included in the measurement of plasma 25-hydroxyvitamin D; quality control for this measurement was performed by an external laboratory by using nonblinded controls. Accordingly, the reported intra- and interassay coefficients of variation would likely have underestimated the true underlying variations. Finally, we did not match cases and controls by season of examination or blood collection. If such matching had been conducted, we could have taken better account of the seasonal variation in plasma 25-hydroxyvitamin D concentrations.

In summary, we found that both plasma 25-hydroxyvitamin D and dietary calcium intake were inversely associated with the prevalence of colorectal adenoma, albeit in a nonlinear manner. We further noted that plasma 25-hydroxyvitamin D levels interacted with the *TaqI* polymorphism of the *VDR* gene but not with dietary calcium intake. These observations highlight the importance of vitamin D in colorectal carcinogenesis, at least in its early stage. Vitamin D might protect against colorectal cancer and adenoma, mainly through mechanisms other than the indirect mechanism via calcium metabolism.

ACKNOWLEDGMENTS

Author affiliations: Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan (Taiki Yamaji, Motoki Iwasaki, Shizuka Sasazuki, Shoichiro Tsugane); and Genetics Division, National Cancer Center Research Institute, Tokyo, Japan (Hiromi Sakamoto, Teruhiko Yoshida).

This work was supported by the Ministry of Health, Labor, and Welfare of Japan (Grant-in-Aid for the Third Term Comprehensive 10-Year-Strategy for Cancer Control); the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Scientific Research on Priority Areas 17015049, Grant-in-Aid for Young Scientists B-22790579); and the National Institute of Biomedical Innovation (the Program for Promotion of Fundamental Studies in Health Sciences) of Japan.

Conflict of interest: none declared.

REFERENCES

1. Grant WB, Garland CF. A critical review of studies on vitamin D in relation to colorectal cancer. *Nutr Cancer.* 2004;48(2):115–123.
2. Huncharek M, Muscat J, Kupelnick B. Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies. *Nutr Cancer.* 2009;61(1):47–69.
3. Wei MY, Garland CF, Gorham ED, et al. Vitamin D and prevention of colorectal adenoma: a meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2008;17(11):2958–2969.
4. Gorham ED, Garland CF, Garland FC, et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *Am J Prev Med.* 2007;32(3):210–216.
5. Yin L, Grandi N, Raum E, et al. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. *Aliment Pharmacol Ther.* 2009;30(2):113–125.
6. Otani T, Iwasaki M, Sasazuki S, et al. Plasma vitamin D and risk of colorectal cancer: the Japan Public Health Center-Based Prospective Study. *Br J Cancer.* 2007;97(3):446–451.
7. Cho E, Smith-Warner SA, Spiegelman D, et al. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *J Natl Cancer Inst.* 2004;96(13):1015–1022.
8. Raimondi S, Johansson H, Maisonneuve P, et al. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Carcinogenesis.* 2009;30(7):1170–1180.
9. Slattery ML. Vitamin D receptor gene (*VDR*) associations with cancer. *Nutr Rev.* 2007;65(8 pt 2):S102–S104.
10. Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev.* 2004;13(9):1502–1508.
11. Wu K, Feskanich D, Fuchs CS, et al. A nested case control study of plasma 25-hydroxyvitamin D concentrations and risk of colorectal cancer. *J Natl Cancer Inst.* 2007;99(14):1120–1129.
12. Levine AJ, Harper JM, Ervin CM, et al. Serum 25-hydroxyvitamin D, dietary calcium intake, and distal colorectal adenoma risk. *Nutr Cancer.* 2001;39(1):35–41.
13. Peters U, McGlynn KA, Chatterjee N, et al. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. *Cancer Epidemiol Biomarkers Prev.* 2001;10(12):1267–1274.
14. Peters U, Hayes RB, Chatterjee N, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor, and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev.* 2004;13(4):546–552.
15. Miller EA, Keku TO, Satia JA, et al. Calcium, dietary, and lifestyle factors in the prevention of colorectal adenomas. *Cancer.* 2007;109(3):510–517.
16. Yamaji T, Iwasaki M, Sasazuki S, et al. Methionine synthase A2756G polymorphism interacts with alcohol and folate intake to influence the risk of colorectal adenoma. *Cancer Epidemiol Biomarkers Prev.* 2009;18(1):267–274.
17. Yamaji T, Iwasaki M, Sasazuki S, et al. Interaction between adiponectin and leptin influences the risk of colorectal adenoma. *Cancer Res.* 2010;70(13):5430–5437.
18. Kudo S, Hirota S, Nakajima T, et al. Colorectal tumours and pit pattern. *J Clin Pathol.* 1994;47(10):880–885.
19. Resources Council, Science and Technology Agency. *Standard Tables of Food Composition in Japan.* 5th revised ed. Tokyo, Japan: Printing Office, the Ministry of Finance; 2000.
20. Yamaji T, Inoue M, Sasazuki S, et al. Fruit and vegetable consumption and squamous cell carcinoma of the esophagus in Japan: the JPHC Study. *Int J Cancer.* 2008;123(8):1935–1940.
21. Ishihara J, Inoue M, Iwasaki M, et al. Dietary calcium, vitamin D, and the risk of colorectal cancer. *Am J Clin Nutr.* 2008;88(6):1576–1583.
22. Willett WC. *Nutritional Epidemiology.* 2nd ed. New York, NY: Oxford University Press; 1998.
23. Gandini S, Boniol M, Haukka J, et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer.* 2011;128(6):1414–1424.
24. Takahashi R, Mizoue T, Otake T, et al. Circulating vitamin D and colorectal adenomas in Japanese men. *Cancer Sci.* 2010;101(7):1695–1700.
25. Shin A, Li H, Shu XO, et al. Dietary intake of calcium, fiber and other micronutrients in relation to colorectal cancer risk: results from the Shanghai Women's Health Study. *Int J Cancer.* 2006;119(12):2938–2942.
26. Wakai K, Hirose K, Matsuo K, et al. Dietary risk factors for colon and rectal cancers: a comparative case-control study. *J Epidemiol.* 2006;16(3):125–135.

27. Mizoue T, Kimura Y, Toyomura K, et al. Calcium, dairy foods, vitamin D, and colorectal cancer risk: the Fukuoka Colorectal Cancer Study. *Cancer Epidemiol Biomarkers Prev.* 2008;17(10): 2800–2807.
28. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr.* 2006;84(1):18–28.
29. Ingles SA, Haile RW, Henderson BE, et al. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. *Cancer Epidemiol Biomarkers Prev.* 1997;6(2):93–98.
30. Beelman CA, Parker R. Degradation of mRNA in eukaryotes. *Cell.* 1995;81(2):179–183.
31. Poynter JN, Jacobs ET, Figueiredo JC, et al. Genetic variation in the vitamin D receptor (VDR) and the vitamin D-binding protein (GC) and risk for colorectal cancer: results from the Colon Cancer Family Registry. *Cancer Epidemiol Biomarkers Prev.* 2010;19(2):525–536.
32. Ukaji M, Saito Y, Fukushima-Uesaka H, et al. Genetic variations of *VDR/NR1I1* encoding vitamin D receptor in a Japanese population. *Drug Metab Pharmacokinet.* 2007; 22(6):462–467.
33. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet.* 2010;376(9736):180–188.
34. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet.* 2010;19(13):2739–2745.
35. Sano Y, Saito Y, Fu KI, et al. Efficacy of magnifying chromoendoscopy for the differential diagnosis of colorectal lesions. *Digest Endosc.* 2005;17(2):105–116.
36. Fu KI, Sano Y, Kato S, et al. Chromoendoscopy using indigo carmine dye spraying with magnifying observation is the most reliable method for differential diagnosis between non-neoplastic and neoplastic colorectal lesions: a prospective study. *Endoscopy.* 2004;36(12):1089–1093.

Human Molecular Genetics, 2011, Vol. 20, No. 24 **4991–4999**
doi:10.1093/hmg/ddr405
Advance Access published on September 9, 2011

Genome-wide association study identifies breast cancer risk variant at 10q21.2: results from the Asia Breast Cancer Consortium

Qiuyin Cai[1], Jirong Long[1], Wei Lu[3], Shimian Qu[1], Wanqing Wen[1], Daehee Kang[4,5,6], Ji-Young Lee[5], Kexin Chen[8], Hongbing Shen[9], Chen-Yang Shen[10], Hyuna Sung[4], Keitaro Matsuo[11], Christopher A. Haiman[12], Ui Soon Khoo[13], Zefang Ren[14], Motoki Iwasaki[18], Kai Gu[3], Yong-Bing Xiang[19], Ji-Yeob Choi[4], Sue K. Park[4,5,6], Lina Zhang[8], Zhibin Hu[9], Pei-Ei Wu[10], Dong-Young Noh[6,7], Kazuo Tajima[11], Brian E. Henderson[12], Kelvin Y.K. Chan[13], Fengxi Su[15], Yoshio Kasuga[20], Wenjing Wang[3], Jia-Rong Cheng[19], Keun-Young Yoo[5], Jong-Young Lee[21], Hong Zheng[8], Yao Liu[9], Ya-Lan Shieh[10], Sung-Won Kim[22], Jong Won Lee[23], Hiroji Iwata[24], Loic Le Marchand[25], Sum Yin Chan[13], Xiaoming Xie[16], Shoichiro Tsugane[18], Min Hyuk Lee[26], Shenming Wang[17], Guoliang Li[1], Shawn Levy[27], Bo Huang[1], Jiajun Shi[1], Ryan Delahanty[1], Ying Zheng[3], Chun Li[2], Yu-Tang Gao[19], Xiao-Ou Shu[1] and Wei Zheng[1,*]

[1]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center and [2]Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, USA, [3]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China, [4]Department of Biomedical Sciences, [5]Department of Preventive Medicine, [6]Cancer Research Institute and [7]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea, [8]Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China, [9]Department of Epidemiology and Biostatistics, Nanjing Medical University, Nanjing, China, [10]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [11]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan, [12]Department of Preventive Medicine, Keck School of Medicine, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA, USA, [13]Department of Pathology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China, [14]School of Public Health, [15]Second Affiliated Hospital, [16]Cancer Center and [17]First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [18]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan, [19]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China, [20]Department of Surgery, Nagano Matsushiro General Hospital, Nagano, Japan, [21]Center for Genome Science, Korea National Institute of Health, KCDC, Osong Health Technology Administration Complex, Chungcheongbuk-do, Korea, [22]Department of Surgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea, [23]Department of Surgery, Ulsan University College of Medicine, Seoul, Korea, [24]Department of Breast Oncology, Aichi Cancer Center Central Hospital, Nagoya, Japan, [25]Epidemiology Program, Cancer Research Center, University of Hawaii, Honolulu, HI, USA, [26]Department of Surgery, Soonchunhyang University Hospital, Korea and [27]HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA

Received July 7, 2011; Revised and Accepted September 5, 2011

*To whom correspondence should be addressed at: Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, 2525 West End Avenue, 8th Floor, TN 37203-1738, Nashville, USA. Tel: +1 6159360682; Fax: +1 6159368241; Email: wei.zheng@vanderbilt.edu

Although approximately 20 common genetic susceptibility loci have been identified for breast cancer risk through genome-wide association studies (GWASs), genetic risk variants reported to date explain only a small fraction of heritability for this common cancer. We conducted a four-stage GWAS including 17 153 cases and 16 943 controls among East-Asian women to search for new genetic risk factors for breast cancer. After analyzing 684 457 SNPs in 2062 cases and 2066 controls (Stage I), we selected for replication among 5969 Chinese women (4146 cases and 1823 controls) the top 49 SNPs that had neither been reported previously nor were in strong linkage disequilibrium with reported SNPs (Stage II). Three SNPs were further evaluated in up to 13 152 Chinese and Japanese women (6436 cases and 6716 controls) (Stage III). Finally, two SNPs were evaluated in 10 847 Korean women (4509 cases and 6338 controls) (Stage IV). SNP rs10822013 on chromosome 10q21.2, located in the zinc finger protein 365 (*ZNF365*) gene, showed a consistent association with breast cancer risk in all four stages with a combined per-risk allele odds ratio of 1.10 (95% CI: 1.07–1.14) (*P*-value for trend = 5.87×10^{-9}). *In vitro* electrophoretic mobility shift assays demonstrated the potential functional significance of rs10822013. Our results strongly implicate rs10822013 at 10q21.2 as a genetic risk variant for breast cancer among East-Asian women.

