPs (*TGFB2 rs2799086*, *TGFB2 rs17047740*, *TGFBR1 rs2026811*, *TGFBR1 rs10733710*, *TGFBR2 rs304822*, *TGFBR3 rs284185*, and *SMAD3 rs7178117*) had some evidence for possible associations with breast cancer risk. However, none of these marginally significant associations remained significant after adjusting for multiple comparisons.

Major strengths of this study include a multistage study design with a large sample size, the population-based design of the SBCGS, and a comprehensive and systematic analysis of genetic variants in 11 TGF-β signaling pathway genes. Limitations to be considered include that only the SMAD-mediated TGF-β signaling pathway was evaluated. Although this is the best characterized mechanism of TGF-β signaling, many studies have also shown that TGF-βs can exert their effects through SMAD-independent pathways, such as phosphoinositide 3-kinase, mitogen–activated protein kinase, protein phosphatase 2, PKB, extracellular signal–regulated kinase, and c-*jun*–NH_2–kinase (7). A further limitation of our study was the relatively low coverage of variants in the *TGFB3* and *SMAD7* genes. However, our coverage was above 75% for 9 included genes (using an r^2 of 0.8) and was on average very good (83.6%).

In conclusion, our finding of an association between *TGFBR2 rs1078985* and a reduced risk of breast cancer among Asian women was not only replicated within the SBCGS, but was also evident among data from 6 collaboration studies. Together, our results support an important role for SNPs in the TGF-β signaling pathway genes in breast cancer susceptibility. These findings may provide new insights into the etiology of breast cancer as well as future potential therapeutic targets.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agents. U.S. Khoo is a consultant and an advisory board member of Vanderbilt University. No potential conflicts of interest were disclosed by the other authors.

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