INTRODUCTION

Breast cancer, one of the most common malignancies among women worldwide, is a complex polygenic disorder for which genetic factors play a significant role in disease etiology (1,2). To date, approximately 20 loci have been associated with breast cancer risk in genome-wide association studies (GWASs) (3–16). With the exception of our study conducted among Chinese women (7,11), all other published GWASs were conducted among women of European ancestry. Only about half of SNPs initially identified in women with European ancestry can be directly replicated in Chinese women, and further, the association with the replicated SNPs is weaker in Chinese women than in women of European ancestry (17,18). Because linkage disequilibrium (LD) structure and allele frequencies of genetic variations differ between women of Chinese and European ancestry, additional risk loci or additional genetic variants in previously identified loci may remain to be discovered by studies conducted among Chinese and other Asian women.

We recently analyzed 684 457 SNPs in 2062 breast cancer cases and 2066 community controls, recruited as part of the Shanghai Breast Cancer Study (SBCS) (7) to identify novel susceptibility loci for breast cancer. We selected the top 49 SNPs that had not been reported previously and that were not in strong LD with any reported SNPs for a fast-track replication conducted through the Asia Breast Cancer Consortium (Table 1 and Fig. 1). By analyzing data from 17 153 cases and 16 943 controls included in the consortium, we found strong evidence for a genetic variant that may contribute to breast cancer susceptibility among East-Asian women.

RESULTS

Of the 49 successfully genotyped SNPs in Stage II (Supplementary Material, Table S1), highly significant associations with breast cancer risk were found for SNPs rs10822013 (10q21.2) (Table 2) and rs2048672 (7q32.3) (Supplementary Material, Table S2). These two SNPs were selected for further validation in Stage III, which included 6436 cases and 6716 controls of Asian ancestry from eight studies participating in the Asia Breast Cancer Consortium (Table 2 and Supplementary Material, Table S2). A third SNP, rs17823421 (16p13.3), also had an association with breast cancer risk at $P < 0.05$ in Stage II (Supplementary Material, Table S3). This SNP, however, was not replicated in three studies included in Stage III; thus, further replication was not conducted.

With the exception of two small studies, the minor T allele of rs10822013 had a consistent positive association with breast cancer risk in the other six studies (Fig. 2, $P = 0.63$ for the heterogeneity test in Stage III). Adjusted odds ratios (ORs) for Stage III were 1.12 [95% confidence interval (CI): 1.03–1.21] and 1.14 (95% CI: 1.03–1.26), respectively, for the CT and TT genotypes (*P*-value for trend = 8.08×10^{-3}) (Table 2). Similarly, the minor T allele of rs10822013 had a consistent positive association with breast cancer risk in all three Stage-IV studies, which were conducted among Korean women (Fig. 2). Adjusted ORs for Stage IV were 1.10 (95% CI: 0.99–1.22) and 1.21 (95% CI: 1.07–1.37), respectively, for the CT and TT genotypes (*P*-value for trend = 2.07×10^{-3}) (Table 2). Pooled analyses of samples from all stages produced ORs of 1.12 (95% CI: 1.06–1.18) and 1.21 (95% CI: 1.13–1.29) for the CT and TT genotypes, respectively (*P*-value for trend = 5.87×10^{-9}) (Table 2). The heterogeneity test for results among all studies was not statistically significant ($P = 0.4817$).

Stratified analyses suggested that the associations of SNP rs10822013 with breast cancer risk were similar among pre- and post-menopausal women (Table 3). No apparent difference was observed between estrogen receptor (ER) (+) and ER (−) breast cancer in relation to rs10822013 (Table 3).

The minor C allele of rs2048672 was associated with an elevated risk of breast cancer in all but one small study included in Stage III (Supplementary Material, Fig. S1). Although none of the study-specific ORs in Stage III was statistically significant, pooling data from Stage III yielded ORs of 1.08 (95% CI: 1.00–1.17) and 1.10 (95% CI: 0.99–1.21), respectively, for the AC and CC genotypes (*P*-value for trend = 0.0545). The minor C allele of rs2048672 was associated with an elevated risk of breast cancer in all three studies included in Stage IV (Supplementary Material, Fig. S1) with pooled ORs of 1.05

Table 1. Selected characteristics of studies participating in the Asia Breast Cancer Consortium

Study/stage	Case	Control	Ethnicity	Study design[a]	Study period	Age (mean)	Menopause (%)	ER (+)[b] (%)
Stage I	2066	2075	Chinese	Population	1996–2005	49.3/49.4	38.7/41.6	63.6
Stage II	4374	1892	Chinese	Population	1996–2005	54.0/52.8[c]	51.1/55.4[c]	64.3
Stage III	6489	6800						
Tianjin	1515	1581	Chinese	Hospital	2004–2008	51.7/51.9	51.7/55.4	44.1
Nanjing	1439	1437	Chinese	Hospital	2004–2008	51.4/51.3	53.3/52.6	54.8
Taiwan	1057	1059	Chinese	Hospital	2004–2007	51.6/47.4[c]	52.4/39.7[c]	66.2
Hong Kong	432	631	Chinese	Hospital	2003–2009	45.9/45.6	52.0/41.2[c]	70.2
Guangzhou	466	518	Chinese	Hospital	2008–2009	47.6/47.6	42.5/47.6	69.8
Nagoya	643	639	Japanese	Hospital	2003–2005	51.4/51.1	48.6/48.6	72.8
Hawaii (MEC)	536	534	Japanese	Population	1993–2008	65.1/60.3[c]	86.7/82.7	85.4
Nagano	401	401	Japanese	Hospital	2001–2005	53.8/54.0	54.9/65.3[c]	74.6
Stage IV	4516	6344						
SeBCS-I	2359	2052	Korean	Hospital	1995–2006	48.1/51.7	37.9/52.0	61.9
SeBCS-II	768	1098	Korean	Hospital	1995–2006	47.5/47.7	36.5/37.2	62.6
KOHBRA/KoGES	1389	3194	Korean	Hospital	2007–2009	40.5/50.3[c]	23.3/NA	62.8
Total	17 445	17 111						

MEC, Multiethnic Cohort Study; SeBCS-I, Seoul Breast Cancer Study Phase I; SeBCS-II, Seoul Breast Cancer Study Phase II; KOHBRA, Korean Hereditary Breast Cancer Study; KoGES, Korean Genome and Epidemiology Study.
[a]With the exception of the MEC (a cohort study), all other studies used the case–control study design with either a population-based or hospital-based approach.
[b]Among cases with ER data.
[c]Significant at $\alpha = 0.01$ level (t-test for continuous variables, χ^2-test for categorical variables).

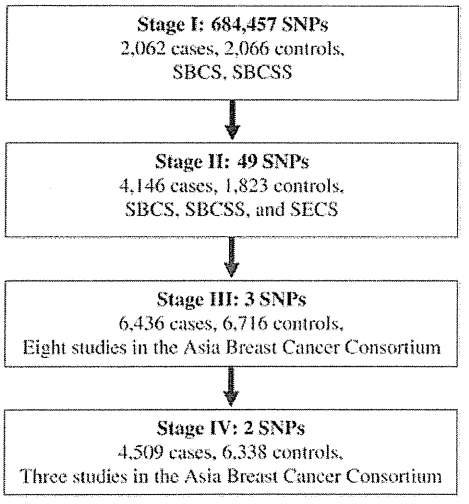

Figure 1. Overview of the study design.

(95% CI: 0.95–1.17) and 1.09 (95% CI: 0.97–1.24), respectively, for the AC and CC genotypes (P-value for trend = 0.1445). Pooled ORs for all stages were 1.11 (95% CI: 1.05–1.17) and 1.15 (95% CI: 1.08–1.23) for the AC and CC genotypes, respectively (P-value for trend = 6.21×10^{-6}) (Supplementary Material, Table S2). The heterogeneity test for results among all studies was not statistically significant ($P = 0.5519$).

SNP rs10822013 is located on 10q21.2 (Fig. 3), a region where a genetic risk variant for breast cancer (rs10995190) was reported recently in a study conducted among women of European ancestry (12). These two SNPs are ~26.7 kb apart and are located in different LD blocks in Chinese (CHB, $r^2 = 0.000$), European (CEU, $r^2 = 0.176$) and African (YRI, $r^2 = 0.005$) populations (Supplementary Material, Fig. S2). Furthermore, rs10995190 has a very low minor allele frequency (MAF) in Chinese (2%) and showed no association with breast cancer risk in our study (OR = 0.84, 95%: 0.63–1.12, $P = 0.23$). Because SNP rs10995190 is not included in the Affymetrix Genome-Wide Human SNP Array 6.0, the genotype frequencies for cases and controls included in the Stage I were imputed (RSQR = 0.9154) using the program MACH (www.sph.umich.edu/csg/abecasis/mach) described in detail previously (7,11). Another SNP (rs16917302) in this locus was also recently associated with breast cancer risk among *BRCA2* mutation carriers, although the P-value (3.8×10^{-5}) was not genome-wide significant (15). SNP rs16917302 is ~9.2 kb away from rs10822013 and these two SNPs are located in different LD blocks in Chinese (CHB, $r^2 = 0.271$), European (CEU, $r^2 = 0.108$.) and African (YRI, $r^2 = 0.075$) populations (Supplementary Material, Fig. S2). SNP rs16917302 was associated with breast cancer risk in the Stage I samples (OR = 0.89, 95%: 0.81–0.98, $P = 0.017$). This SNP, however, was not selected for replication due to a relatively large P-value in the Stage-I scan.

Luciferase reporter assays showed no differences between the empty vector and the pGL3 basic or pGL3 enhancer vectors harboring rs10822013 fragments, indicating that rs10822013 fragments may not have intrinsic promoter activity (data not shown). In contrast, in the pGL3 promoter vector, fragments containing rs10822013 showed reduced luciferase activity, and the reduction was slightly more apparent in fragments containing the reference allele C than those containing the risk allele T (9% difference). However, the difference was not statistically significant. To further investigate whether the DNA sequence containing rs10822013 interacts with nuclear proteins, and if so, whether a single-nucleotide change in the rs10822013 site alters protein–DNA interactions, we performed electrophoretic mobility shift assays. In these assays,

Table 2. Association of SNP rs10822013 (10q21.2) with breast cancer risk in Stages I–IV

Stage	Cases/controls	MAF[a] (%)	OR[b] (95% CI) CT	TT	*P*-value for trend[b]
I	2062/2066	47.0	1.13 (0.97–1.31)	1.37 (1.15–1.63)	4.33×10^{-4}
II	4146/1823	46.9	1.13 (0.99–1.29)	1.26 (1.08–1.48)	3.74×10^{-3}
III	6436/6716	47.8	1.12 (1.03–1.21)	1.14 (1.03–1.26)	8.08×10^{-3}
IV	4509/6338	46.4	1.10 (0.99–1.22)	1.21 (1.07–1.37)	2.07×10^{-3}
Combined	17 153/16 943	47.1	1.12 (1.06–1.18)	1.21 (1.13–1.29)	5.87×10^{-9}
Chinese	11 069/9045	47.6	1.12 (1.04–1.19)	1.22 (1.12–1.32)	1.92×10^{-6}
Japanese	1575/1560	47.2	1.17 (0.98–1.38)	1.15 (0.94–1.40)	1.62×10^{-1}
Korean	4509/6338	46.4	1.10 (0.99–1.22)	1.21 (1.07–1.37)	2.07×10^{-3}

[a]Effect allele frequency (T) in controls.
[b]Adjusted for age and study site.

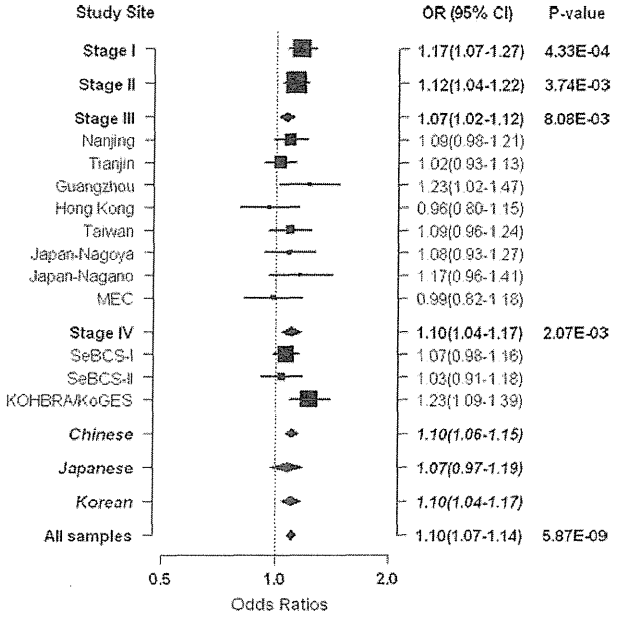

Figure 2. ORs per risk allele of SNP rs10822013 and 95% CIs for breast cancer by study site, ethnicity and study stage. The size of the box is proportional to the number of cases and controls in each study. The heterogeneity test for results among all studies was not statistically significant ($P = 0.4817$).

oligonucleotide probes corresponding to the reference allele C or the risk allele T were incubated with nuclear protein extracts from the MCF7 breast cancer cell line and from HEK293 cells. Compared with the reference allele C, the risk allele T of rs10822013 produced increased DNA–protein complex intensity (II) in both MCF7 and HEK293 cells (Fig. 4).

DISCUSSION

In this large GWAS conducted among East-Asian women and including 17 153 cases and 16 943 controls, we found strong evidence for a novel susceptibility variant for breast cancer, rs10822013 at 10q21.2.

SNP rs10822013 at 10q21.2 (Fig. 3) is located in an intronic region of the zinc finger protein 365 (*ZNF365*). This SNP is ~26.7 kb upstream of SNP rs10995190, which was recently reported in a GWAS conducted among women of European ancestry, and 9.2 kb upstream of SNP rs16917302, which was recently reported in a GWAS conducted among *BRCA2* mutation carriers. However, LD between rs10995190 and rs10822013 is virtually absent in European populations and completely absent in Chinese and African populations (Supplementary Material, Fig. S2). In addition, LD between rs16917302 and rs10822013 is very weak in Chinese and European populations and completely absent in African populations (Supplementary Material, Fig. S2). Furthermore, rs10995190 has a very low MAF in Chinese populations (2%) and we found no association of rs10995190 with breast cancer risk in our study, indicating that rs10822013 is likely a new risk variant for breast cancer.

SNP rs10822013 and its variant could affect alternative splicing or isoform transcription of the *ZNF365* gene. A database search (www.cbrc.jp/research/db/TFSEARCH.html) for transcription-factor-binding sites showed that sequences at rs10822013 have a high degree of similarity with consensus elements that can be differentially recognized by the transcription factor Cap. Our *in vitro* assay data showed that the risk allele T of rs10822013 produced increased DNA–protein complex intensity in both MCF7 breast cancer cells and HEK293 cells. The *ZNF365* gene encodes the zinc finger centrosomal protein, which is essential for cell division. The *ZNF365* gene is highly expressed in a number of cancer cell lines. Ectopic expression of the *ZNF365* gene can cause centrosome alterations and abnormal mitosis, which could lead to abnormal chromosome segregation and subsequently contribute to aneuploidy and malignant transformation (19). Several alternatively spliced variants, encoding distinct proteins, have been identified. These isoforms have different expression patterns, and their functions are largely unknown (www.ncbi.nlm.nih.gov/gene/22891). It has been reported that mutation in the *ZNF365* gene may be associated with uric acid nephrolithiasis (20). Taken together, these data suggest that rs10822013 may be a functional variant.

We also conducted a database search (SCAN, www.scandb.org) for expression quantitative trait locus (eQTL) genes associated with rs10822013 and the 26 SNPs that are in strong LD with rs10822013. Significant associations ($P < 10^{-4}$) between

Table 3. Association of SNP rs10822013 with breast cancer risk by menopausal status and ER status in Stages I–IV[a]

Genotype	Cases/controls	OR (95% CI)
Pre-menopausal women		
CC	1923/1466	1.00 (reference)
CT	3824/2638	1.14 (1.04–1.24)
TT	1907/1232	1.19 (1.07–1.32)
Per T allele	7654/5336	1.09 (1.04–1.15)
P-value for trend		1.14×10^{-3}
Post-menopausal women		
CC	1719/1507	1.00 (reference)
CT	3518/2725	1.11 (1.01–1.21)
TT	1687/1185	1.21 (1.09–1.35)
Per T allele	6924/5417	1.10 (1.05–1.16)
P-value for trend		3.17×10^{-4}
Heterogeneity test (pre- versus post-menopause): $P = 0.7343$		
ER (+) breast cancer		
CC	1956/4742	1.00 (reference)
CT	3961/8440	1.12 (1.04–1.20)
TT	1928/3761	1.21 (1.11–1.31)
Per T allele	7845/16 943	1.10 (1.05–1.15)
P-value for trend		1.05×10^{-5}
ER (−) breast cancer		
CC	1176/4742	1.00 (reference)
CT	2339/8440	1.11 (1.02–1.21)
TT	1154/3761	1.23 (1.12–1.36)
Per T allele	4669/16 943	1.11 (1.06–1.17)
P-value for trend		3.68×10^{-5}

[a]Adjusted for age and study site.

SNPs and gene expression were identified for 32 genes that were associated with two or more of these SNPs. The most interesting of these is a *trans*-eQTL gene, the ferritin, heavy polypeptide 1 (*FTH1*) gene, located on chromosome 11q13, for which small *P*-values were found for three SNPs (rs10822013, $P = 5 \times 10^{-6}$; rs10509168, $P = 1 \times 10^{-5}$; and rs2393886, $P = 2 \times 10^{-5}$). The *FTH1* gene encodes the heavy subunit of ferritin, the major intracellular iron storage protein in prokaryotes and eukaryotes. Overexpression of the *FTH1* gene was associated with a reduction of cellular labile iron, oxidative stress and inhibition of apoptosis (21,22). Gene expression levels of *FTH1* were up-regulated in breast cancer cells with an aggressive mesenchymal phenotype (23). We also found significant associations with the eQTL genes *CCR7* and *CFLAR*, both of which may be involved in cancer development (24–27).

In summary, in this large GWAS conducted as part of the Asia Breast Cancer Consortium, we found strong evidence of an association of a genetic variant, rs10822013 (10q21.2), with breast cancer risk. Our study further demonstrates the utility of conducting GWASs in non-European populations to identify novel genetic risk factors for breast cancer.

MATERIALS AND METHODS

Ethics statement

The study protocol was approved by the institutional review boards at Vanderbilt University Medical Center and at each collaborating institute. Informed consent was obtained from all participants.

Study population

This study consisted of a discovery stage and three validation stages. The overall study design is presented in Figure 1. Fifteen studies contributing a total of 17 153 breast cancer cases and 16 943 controls participated in this consortium. Detailed descriptions of participating studies are included in Supplementary Material. Briefly, the consortium included 20 114 Chinese women from eight studies conducted in the following locations: Shanghai, China [$n = 10\,097$; SBCS Phase I (SBCS-I) (7,28) and Phase II (SBCS-II) (7), Shanghai Breast Cancer Survival Study (SBCSS)/Shanghai Endometrial Cancer Study (SECS, only SECS controls were included in the current study) (7)], Tianjin, China [$n = 3095$ (29)], Nanjing, China [$n = 2816$ (30,31)], Taiwan [$n = 2080$ (32,33)], Hong Kong [$n = 1047$ (34)] and Guangzhou, China ($n = 979$); 3135 Japanese women from three studies conducted in Nagoya, Japan [$n = 1273$, (35)], Hawaii, USA [$n = 1061$; Multiethnic Cohort Study (MEC) (36,37)] and Nagano, Japan [$n = 801$, (38)]; and 10 847 women from three studies conducted in Korea [Seoul Breast Cancer Study (SeBCS) (39,40), Korean Hereditary Breast Cancer Study (KOHBRA) (41) and Korean Genome and Epidemiology Study (KoGES) (42)] (Table 1).

SNP selection for validation in Stage II

In the present study, we selected 49 promising SNPs for Stage-II replication among 4146 cases and 1823 controls recruited in the Shanghai studies. Only SNPs included in the Affymetrix 6.0 SNP arrays were selected for Stage-II evaluation. Selection criteria for these SNPs were: (i) MAF $\geq$ 10%, (ii) very clear genotyping clusters, (iii) not in strong LD ($r^2 \leq 0.5$) with any previously confirmed breast cancer genetic risk variants, (iv) consistent with Hardy–Weinberg equilibrium (HWE) with $P > 0.01$ in controls, and (v) $P < 0.005$ in the SBCS GWA scan data for SNPs on Affymetrix 6.0 array (7,11). If there were multiple SNPs in strong LD with a strong association in one region, the SNP with the smallest *P*-value was selected.

Genotyping

Genotyping for Stage I has been described previously (7,11). Briefly, the initial 300 subjects were genotyped using the Affymetrix GeneChip Mapping 500K Array Set, and the remaining 3918 subjects were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. We included one negative control and three positive quality-control (QC) samples from Coriell Cell Repositories (http://ccr.coriell.org/) in each of the 96-well plates for Affymetrix SNP Array 6.0 genotyping. A total of 127 positive QC samples were successfully genotyped, and the average concordance rate was 99.9% with a median value of 100%. The sex of all study samples was confirmed to be female. The identity-by-descent analysis based on identity-by-state was conducted to detect first-degree cryptic relationships using PLINK, version 1.06. All samples with a call rate <95% were excluded. SNPs were excluded if: (i) MAF < 1%, (ii) call rate < 95%, or (iii) genotyping

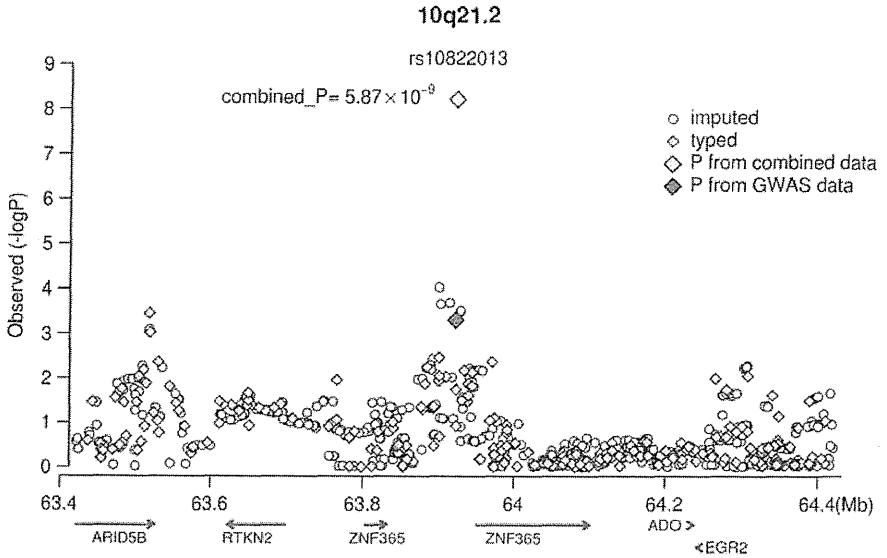

Figure 3. A regional plot of the $-\log P$-values for SNPs at 10q21.2. Results ($-\log P$) are shown for directly genotyped (blue diamonds) and imputed (white circles) SNPs for a 1 Mb region centered on SNP rs10822013. SNP rs10822013 is shown in red diamonds for Stage-I data and yellow diamonds for all four stages combined. Gene locations are from the March 2006 UCSC Genome Browser assembly.

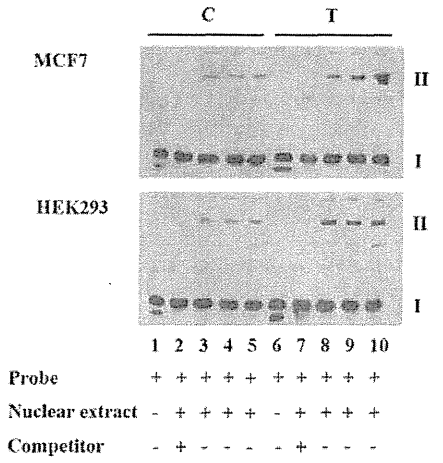

Figure 4. Electrophoretic mobility shift assays of SNP rs10822013. Nuclear protein extracts from MCF7 (top panel) and HEK293 (bottom panel) cells were incubated with biotin-labeled probes corresponding to the reference allele (lanes 1–5) or risk allele (lanes 6–10) of rs10822013 in the absence or presence of competitors. Lanes 1 and 6, no nuclear extracts; lanes 2 and 7, unlabeled competitor in 200-fold molar excess; lanes 3 and 8 (5 mM $MgCl_2$); lanes 4 and 9 (2.5 mM $MgCl_2$); and lanes 5 and 10 (1.25 mM $MgCl_2$), no competitor. I, free biotin-labeled probes; II, specific DNA–protein complex bands.

concordance rate < 95% in QC samples. The final data set included 2062 cases and 2066 controls for 684 457 markers.

Genotyping for Stage II was completed using the iPLEX Sequenom MassArray platform (7,11). Included in each 96-well plate as QC samples were one negative control (water), two blinded duplicates and two samples from the HapMap project. The mean concordance rate was 99.7% for the blinded duplicates and 98.8% for HapMap samples. To compare the consistency between Affymetrix 6.0 and Sequenom platforms, we also genotyped 124 samples included in Stage I in the Stage II Sequenom genotyping; the concordance rate was 98.6% between the two platforms.

Genotyping for eight of the studies included in Stage III was performed at five different centers using TaqMan assays. The genotyping assay protocol was developed and validated at the Vanderbilt Molecular Epidemiology Laboratory, and TaqMan genotyping assay reagents were provided to the investigators of the Tianjin study (Tianjin Cancer Institute and Hospital), Nanjing study (Nanjing Medical University), Guangzhou study (Sun Yat-sen University) and MEC study (University of Southern California), who conducted genotyping assays at their own laboratories. Samples from the four other studies included in Stage III were genotyped at the Vanderbilt Molecular Epidemiology Laboratory. For TaqMan genotyping assays conducted at the Vanderbilt Molecular Epidemiology Laboratory, one negative control and two samples from the HapMap project were included in each 96-well plate, along with 30 unrelated European and 45 Chinese samples from the HapMap project for QC purposes. The consistency rate was 100% for the HapMap samples comparing the genotyping data obtained from the current study with the data obtained from the HapMap project. Each of the non-Vanderbilt laboratories was asked to genotype a trial plate containing DNA from 70 Chinese-ancestry samples before the main study genotyping was conducted. The consistency rate across all centers for these trial samples was 100% compared with genotypes previously determined at Vanderbilt. In addition, replicate samples comparing 3–7% of all study samples were dispersed among the genotyping plates at all centers.

Data from the SeBCS phase I (SeBCS-I) analyzed in Stage IV were extracted from GWA scan data generated using the Affymetrix Genome-Wide Human SNP Array 6.0. A total of 30 QC samples were successfully genotyped, and the concordance rate was 99.83%. The sex of all samples was

confirmed to be female. SNPs were excluded if: (i) genotype call rate < 95%, (ii) MAF < 1% in either the cases or controls, (iii) deviation from HWE at $P < 10^{-6}$, and (iv) poor cluster plot in either cases or controls. Genotyping for other Stage IV samples [SeBCS phase II (SeBCS-II), KOHBRA and KoGES] was completed using the iPLEX Sequenom MassArray platform as described above (7,11). The mean concordance rate was 99.7% for the blinded duplicates, 98.9% for HapMap samples and 99.5% between Sequenom and Affymetrix 6.0 genotyping.

Plasmid constructs and luciferase assays

A 2.2 kb DNA fragment was PCR-amplified by using human genomic DNA containing either the major allele (C) or the minor allele (T) of rs10822013 with forward primer 5′-GTTACGCGTCAC CTA TAG AAA AGG GCC TGG TTG-3′ and backward primer 5′-GTTCTCGAGCTC TTT ACC TAG GGC AGA GGA GC-3′. The fragments were cloned upstream of the luciferase reporter vectors pGL3 basic, pGL3 promoter or pGL3 enhancer (Promega, WI, USA) between the *Nhe*1 and *Xho*1 restriction sites. All DNA constructs were verified by sequencing analysis. Enhancer and promoter activity was determined by transient transfection followed by an *in vitro* luciferase reporter assay in HEK293 cells. Transfection was performed with the use of FuGene 6 Transfection Reagent (Roche Diagnostics, Indianapolis, IN, USA) in triplicate for each of the constructs. Briefly, 2×10^5 cells were seeded in 24-well plates and co-transfected with pGL4.73, a *Renilla* expressing vector, which served as a reference for transfection efficiency. Thirty-six to 48 h later, the cells were lysed with passive lysis buffer, and luminescence (relative light units) was measured using the Dual-Luciferase Assay System (Promega). The rs10822013 regulatory activity was measured as a ratio of firefly luciferase activity to *Renilla* luciferase activity, and the mean was calculated from three independent experiments with triplicate assays in each experiment.

Electrophoretic mobility shift assay

Biotin-labeled, double-stranded oligonucleotide probes 5′-TGG CAC AAG AAA ATG CGT TGT GAA CAA ACT-3′ and 5′-AGT TTG TTC ACA ACG CAT TTT CTT GTG CCA-3′, and 5′-TGG CAC AAG AAA ATG TGT TGT GAA CAA ACT-3′ and 5′-AGT TTG TTC ACA ACA CAT TTT CTT GTG CCA-3′ containing either the major or minor allele sequence were synthesized. The probes were incubated with nuclear protein extracts from HEK293 and MCF7 cells, in the presence or absence of competitors, i.e. unlabelled probes. Protein–DNA complexes were resolved by polyacrylamide gel electrophoresis and detected using a LightShift Chemiluminescent EMSA kit (Pierce Biotechnology, Rockford, IL, USA).

Statistical analyses

PLINK version 1.06 was used to analyze genome-wide data obtained in Stage I. A set of 4305 SNPs with an MAF ≥35% and a distance ≥100 kb between two adjacent SNPs was selected to evaluate the population structure. The inflation factor λ was estimated to be 1.038, suggesting that any population substructure, if present, should not have any appreciable effect on the results.

Individual data were obtained from each study for a pooled analysis. Case–control differences in selected demographic characteristics and major risk factors were evaluated using *t*-tests (for continuous variables) or χ^2-tests (for categorical variables). Associations between SNPs and breast cancer risk were assessed using ORs and 95% CIs derived from logistic regression models. ORs were estimated for heterozygotes and homozygotes for the variant allele compared with homozygotes for the common allele. ORs also were estimated for the variant allele based on a log-additive model and adjusted for age, study site and ethnicity, when appropriate. Heterogeneity across studies and between ethnicities was assessed with likelihood ratio tests. Stratified analyses by ethnicity, menopausal status and ER status were carried out.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

The authors wish to thank the study participants and research staff for their contributions and commitment to this project, Regina Courtney and Jie Wù for DNA preparation, and Bethanie Rammer and Mary Jo Daly for clerical support in the preparation of this manuscript.

Conflict of Interest statement. None declared.

FUNDING

This research was supported in part by US National Institutes of Health grants R01CA124558, R01CA064277, R37CA070867, R01CA090899 and R01CA100374, as well as Ingram Professorship and Research Reward funds awarded to W.Z.; R01CA118229, R01CA092585, and Department of Defense (DOD) Idea Award BC011118 awarded to X.-O.S.; and R01CA122756 and DOD Idea Award BC050791 awarded to Q.C. Participating studies (principal investigator, grant support) in the consortium are as follows: the Shanghai Breast Cancer Study (W.Z., R01CA064277), the Shanghai Breast Cancer Survival Study (X.-O.S., R01CA118229), the Shanghai Endometrial Cancer Study (X.-O.S., R01CA092585, contributed only controls to the consortium), the Seoul Breast Cancer Study [D.K., Basic Research Laboratory (BRL) program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology, 2011-0001564], the Korean Hereditary Breast Cancer Study/Korean Genome and Epidemiology Study (S.-W.K., the National R&D Program for Cancer Control, Ministry for Health, Welfare and Family Affairs, Republic of Korea, 1020350), the Tianjin Study (K.C., the National Natural Science Foundation of China Grant No. 30771844), the Nanjing Study (H.S., 09KJA330001, Jiangsu, China), the Taiwan Biobank Study

(C.-Y.S., DOH97-01), the Hong Kong Study (U.S.K., Research Grant Council, Hong Kong SAR, China, HKU 7520/05M and 76730M), the Guangzhou Breast Cancer Study (Z.R., the National Natural Science Foundation of China, 81072383), the Multiethnic Cohort Study (B.E.H., CA063464; L.K., CA054281; and C.H., CA132839), the Nagano Breast Cancer Study (S.T., Grants-in-Aid for the Third Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan, for Scientific Research on Priority Areas, 17015049 and for Scientific Research on Innovative Areas, 221S0001, from the Ministry of Education, Culture, Sports, Science, and Technology of Japan) and the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (K.T., Grants-in-Aid for Scientific Research on Priority Areas, 17015052, from the Ministry of Education, Culture, Sports, Science, and Technology of Japan H.T., Grants-in-Aid for the Third Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan, H20-002). Sample preparation and genotyping assays at Vanderbilt were conducted at the Survey and Biospecimen Shared Resources and Vanderbilt Microarray Shared Resource, which are supported in part by Vanderbilt-Ingram Cancer Center (P30 CA068485).

REFERENCES

1. Nathanson, K.L., Wooster, R. and Weber, B.L. (2001) Breast cancer genetics: what we know and what we need. *Nat. Med.*, **7**, 552–556.
2. Balmain, A., Gray, J. and Ponder, B. (2003) The genetics and genomics of cancer. *Nat. Genet.*, **33** (suppl.), 238–244.
3. Easton, D.F., Pooley, K.A., Dunning, A.M., Pharoah, P.D., Thompson, D., Ballinger, D.G., Struewing, J.P., Morrison, J., Field, H., Luben, R. *et al.* (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*, **447**, 1087–1093.
4. Hunter, D.J., Kraft, P., Jacobs, K.B., Cox, D.G., Yeager, M., Hankinson, S.E., Wacholder, S., Wang, Z., Welch, R., Hutchinson, A. *et al.* (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.*, **39**, 870–874.
5. Stacey, S.N., Manolescu, A., Sulem, P., Thorlacius, S., Gudjonsson, S.A., Jonsson, G.F., Jakobsdottir, M., Bergthorsson, J.T., Gudmundsson, J., Aben, K.K. *et al.* (2008) Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat. Genet.*, **40**, 703–706.
6. Gold, B., Kirchhoff, T., Stefanov, S., Lautenberger, J., Viale, A., Garber, J., Friedman, E., Narod, S., Olshen, A.B., Gregersen, P. *et al.* (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. *Proc. Natl Acad. Sci. USA*, **105**, 4340–4345.
7. Zheng, W., Long, J., Gao, Y.T., Li, C., Zheng, Y., Xiang, Y.B., Wen, W., Levy, S., Deming, S.L., Haines, J.L. *et al.* (2009) Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat. Genet.*, **41**, 324–328.
8. Thomas, G., Jacobs, K.B., Kraft, P., Yeager, M., Wacholder, S., Cox, D.G., Hankinson, S.E., Hutchinson, A., Wang, Z., Yu, K. *et al.* (2009) A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat. Genet.*, **41**, 579–584.
9. Ahmed, S., Thomas, G., Ghoussaini, M., Healey, C.S., Humphreys, M.K., Platte, R., Morrison, J., Maranian, M., Pooley, K.A., Luben, R. *et al.* (2009) Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat. Genet.*, **41**, 585–590.
10. Stacey, S.N., Manolescu, A., Sulem, P., Rafnar, T., Gudmundsson, J., Gudjonsson, S.A., Masson, G., Jakobsdottir, M., Thorlacius, S., Helgason, A. *et al.* (2007) Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat. Genet.*, **39**, 865–869.
11. Long, J., Cai, Q., Shu, X.O., Qu, S., Li, C., Zheng, Y., Gu, K., Wang, W., Xiang, Y.B., Cheng, J. *et al.* (2010) Identification of a functional genetic variant at 16q12.1 for breast cancer risk: results from the Asia Breast Cancer Consortium. *PLoS Genet.*, **6**, e1001002.
12. Turnbull, C., Ahmed, S., Morrison, J., Pernet, D., Renwick, A., Maranian, M., Seal, S., Ghoussaini, M., Hines, S., Healey, C.S. *et al.* (2010) Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat. Genet.*, **42**, 504–507.
13. Antoniou, A.C., Wang, X., Fredericksen, Z.S., McGuffog, L., Tarrell, R., Sinilnikova, O.M., Healey, S., Morrison, J., Kartsonaki, C., Lesnick, T. *et al.* (2010) A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. *Nat. Genet.*, **42**, 885–892.
14. Fletcher, O., Johnson, N., Orr, N., Hosking, F.J., Gibson, L.J., Walker, K., Zelenika, D., Gut, I., Heath, S., Palles, C. *et al.* (2011) Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. *J. Natl Cancer Inst.*, **103**, 425–435.
15. Gaudet, M.M., Kirchhoff, T., Green, T., Vijai, J., Korn, J.M., Guiducci, C., Segre, A.V., McGee, K., McGuffog, L., Kartsonaki, C. *et al.* (2010) Common genetic variants and modification of penetrance of BRCA2-associated breast cancer. *PLoS Genet.*, **6**, e1001183.
16. Zhang, B., Beeghly-Fadiel, A., Long, J. and Zheng, W. (2011) Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. *Lancet Oncol.*, **12**, 477–488.
17. Long, J., Shu, X.O., Cai, Q., Gao, Y.T., Zheng, Y., Li, G., Li, C., Gu, K., Wen, W., Xiang, Y.B. *et al.* (2010) Evaluation of breast cancer susceptibility loci in Chinese women. *Cancer Epidemiol. Biomarkers Prev.*, **19**, 2357–2365.
18. Zheng, W., Wen, W., Gao, Y.T., Shyr, Y., Zheng, Y., Long, J., Li, G., Li, C., Gu, K., Cai, Q. *et al.* (2010) Genetic and clinical predictors for breast cancer risk assessment and stratification among Chinese women. *J. Natl Cancer Inst.*, **102**, 972–981.
19. Wang, Q., Du, X., Meinkoth, J., Hirohashi, Y., Zhang, H., Liu, Q., Richter, M. and Greene, M.I. (2006) Characterization of Su48, a centrosome protein essential for cell division. *Proc. Natl Acad. Sci. USA*, **103**, 6512–6517.
20. Gianfrancesco, F., Esposito, T., Ombra, M.N., Forabosco, P., Maninchedda, G., Fattorini, M., Casula, S., Vaccargiu, S., Casu, G., Cardia, F. *et al.* (2003) Identification of a novel gene and a common variant associated with uric acid nephrolithiasis in a Sardinian genetic isolate. *Am. J. Hum. Genet.*, **72**, 1479–1491.
21. Berberat, P.O., Katori, M., Kaczmarek, E., Anselmo, D., Lassman, C., Ke, B., Shen, X., Busuttil, R.W., Yamashita, K., Csizmadia, E. *et al.* (2003) Heavy chain ferritin acts as an antiapoptotic gene that protects livers from ischemia reperfusion injury. *FASEB J.*, **17**, 1724–1726.
22. Epsztejn, S., Glickstein, H., Picard, V., Slotki, I.N., Breuer, W., Beaumont, C. and Cabantchik, Z.I. (1999) H-ferritin subunit overexpression in erythroid cells reduces the oxidative stress response and induces multidrug resistance properties. *Blood*, **94**, 3593–3603.
23. Shpyleva, S.I., Tryndyak, V.P., Kovalchuk, O., Starlard-Davenport, A., Chekhun, V.F., Beland, F.A. and Pogribny, I.P. (2011) Role of ferritin alterations in human breast cancer cells. *Breast Cancer Res. Treat.*, **126**, 63–71.
24. Wu, X., Lee, V.C., Chevalier, E. and Hwang, S.T. (2009) Chemokine receptors as targets for cancer therapy. *Curr. Pharm. Des.*, **15**, 742–757.
25. Mburu, Y.K., Wang, J., Wood, M.A., Walker, W.H. and Ferris, R.L. (2006) CCR7 mediates inflammation-associated tumor progression. *Immunol. Res.*, **36**, 61–72.
26. Yu, J.W. and Shi, Y. (2008) FLIP and the death effector domain family. *Oncogene*, **27**, 6216–6227.
27. Safa, A.R., Day, T.W. and Wu, C.H. (2008) Cellular FLICE-like inhibitory protein (C-FLIP): a novel target for cancer therapy. *Curr. Cancer Drug Targets*, **8**, 37–46.
28. Gao, Y.T., Shu, X.O., Dai, Q., Potter, J.D., Brinton, L.A., Wen, W., Sellers, T.A., Kushi, L.H., Ruan, Z., Bostick, R.M. *et al.* (2000) Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. *Int. J. Cancer*, **87**, 295–300.
29. Zhang, L., Gu, L., Qian, B., Hao, X., Zhang, W., Wei, Q. and Chen, K. (2009) Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes CYP17 and CYP19 with breast cancer risk in Chinese women. *Breast Cancer Res. Treat.*, **114**, 327–338.

30. Liang, J., Chen, P., Hu, Z., Zhou, X., Chen, L., Li, M., Wang, Y., Tang, J., Wang, H. and Shen, H. (2008) Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women. *Carcinogenesis*, **29**, 2341–2346.
31. Wang, Y., Hu, Z., Liang, J., Wang, Z., Tang, J., Wang, S., Wang, X., Qin, J., Wang, X. and Shen, H. (2008) A tandem repeat of human telomerase reverse transcriptase (hTERT) and risk of breast cancer development and metastasis in Chinese women. *Carcinogenesis*, **29**, 1197–1201.
32. Ding, S.L., Yu, J.C., Chen, S.T., Hsu, G.C., Kuo, S.J., Lin, Y.H., Wu, P.E. and Shen, C.Y. (2009) Genetic variants of BLM interact with RAD51 to increase breast cancer susceptibility. *Carcinogenesis*, **30**, 43–49.
33. Hsu, H.M., Wang, H.C., Chen, S.T., Hsu, G.C., Shen, C.Y. and Yu, J.C. (2007) Breast cancer risk is associated with the genes encoding the DNA double-strand break repair Mre11/Rad50/Nbs1 complex. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 2024–2032.
34. Chan, K.Y., Liu, W., Long, J.R., Yip, S.P., Chan, S.Y., Shu, X.O., Chua, D.T., Cheung, A.N., Ching, J.C., Cai, H. *et al.* (2009) Functional polymorphisms in the BRCA1 promoter influence transcription and are associated with decreased risk for breast cancer in Chinese women. *J. Med. Genet.*, **46**, 32–39.
35. Hamajima, N., Matsuo, K., Saito, T., Hirose, K., Inoue, M., Takezaki, T., Kuroishi, T. and Tajima, K. (2001) Gene-environment interactions and polymorphism studies of cancer risk in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II). *Asian Pac. J. Cancer Prev.*, **2**, 99–107.
36. Haiman, C.A., Garcia, R.R., Hsu, C., Xia, L., Ha, H., Sheng, X., Le, M.L., Kolonel, L.N., Henderson, B.E., Stallcup, M.R. *et al.* (2009) Screening and association testing of common coding variation in steroid hormone receptor co-activator and co-repressor genes in relation to breast cancer risk: the Multiethnic Cohort. *BMC Cancer*, **9**, 43.
37. Kolonel, L.N., Henderson, B.E., Hankin, J.H., Nomura, A.M., Wilkens, L.R., Pike, M.C., Stram, D.O., Monroe, K.R., Earle, M.E. and Nagamine, F.S. (2000) A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am. J. Epidemiol.*, **151**, 346–357.
38. Itoh, H., Iwasaki, M., Hanaoka, T., Kasuga, Y., Yokoyama, S., Onuma, H., Nishimura, H., Kusama, R. and Tsugane, S. (2009) Serum organochlorines and breast cancer risk in Japanese women: a case-control study. *Cancer Causes Control*, **20**, 567–580.
39. Choi, J.Y., Lee, K.M., Park, S.K., Noh, D.Y., Ahn, S.H., Yoo, K.Y. and Kang, D. (2005) Association of paternal age at birth and the risk of breast cancer in offspring: a case control study. *BMC Cancer*, **5**, 143.
40. Lee, K.M., Choi, J.Y., Park, S.K., Chung, H.W., Ahn, B., Yoo, K.Y., Han, W., Noh, D.Y., Ahn, S.H., Kim, H. *et al.* (2005) Genetic polymorphisms of ataxia telangiectasia mutated and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 821–825.
41. Han, S.A., Park, S.K., Hyun, A.S., Hyuk, L.M., Noh, D.Y., Kim, L.S., Noh, W.C., Jung, Y., Sang, K.K. and Kim, S.W., Korean Breast Cancer Study Group (2011) The Korean Hereditary Breast Cancer (KOHBRA) Study: protocols and interim report. *Clin. Oncol. (R. Coll. Radiol.)*, **23**, 434–441.
42. Cho, Y.S., Go, M.J., Kim, Y.J., Heo, J.Y., Oh, J.H., Ban, H.J., Yoon, D., Lee, M.H., Kim, D.J., Park, M. *et al.* (2009) A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat. Genet.*, **41**, 527–534.

Original Article

Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption

Ribeka Takachi PhD[1,2], Yoshitaka Tsubono PhD[1], Keisuke Baba[3], Manami Inoue PhD[1], Shizuka Sasazuki PhD[1], Motoki Iwasaki PhD[1], and Shoichiro Tsugane PhD[1] for the Japan Public Health Center-based Prospective Study Group

[1]*Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan*
[2]*Department of Community Preventive Medicine, Division of Social and Environmental Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*
[3]*Tohoku University School of Medicine, Miyagi, Japan*

Asian populations have changed from traditional to Westernized diets, with increased red meat intake. They are suggested to be particularly susceptible to the adverse effects of red meat on the development of colorectal cancers, however, few prospective studies of this putative link have been conducted. We examined associations between the consumption of red and processed meat and the risk of subsite-specific colorectal cancer by gender in a large Japanese cohort. During 1995-1998, a validated food frequency questionnaire was administered to 80,658 men and women aged 45-74 years. During 758,116 person-years of follow-up until the end of 2006, 1,145 cases of colorectal cancer were identified. Higher consumption of red meat was significantly associated with a higher risk of colon cancer among women [multivariate hazard ratios (95%CIs) for the highest versus lowest quintiles (HR): 1.48 (1.01, 2.17; trend p=0.03)], as was higher consumption of total meat among men [HR=1.44 (1.06, 1.98; trend p=0.07)]. By site, these positive associations were found for the risk of proximal colon cancer among women and for distal colon cancer among men. No association was found between the consumption of processed meat and risk of either colon or rectal cancer. In conclusion, red meat intake may modestly increase the risk of colon cancer in middle-aged Japanese, although the highest quintile of red meat consumption could be considered moderate by Western standards.

Key Words: meat, colon cancer, rectal cancer, prospective studies, Japan

INTRODUCTION

The linear increase in the incidence and mortality of colon cancer between 1970 and the mid-1990s among Japanese of both sexes occurred in parallel with an increase in the intake of meat, such as beef and pork products.[1-4] Despite this increase, however, intake is still lower in Japanese than Western populations (approx 78, 130, 160, 185, and 200 g per capita per day in Japan, UK, Italy, France, and US, respectively, according to the FAO food supply database, 1995).[3] Given findings that descendents of Japanese migrants to the US have a higher incidence of colorectal cancer than US-born Caucasians,[5,6] individuals of Asian ethnicity may be particularly susceptible to the adverse effects of the Westernized diet, including red meat intake, owing to exposure to other lifestyle risk factors, the modifying influence of genetic biological susceptibility factors, or both.

A recent joint report by the World Cancer Research Fund/American Institute for Cancer Research concluded that the evidence that red and processed meats are a cause of colorectal cancer is convincing.[7] Most prospective studies to date have been conducted in Western countries,[8-10] however, and we are aware of only five in Asian populations, including the Japanese,[11-15] most of which failed to demonstrate a clear positive association between red or processed meat intake and colorectal cancer risk.

Asian populations tend to differ from Western populations in colonic anatomy and pattern of intracolonic bacteria,[16,17] the latter of which relates to the production of secondary bile acids from primary bile acids (which are required to digest animal fat) and of endogenous *N*-nitroso compounds (NOC).[7,18,19] A number of potential differences in the distribution of possible confounders is also likely, with Asians having a higher distribution of smok-

Corresponding Author: Dr Shizuka Sasazuki, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
Tel: +81-3-3542-2511 ext. 3378; Fax: +81-3-3547-8578
Email: ssasazuk@ncc.go.jp
Manuscript received 8 December 2010. Initial review completed 9 March 2011. Revision accepted 16 June 2011.

ers (among men) and higher consumption of salt-preserved fish, the major sources of exogenous NOC,[7,20] as well as a lower prevalence of obesity. Moreover, few prospective studies have evaluated the effect of red meat consumption on the risk of subsite-specific colon cancers, separately by gender,[21-23] although risk factors for and biological pathways of proximal and distal colon carcinogenesis have been suggested to differ. In Japan, incidence rates for colorectal cancer have reached those in Western countries (GLOBOCAN 2002). These findings highlight the importance of studies aimed at characterizing the influence of red meat consumption on the risk of colorectal cancer by sub-site in Asian populations.

In this study, we used a validated comprehensive food frequency questionnaire to examine associations between red meat and the risk of colorectal cancer in a population-based prospective cohort study in Japan. Particular focus was placed on the risk of colorectal cancer according to sub-site in relation to red meat intake.

MATERIALS AND METHODS

Study population

The Japan Public Health Center-based Prospective (JPHC) Study was conducted in two cohorts (Cohort I and II), initiated in 1990-1994. The study population was defined as all registered Japanese inhabitants aged 40-69 years in 11 public health center areas, as identified from the population registries maintained by the local municipalities. The study design has been described in detail previously.[24] The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Participants in the present study were subjects in the JPHC study who responded to a self-administered 5-year follow-up questionnaire, which included comprehensive information on food intake and lifestyle factors, in 1995-1999, at age 45-74 years. This follow-up survey was used as the starting point in the present study. One public health center area (Tokyo) was excluded from the present analysis because cancer incidence data were not available.

After exclusion of 11,943 persons who had died, moved out of a study area, or were lost to follow-up before the starting point of the present study (1995-1999), the remaining 121,134 subjects were eligible for participation. Of these, 98,514 subjects responded to the questionnaire survey (46,029 men, 52,485 women; response rate: 81.3%) and were included in the present study.

Follow-up

Subjects were followed from the starting point (time that the FFQ for 5-year follow-up survey was completed) until December 31, 2006. Changes in residence status, including survival, were obtained annually from the residential registry in each area; or for those who had moved out of the study area, through the municipal office in the area to which they had moved. Mortality data for persons in the residential registry are forwarded to the Ministry of Health, Labour and Welfare, and are coded for inclusion in the national Vital Statistics. Residency registration and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period in the present study, 7,658 (7.8%) subjects died, 3,970 (4.0%) moved out of the study area, and 318 (0.3%) were lost to follow-up.

The occurrence of cancer was identified by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries, with permission from the local governments responsible for the cancer registries. Colorectal cancer cases were coded according to the International Classification of Diseases for Oncology, Third Edition (C18-C20), with colon cancer as C18 (C18.0-C18.5 for proximal colon cancer and C18.6-C18.7 for distal colon cancer) and rectal cancer as C19 and C20.[25] In our cancer registry system, the proportion of cases for which information was available from death certificates only was 2.6% of colorectal cancers. We confirmed 1,435 cases of newly diagnosed colorectal cancer among the 98,514 subjects by December 31, 2006.

Of the 98,514 respondents, we excluded subjects with a history of cancer (n=4,008), those who did not complete the diet component of the questionnaire (n=1,030), and those with extreme self-reported height or weight (≥200 cm, <20 kg; n=2,456). A history of cancer was defined as a diagnosis of cancer before the starting point or a self-report of cancer in the questionnaires. Of the remaining 91,020 subjects, 4,550 who reported extreme total energy intake (lower and upper 2.5 percentiles: 913 and 3,954 kcal/day, respectively), and subjects for whom values for any of the potential confounders were missing (n=5,812) were excluded, leaving 80,658 subjects (38,462 men, 42,196 women) for final analysis, including 1,145 with colorectal cancer (481 colon and 233 rectal cancer cases in men, and 307 colon and 124 rectal cancer cases in women). By sub-site, proximal and distal colon cancer accounted for 200 and 257 cases in men and 179 and 110 in women, respectively.

Food frequency questionnaire (FFQ)

The FFQ asked about the usual consumption of 138 foods and beverages during the previous year in standard portions/units and nine frequency categories.[26] The FFQ enquired about 16 meat items. The red meat items included 3 beef dishes (steak, grilled beef, and stewed beef), 6 pork dishes (stir-fried pork, deep-fried pork, stewed pork in Western style, stewed pork in Japanese style, pork in soup, and pork liver), 4 processed meat products (ham, sausage or Weiner sausage, bacon, and luncheon meat), and chicken liver. Poultry items included two chicken meals (grilled chicken and deep-fried chicken). Standard portion sizes were specified for each food item in three amount choices: small (50% smaller than standard), medium (same as standard) and large (50% larger). The amount of each food consumed (grams/day) was calculated from the responses. Energy and nutrient intake, excluding heme iron, were calculated using the Standardized Tables of Food Composition, 5th revised edition.[27] Heme iron intake was computed using the following proportions of iron for each type of meat: 69% for beef; 39% for pork, ham, bacon, and luncheon meats; 26% for chicken and fish (19 items); and 21% for liver.

The validity of the FFQ for the assessment of meat intake has been confirmed.[28,29] Spearman's correlation coefficients between energy-adjusted meat intake based on

the FFQ and those based on 28-day (or 14-day for one public health center area) dietary records among subsamples of men and women were 0.50 and 0.45 for Cohort I and 0.48 and 0.44 for Cohort II, respectively. Correlation coefficients for the reproducibility of the FFQ administered 1 year apart for men and women were 0.52 and 0.52 for Cohort I and 0.52 and 0.41 for Cohort II, respectively.[29,30] Correlation coefficients for the validity of the FFQ for assessment of specific meats for men were as follows: beef; 0.43, pork; 0.42, processed meat; 0.45, chicken; 0.20. For women as compared with men, the validity of the FFQ was comparable (unpublished data, Nanri, et al).

Statistical analysis

Person-years of follow-up were calculated for each subject from the starting point to the date of diagnosis, date of emigration from the study area, date of death, or end of the follow-up period (December 31, 2006), whichever occurred first. Subjects lost to follow-up were censored on the last confirmed date of presence in the study area. A total of 354,987 and 403,129 person-years for men and women, respectively, were accrued for the present analysis.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for energy-adjusted meat consumption categories in quintiles based on the sex-specific distributions for men and women separately, with the lowest consumption category as the reference, using Cox proportional hazards models with adjustment for potential confounding variables according to the SAS PHREG procedure (SAS software, version 9.1, SAS Institute, Inc., Cary, North Carolina). The assumption of proportional hazards was established graphically; no deviation from proportionality was found. Person-years (follow-up time) was used for the underlying time metric in the Cox regressions. A residual model was used for energy adjustment.[31]

We conducted initial analyses by adjusting for age at the starting point (continuous) and study area (10 PHC districts). In the second multivariate model, we further adjusted for body mass index in kg/m^2 (<19, 19-22.9, 23-24.9, 25-26.9, and ≥27), smoking status (never, past, and current), alcohol consumption (none, occasional, 1-149, 150-299, 300-449, and ≥450 g of ethanol/week), physical activity in metabolic equivalent task-hours/day (<30, 30-34.9, 35-39.9, and ≥40), diabetes who either report of medication use for diabetes or a history of diabetes, screening examinations (yes/no) for fecal occult blood test, barium enema, and colonoscopy and quintiles of total energy, calcium, vitamins D and B-6, folate, and dietary fiber. This multivariate model was further adjusted for dried and salted fish intake in quintiles as a potential proxy for the intake of *N*-nitroso compounds.[20,32,33] Subjects for whom values for any of the potential confounders were missing were excluded from the final analysis, because findings did not materially differ when subjects with missing values were retained in the analyses (n=86,470) by assigning dummy variables for missing responses. Further, we conducted an additional analysis with the sub-site of colon cancer (proximal and distal) as endpoints. We also assessed linear associations (trend p-values) using the median values of meat intake for each quintile in the hazard models.

We additionally performed sub-group analyses according to age (<60 or ≥60 years), smoking status ("never" for nonsmokers or "past" and "current smoker" for ever smokers), body mass index (<25 or ≥25 kg/m^2), alcohol intake (<150 or ≥150 g ethanol/week, for men only), and Cohort (I or II). We sought to confirm whether extremely high meat affects the risk of colorectal cancer compared with very low meat intake. HRs were calculated for meat consumption categories in deciles. Throughout this paper, all p-values are two-sided, and statistical significance was determined at the p <0.05 level.

RESULTS

Red meat intake for men and women ranged from a median value of 15.4 and 13.6 g/day, respectively, in the lowest quintile to 102 and 93.0 g/day, respectively, in the highest. Subjects with higher red meat consumption were slightly younger.

Table 1 shows age-adjusted values for subject characteristics according to quintile of red meat consumption. For both men and women, subjects with higher red meat consumption were more likely to be overweight, and less likely to be heavy drinkers or participate in fecal occult blood test screening. They were also more likely to consume lower levels of calcium, dietary fiber, as well as dried and salted fish. Higher red meat intake was not associated with the prevalence of ever smoking, history of diabetes, or level of physical activity.

As shown in Table 2, higher consumption of red meat was significantly associated with a higher risk of colon cancer among women. Although a statistically significant association was not found between red meat consumption and colon cancer among men (point estimates of multivariate HRs increased), a significant association was seen for higher consumption of total meat. A significant association was seen between higher consumption of beef and pork and the risk of colon cancer among women. No association between the consumption of processed meat and risk of colon cancer was seen among either men or women. Positive associations of red meat, beef, and pork with the risk of colon cancer were more clearly seen after adjustment for dried and salted fish as a potential confounding factor than without this adjustment among women, but not among men (data not shown). No association was found between total meat, red meat or specific meat consumption and the risk of rectal cancer in either gender (Table 2). These results were not different substantially from those using gender combined quintiles (data not shown).

HRs for colon cancer among men with higher total meat intake were attenuated by further adjustment for heme iron, but were not substantially changed by further adjustment for saturated fatty acid intake, with corresponding multivariate HRs for the highest versus lowest quintile of 1.30 (95% CI: 0.87, 1.93; trend p=0.38) and 1.43 (95% CI: 0.95, 2.16; trend p=0.19), respectively. HRs for colon cancer among women with higher red meat intake were not substantially changed by further adjustment for heme iron, but were attenuated by further adjustment for saturated fatty acid intake, with corresponding HRs (95% CI) of 1.62 (1.01, 2.61; trend p=0.02) and 1.38 (0.84, 2.27; trend p=0.18). We further adjusted for

Table 1. Characteristics of subjects according to quintile (Q) of red meat intake for men and women: the JPHC Study, 1995 and 1998 (n= 80,658)

	Men						Women					
	Q1	Q2	Q3	Q4	Q5		Q1	Q2	Q3	Q4	Q5	
Median intake (g)	15	31	46	65	102		14	29	43	60	93	
Range	<24.1	≥24.1, <38.8	≥38.8, <54.6	≥54.6, <78.7	≥78.7		<22.0	≥22.0, <35.8	≥35.8, <50.4	≥50.4, <71.8	≥71.8	
No. of subjects	7,692	7,693	7,692	7,693	7,692		8,439	8,439	8,440	8,439	8,439	
Age	58.1	56.8	56.2	56.0	56.0		58.1	56.9	56.3	56.0	56.0	
(SD†)	(7.7)	(7.7)	(7.7)	(7.8)	(7.7)		(7.6)	(7.6)	(7.6)	(7.8)	(7.8)	
Meat intake (g/day, mean§)												
Total meat	20	39	56	77	127		17	36	52	71	115	
Red meat												
Beef	4	9	13	19	31		3	7	10	15	24	
Pork	8	18	26	37	67		8	18	26	36	65	
Processed meat	2	4	6	8	13		2	4	6	8	12	
Chicken	5	8	10	11	14		5	7	9	10	12	
BMI ≥25kg/m^2 (%¶)	25.7	26.6	27.4	29.7	33.0	**	27.4	27.0	26.6	27.8	31.9	**
Past smoker (%¶)	19.0	18.3	19.1	18.6	17.0		0.8	1.2	1.1	1.3	1.2	
Current smoker (%¶)	47.0	48.5	48.3	47.0	45.1	**	5.8	5.6	5.1	5.7	6.4	
Moderate drinker (>0, <300 g alc/w, %¶)	32.6	36.5	39.0	38.8	37.6	**	11.4	12.7	13.3	12.7	10.2	**
Heavy drinker (≥300 g alc/w, %¶)	39.5	35.7	31.1	27.8	19.5	**	1.9	1.4	1.2	0.7	0.4	**
Physical activity (MET-h‡/day, mean§)	32.9	32.8	32.7	32.5	32.1	**	31.8	32.0	32.0	31.8	31.5	**
Screening examination (yes, %¶)												
Fecal occult blood test	29.8	29.9	30.0	28.2	23.7	**	28.5	29.2	29.9	28.4	23.2	**
Barium enema	6.9	7.5	7.2	7.3	7.2		6.3	5.9	6.0	5.9	7.1	
Colonoscopy	8.6	8.4	8.5	7.9	7.0	**	6.8	6.4	6.7	6.3	6.2	
History of diabetes (%¶)	7.6	7.3	7.0	7.2	6.9		3.8	3.6	3.4	3.2	3.7	
Dietary intake (mean§)												
Total energy, kcal/d	2,132	2,146	2,133	2,105	2,048	**	1,893	1,891	1,872	1,863	1,838	**
Calcium, mg/d	554	534	522	505	462	**	656	624	592	557	491	**
Vitamin D, μg/d	10.1	10.1	10.3	10.4	9.8		10.7	10.4	10.5	10.3	9.3	**
Vitamin B_6, mg/d	1.57	1.58	1.59	1.61	1.60	**	1.54	1.53	1.53	1.53	1.50	**
Folate, μg/day	386	391	393	393	379		452	445	435	425	394	**
Dietary fiber, g/d	12.8	12.6	12.4	12.0	11.0	**	16.0	15.1	14.4	13.6	11.9	**
Dried and salted fish, g/d	20.1	19.2	18.8	18.4	15.4	**	21.1	20.2	19.4	18.6	15.0	**
Saturated fatty acid, g/d	12.7	14.4	16.0	17.89	22.28	**	14.5	16.0	17.1	18.4	22.0	**
Heme iron, mg/d	0.31	0.40	0.48	0.58	0.78	**	0.30	0.38	0.45	0.53	0.70	**

† SD, standard deviation; ‡ MET-h, metabolic equivalent task hours.
§ Values are age-adjusted least square means. ¶ Values are age-standardized proportions. **p<0.01; Trend tests across categories of red meat consumption were calculated by analysis of covariance for age-adjusted means and the Cochran-Mantel-Haenszel test for age-adjusted proportions.

cholesterol-lowering medications (4% and 7% user for men and women, respectively) or hormone replacement therapy (2.5% current user for women) in the multivariate analysis, but results for colon and rectal cancer did not substantially changed (data not shown).

On further analysis using of colon cancer sub-sites (proximal and distal) as endpoints, as shown in Table 3, higher consumption of total meat, red meat, and beef was marginally associated with a higher risk of distal colon cancer but not with the risk of proximal colon cancer among men. In contrast, higher consumption of red meat and beef was associated with a higher risk of proximal colon cancer but not with the risk of distal colon cancer among women. Higher consumption of processed meat was not associated with either proximal or distal colon cancer for either gender.

Stratified analyses according to age (<60 or ≥60 years) showed a clearer association between red meat intake and the risk of colon cancer among the older age group than the younger group for both men and women. Corresponding HRs (95% CI) for the older and younger age groups were 1.46 (0.95, 2.23; trend p=0.07) and 1.05 (0.66, 1.68; trend p=0.87), respectively, among men (259 and 222 cases, respectively), and 1.64 (0.95, 2.82; trend p=0.06) and 1.34 (0.78, 2.30; trend p=0.20), respectively, among women (152 and 155 cases, respectively). Further, significant positive associations were found between the consumption of total or processed meat for men, and beef or pork for women, and the risk of colon cancer among the older age group only (data not shown), although tests of interaction were not statistically significant between age and red meat, or any meat intake for the risk of colon cancer (data not shown). Stratified analyses according to smoking status (never or ever smoker) showed a clearer positive association between processed meat intake and the risk of colon cancer among male nonsmokers (HR: 1.79; 95% CI: 1.04, 3.10; trend p=0.02) than male ever-smokers (HR: 1.10; 95% CI: 0.77, 1.58; trend p=0.62), although tests of interaction were not statistically significant. The main results [positive association between total meat (among men), and red meat including beef and pork (among women) and the risk of colon cancer; and no association between meats (combined or separated) and the risk of rectal cancer among either gender] did not sub-

stantially changed in analyses stratified by body mass index, alcohol intake, or cohort (data not shown). Also, the results did not substantially changed in the analyses that excluded cases diagnosed during the first two years of follow-up (data not shown). When colon cancer was limited to invasive cases (269 cases in men and 186 in women), point estimates of multivariate HRs increased with red meat intake but did not reach statistically significant levels, with multivariate HRs (95%CIs) for the highest versus lowest quintiles of intake of 1.19 (0.78, 1.82; trend p=0.37) for men, 1.39 (0.85, 2.28; trend p=0.18) for women, and 1.30 (0.94, 1.78; trend p=0.08) for the two genders combined.

Finally, in analyses by deciles of meat consumption, higher processed meat intake showed a marginally significant association with the risk of colon cancer for men but not for women, with multivariate HRs for the highest versus lowest decile of 1.37 (95% CI: 0.92, 2.03; trend p=0.05) and 1.67 (95% CI: 0.97, 2.88; trend p=0.36), respectively.

Table 2. Hazard ratios and 95% confidence intervals for colon and rectal cancer according to quintiles of meat consumptions for men and women: the JPHC Study, 1995 and 1998–2006 (n=38,462 and 42,196 for men and women, respectively)

	Men						Women					
	Colon (481 cases)			Rectal (233 cases)			Colon (307 cases)			Rectal (124 cases)		
	Median (g/d)	Cases	HR† (95%CI‡)	Cases	HR† (95%CI‡)		Median (g/d)	Cases	HR† (95%CI‡)	Cases	HR† (95%CI‡)	
Total meat												
Q1	20	98	1.00 (reference)	60	1.00 (reference)		18	63	1.00 (reference)	31	1.00 (reference)	
Q2	39	107	1.25 (0.95, 1.65)	43	0.78 (0.53, 1.16)		36	65	1.14 (0.80, 1.62)	19	0.63 (0.35, 1.12)	
Q3	56	99	1.27 (0.95, 1.69)	46	0.89 (0.60, 1.32)		52	46	0.82 (0.56, 1.21)	25	0.89 (0.52, 1.53)	
Q4	77	82	1.12 (0.83, 1.52)	47	0.94 (0.63, 1.40)		70	67	1.26 (0.88, 1.81)	28	1.02 (0.59, 1.74)	
Q5	117	95	1.44 (1.06, 1.98)	37	0.83 (0.52, 1.30)		107	66	1.35 (0.92, 1.98)	21	0.78 (0.41, 1.46)	
trend p			0.07		0.64				0.10		0.83	
Red meat												
Q1	15	103	1.00 (reference)	53	1.00 (reference)		14	63	1.00 (reference)	31	1.00 (reference)	
Q2	31	103	1.14 (0.87, 1.50)	46	0.96 (0.64, 1.43)		29	67	1.19 (0.84, 1.69)	20	0.67 (0.38, 1.19)	
Q3	46	90	1.08 (0.81, 1.44)	48	1.06 (0.71, 1.58)		43	39	0.70 (0.47, 1.06)	30	1.08 (0.65, 1.81)	
Q4	65	94	1.19 (0.89, 1.60)	50	1.16 (0.78, 1.74)		60	68	1.30 (0.91, 1.86)	21	0.77 (0.43, 1.37)	
Q5	102	91	1.27 (0.93, 1.74)	36	0.93 (0.58, 1.49)		93	70	1.48 (1.01, 2.17)	22	0.81 (0.43, 1.52)	
trend p			0.15		0.99				0.03		0.63	
Beef												
Q1	0.2	102	1.00 (reference)	53	1.00 (reference)		0.1	59	1.00 (reference)	27	1.00 (reference)	
Q2	6.0	83	0.88 (0.65, 1.18)	46	0.89 (0.60, 1.33)		3.9	67	1.37 (0.96, 1.94)	30	1.27 (0.75, 2.15)	
Q3	11	101	1.23 (0.93, 1.63)	38	0.82 (0.54, 1.25)		8.8	61	1.31 (0.91, 1.89)	24	1.06 (0.61, 1.86)	
Q4	19	108	1.35 (1.02, 1.78)	46	1.02 (0.68, 1.53)		15	54	1.26 (0.86, 1.84)	20	0.94 (0.52, 1.72)	
Q5	34	87	1.15 (0.85, 1.55)	50	1.16 (0.77, 1.74)		28	66	1.62 (1.12, 2.34)	23	1.11 (0.61, 2.02)	
trend p			0.10		0.28				0.04		0.95	
Pork												
Q1	6.5	112	1.00 (reference)	54	1.00 (reference)		6.1	65	1.00 (reference)	24	1.00 (reference)	
Q2	15	95	0.94 (0.71, 1.24)	54	1.08 (0.74, 1.58)		15	54	0.92 (0.64, 1.32)	28	1.18 (0.68, 2.05)	
Q3	24	86	0.89 (0.67, 1.18)	34	0.71 (0.46, 1.10)		24	62	1.04 (0.73, 1.49)	23	0.97 (0.54, 1.73)	
Q4	36	96	1.01 (0.77, 1.34)	50	1.08 (0.72, 1.60)		35	48	0.81 (0.55, 1.20)	25	1.06 (0.60, 1.90)	
Q5	62	92	1.06 (0.78, 1.42)	41	0.97 (0.63, 1.51)		59	78	1.42 (0.99, 2.04)	24	1.06 (0.57, 1.97)	
trend p			0.53		0.97				0.05		0.97	
Processed meat												
Q1	0.2	106	1.00 (reference)	66	1.00 (reference)		0.4	61	1.00 (reference)	27	1.00 (reference)	
Q2	1.9	106	1.11 (0.85, 1.46)	49	0.84 (0.58, 1.21)		2.2	69	1.26 (0.89, 1.79)	27	1.09 (0.64, 1.87)	
Q3	3.9	81	0.91 (0.68, 1.22)	35	0.64 (0.42, 0.97)		4.3	60	1.10 (0.76, 1.58)	21	0.85 (0.47, 1.52)	
Q4	7.3	89	1.05 (0.79, 1.41)	48	0.91 (0.62, 1.33)		7.6	58	1.12 (0.77, 1.62)	27	1.19 (0.68, 2.08)	
Q5	16	99	1.27 (0.95, 1.71)	35	0.70 (0.45, 1.09)		15	59	1.19 (0.82, 1.74)	22	0.98 (0.53, 1.79)	
trend p			0.10		0.25				0.64		1.00	
Chicken												
Q1	0.5	103	1.00 (reference)	59	1.00 (reference)		0.5	66	1.00 (reference)	21	1.00 (reference)	
Q2	4.3	95	0.99 (0.75, 1.31)	47	0.82 (0.55, 1.20)		4.0	55	0.90 (0.62, 1.29)	29	1.35 (0.76, 2.38)	
Q3	7.4	106	1.13 (0.86, 1.49)	40	0.72 (0.48, 1.08)		6.8	75	1.26 (0.90, 1.77)	28	1.33 (0.75, 2.37)	
Q4	11	88	1.06 (0.79, 1.42)	48	0.90 (0.61, 1.34)		11	50	0.83 (0.57, 1.21)	20	0.97 (0.52, 1.82)	
Q5	21	89	1.11 (0.83, 1.49)	39	0.72 (0.47, 1.09)		19	61	1.01 (0.70, 1.46)	26	1.27 (0.69, 2.32)	
trend p			0.44		0.22				0.91		0.80	

† HR, hazard ratio; ‡ CI, confidence interval. Hazard ratio was adjusted for age (continuous), Public Health Center area, Body Mass Index in kg/m^2 (<19, 19–22.9, 23–24.9, 25–26.9, and ≥27), smoking status (never, past, and current), alcohol consumption (non, occasional, 1–149, 150–299, 300–449, and ≥450g ethanol/week), physical activity in metabolic equivalent task-hours/day (<30, 30–34.9, 35–39.9, ≥40), medication use for diabetes, history of diabetes, screening examinations (fecal occult blood test; barium enema; colonoscopy), and quintiles of intake of energy, calcium, vitamin D, vitamin B_6, folate, dietary fiber, and dried and salted fish. Linear trends across quintiles of red meat or other meat intake were tested using the derived variable based on median consumption for each quintile as a continuous variable.

Table 3. Hazard ratios and 95% confidence intervals for colon cancer by sub-site according to quintiles of meat consumptions for men and women, the JPHC Study, 1995 and 1998–2006

	Men						Women					
	Proximal colon (200 cases)			Distal colon (257 cases)			Proximal colon (179 cases)			Distal colon (110 cases)		
	Cases	HR†	(95%CI‡)	Cases	HR†	(95%CI‡)	Cases	HR†	(95%CI‡)	Cases	HR†	(95%CI‡)
Total meat												
Q1	42	1.00	(reference)	52	1.00	(reference)	40	1.00	(reference)	18	1.00	(reference)
Q2	47	1.32	(0.87, 2.01)	52	1.14	(0.78, 1.69)	37	1.01	(0.65, 1.59)	25	1.54	(0.84, 2.85)
Q3	37	1.12	(0.71, 1.76)	56	1.36	(0.92, 2.00)	26	0.72	(0.43, 1.18)	17	1.07	(0.55, 2.1)
Q4	41	1.33	(0.85, 2.08)	40	1.03	(0.67, 1.58)	37	1.07	(0.67, 1.71)	28	1.78	(0.96, 3.3)
Q5	33	1.21	(0.73, 2.01)	57	1.65	(1.09, 2.52)	39	1.23	(0.75, 2.01)	22	1.41	(0.71, 2.79)
trend p		0.52			0.04			0.34			0.35	
Red meat												
Q1	47	1.00	(reference)	52	1.00	(reference)	36	1.00	(reference)	22	1.00	(reference)
Q2	43	1.06	(0.70, 1.61)	54	1.19	(0.81, 1.74)	39	1.21	(0.77, 1.91)	24	1.22	(0.68, 2.18)
Q3	36	0.96	(0.61, 1.49)	49	1.17	(0.79, 1.74)	26	0.82	(0.49, 1.37)	12	0.61	(0.30, 1.25)
Q4	40	1.12	(0.72, 1.73)	51	1.29	(0.87, 1.94)	36	1.21	(0.75, 1.96)	29	1.50	(0.84, 2.68)
Q5	34	1.07	(0.66, 1.75)	51	1.42	(0.92, 2.19)	42	1.57	(0.95, 2.58)	23	1.21	(0.63, 2.32)
trend p		0.74			0.12			0.08			0.41	
Beef												
Q1	50	1.00	(reference)	49	1.00	(reference)	28	1.00	(reference)	29	1.00	(reference)
Q2	36	0.78	(0.50, 1.20)	42	0.92	(0.60, 1.39)	42	1.95	(1.20, 3.16)	21	0.82	(0.46, 1.44)
Q3	42	1.06	(0.70, 1.61)	54	1.35	(0.91, 2.01)	39	1.91	(1.17, 3.12)	18	0.73	(0.40, 1.32)
Q4	40	1.06	(0.69, 1.63)	62	1.58	(1.07, 2.34)	26	1.39	(0.81, 2.40)	24	1.05	(0.60, 1.84)
Q5	32	0.89	(0.56, 1.41)	50	1.36	(0.90, 2.06)	44	2.52	(1.53, 4.14)	18	0.78	(0.42, 1.44)
trend p		0.95			0.04			0.01			0.69	
Pork												
Q1	45	1.00	(reference)	62	1.00	(reference)	36	1.00	(reference)	22	1.00	(reference)
Q2	41	1.02	(0.67, 1.56)	50	0.89	(0.61, 1.29)	40	1.23	(0.78, 1.93)	13	0.65	(0.32, 1.29)
Q3	38	1.01	(0.65, 1.57)	45	0.82	(0.55, 1.21)	34	1.03	(0.64, 1.65)	25	1.22	(0.68, 2.19)
Q4	37	0.99	(0.63, 1.55)	50	0.94	(0.64, 1.38)	24	0.72	(0.42, 1.22)	22	1.06	(0.58, 1.96)
Q5	39	1.17	(0.74, 1.87)	50	1.01	(0.68, 1.52)	45	1.42	(0.88, 2.30)	28	1.42	(0.77, 2.61)
trend p		0.52			0.75			0.32			0.11	
Processed meat												
Q1	36	1.00	(reference)	64	1.00	(reference)	31	1.00	(reference)	26	1.00	(reference)
Q2	51	1.60	(1.04, 2.46)	53	0.92	(0.64, 1.33)	42	1.51	(0.95, 2.42)	23	0.98	(0.55, 1.73)
Q3	37	1.20	(0.75, 1.91)	39	0.73	(0.49, 1.10)	37	1.33	(0.82, 2.16)	19	0.79	(0.43, 1.44)
Q4	39	1.31	(0.82, 2.08)	46	0.93	(0.63, 1.38)	38	1.42	(0.87, 2.31)	18	0.77	(0.42, 1.44)
Q5	37	1.38	(0.85, 2.25)	55	1.19	(0.80, 1.77)	31	1.23	(0.73, 2.07)	24	1.03	(0.57, 1.87)
trend p		0.54			0.19			0.87			0.88	
Chicken												
Q1	42	1.00	(reference)	56	1.00	(reference)	40	1.00	(reference)	21	1.00	(reference)
Q2	38	1.00	(0.64, 1.56)	51	0.95	(0.65, 1.40)	32	0.85	(0.53, 1.37)	20	1.03	(0.55, 1.91)
Q3	43	1.12	(0.73, 1.73)	57	1.14	(0.78, 1.65)	43	1.19	(0.76, 1.84)	28	1.47	(0.83, 2.62)
Q4	35	1.04	(0.66, 1.65)	49	1.08	(0.73, 1.60)	28	0.73	(0.45, 1.20)	21	1.10	(0.59, 2.06)
Q5	42	1.34	(0.85, 2.09)	44	0.99	(0.66, 1.48)	36	0.95	(0.59, 1.51)	20	1.01	(0.53, 1.92)
trend p		0.18			0.96			0.70			0.91	

† HR, hazard ratio; ‡ CI, confidence interval. Hazard ratio was adjusted for age (continuous), Public Health Center area, Body Mass Index in kg/m^2 (<19, 19–22.9, 23–24.9, 25–26.9, and ≥27), smoking status (never, past, and current), alcohol consumption (non, occasional, 1–149, 150–299, 300–449, and ≥450g ethanol/week), physical activity in metabolic equivalent task-hours/day (<30, 30–34.9, 35–39.9, ≥40), medication use for diabetes, history of diabetes, screening examinations (fecal occult blood test; barium enema; colonoscopy), and quintiles of intake of energy, calcium, vitamin D, vitamin B-6, folate, dietary fiber, and dried and salted fish. Linear trends across quintiles of red meat or other meat intake were tested using the derived variable based on median consumption for each quintile as a continuous variable.

The lack of association between red meat or processed meat intake and rectal cancer did not change substantially in the decile analyses, with multivariate HRs (95% CI) for the highest versus lowest decile among men and women of 0.83 (0.42, 1.64; trend p=0.80) and 1.33 (0.60, 2.95; trend p=0.83), respectively, for red meat intake, and 0.68 (0.37,1.24; trend p=0.26) and 1.28 (0.55, 2.96; trend p=0.90), respectively, for processed meat intake.

DISCUSSION

In this population-based prospective cohort study in Japan, we observed that higher consumption of red meat, including beef and pork, was associated with an increased risk

of colon cancer among women, and that higher total meat consumption was associated with this cancer among men. By site, these positive associations were found for the risk of distal colon cancer among men and proximal colon cancer among women. No association was found between the consumption of red meat and the risk of rectal cancer in either gender, or between processed meat and the risk of either colon or rectal cancer. The highest quintile of red meat consumption in our cohort (120 and 105 g per day for men and women, respectively, based on a corrected median value according to weighed dietary records among sub-samples[28,29]) could be considered moderate by Western standards, at least.[3]

A number of mechanisms to explain the association between red meat or processed meat and colorectal cancer have been proposed. First, secondary bile acids produced by anaerobic bacteria in the large bowel from primary bile acids, which are essential to the digestion of animal fat, are thought to be colonic irritants and to have hyperproliferative effects.[34] Second, red meat is a major source of heme iron, which has high bioavailability, and iron is thought to be carcinogenic as a prooxidant.[7] Third, red meat intake enhances the production of endogenous NOC by gut bacteria, depending on pH and substrate availability.[7,18,19] Fourth, processed meat is also a candidate exogenous source of NOC, which is formed during the curing process.[7] Finally, potentially carcinogenic heterocyclic amines are formed when muscle meats such as beef, pork, or fish are cooked at high temperatures.[7]

These possible mechanisms of the association between red meat and colon cancer might also explain the association between total meat and colon cancer among men. Point estimates of multivariate HRs increased with red meat intake (but did not reach statistically significant levels), for men. Furthermore, red meat intake accounted for 85% of total meat consumption. Thus, observed results of colon cancer in men might not essentially differ from the results in women. In this study, positive associations between meat and colon cancer were clearer for the older than the younger group. These age differences in association may be partly due to changes in bacterial flora, such as the decline in beneficial bifidobacteria numbers or the increase in pH in the elderly gut,[35,36] both of which affect the production of secondary bile acids or endogenous NOC.

A number of potential differences in the impact of dietary intake on the risk of proximal or distal colon cancers have been suggested. Levels of bile acid metabolites are higher in the right than left colon, while those of a marker of exposure to potentially carcinogenic NOC are higher in the distal than proximal colonic DNA of colorectal cancer patients.[8,37] Gender differences in the risk of subsite-specific colon cancers have also been suggested [37-39] due to the higher intracolonic pH or longer bowel transit time in women than in men, which in turn affects the production of secondary bile acid or NOC. In this study, the association between meat and colon cancer were partly explained by saturated fatty acid for women and heme iron for men. On the other hand, larger number of distal colon cancer cases in men, and proximal colon in women, than opposite sub-site of colon cancer cases might possibly clearly reflect the results of total colon cancer among either gender.

To our knowledge, seven studies have independently reported associations between red meat consumption and the risk of proximal or distal colon cancer.[11,21-23,40-42] Results have shown a relatively consistent stronger positive association for the distal colon: five studies showed a stronger association for distal than proximal colon cancer[11,21-23,40] among men[21,22], women[23], or combined[11,40]; one showed a stronger association for proximal colon cancer[41]; and one found no difference for men and women combined.[42] Only a few prospective studies have evaluated the effect of red meat consumption on the risk of subsite-specific colon cancers separately by gender (men[21,22] or women[23]). Our results for the distal colon in men are consistent with one of these previous studies.[21] The observed site-specific differences in risk between genders, however, suggest possible differences in the etiology of proximal and distal colon cancers that are consistent with women's higher incidence of proximal colon tumors and adenomas in the present and Western populations.[43]

The major strength of the present study is its prospective design, which avoids exposure recall bias. Other strengths include the following: study subjects were selected from the general population; response rate to the questionnaire in this general population setting (81%) was high; and the proportion of losses to follow-up (0.3%) was negligible. Further, the number of exclusions due to missing data on red meat consumption, extreme values of energy as a proxy for dietary information, and extreme values for height and weight was not particularly large (8 percent). Although a difference in incidence among subjects with and without missing or extreme information had the potential to influence the results, no such notable difference was seen. Finally, variation among subjects in red meat consumption was sufficiently large, with a 7-fold difference in median intake between the highest (102 and 93 g for men and women, respectively) and lowest quartile groups (15 and 14 g, respectively) (Table 1). This difference was similar to or greater than those in the 7 [21,23,40,44-48] of 11 studies [21,23,40-42,44-49] which found a significant positive association between red meat intake and the risk of colon and/or rectal cancer in Western countries.

Our study has several potential limitations. First, the validity of the FFQ for meat intake was moderate at best (r=0.48-0.50 for men, r=0.44-0.45 for women),[28,29] and was not substantially different by types of meat. It could be suggested that the observed association with the risk of colon cancer might have underestimated the true magnitude of association consequent to misclassification in the FFQ. The potential attenuation might be equivalent by types of meat. However, this bias may have operated in the same direction for subsite-specific cancers between men and women. On this basis, the contrary results for subsite-specific colon cancer between men and women might not be attributable to the validity of the FFQ. Second, we did not note substantial associations for processed meat, and consumption in the highest category (median 16 and 15 g per day for men and women, respectively) was substantially lower than those for studies in Western countries which found a significant positive association with the risk of colon and/or rectal cancer.[22,40,44,46,47,49] Consumption of processed meat in our cohort was likely

not large enough to observe a positive association, and the possibility of an adverse effect on the colon cancer from a greater intake than in our highest quartiles of processed meat cannot be excluded. The different results between pork and processed meat might be partly attributable to relatively low level of processed meat intake among the Japanese. In this study, the results did not support a hypothesis that higher processed meat or other meat intake increases the risk of rectal cancer with these levels. Third, although we measured and adjusted for possible confounding variables to the extent possible, the possibility of confounding by unmeasured variables cannot be totally disregarded. Also, it is possible that some of the significant findings may be due to chance.

In conclusion, in this large-scale, population-based prospective cohort study among middle-aged Japanese men and women, whose consumption of red meat was considered moderate by Western standards, we found that higher consumption of red meat was associated with an increased risk of colon cancer among women, as was higher consumption of total meat among men. The positive associations for subsite-specific colon cancers appeared to differ by gender. The Japanese may be particularly susceptible to the adverse effects of red meat intake in the development of colon cancers.

AUTHOR DISCLOSURES

This research was supported by Grants in Aid for Cancer Research and for the Third-Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan. The funding agencies had no role in the research presented herein, and the researchers were fully independent in pursuing this research. None of the authors declares a personal or financial conflict of interest.

REFERENCES

1. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare: Vital Statistics of Japan 2000, Tokyo: Health and Welfare Statistics Association; 2002.
2. Cancer incidence and incidence rates in Japan in 1998: estimates based on data from 12 population-based cancer registries. Jpn J Clin Oncol. 2003;33:241-5.
3. FAO (2007) FAOSTAT: Production, ProSTAT, Crops: Food and Agriculture Organization of the United Nations. [cited 2010/9/27]; Available from: http://faostat.fao.org/
4. Ministry of Health, Labour and Welfare/ Society for the information on Health and Nutrition. The National Health and Nutrition Survey in Japan, 2005. Tokyo: Daiichi Publishing; 2008.
5. Flood DM, Weiss NS, Cook LS, Emerson JC, Schwartz SM, Potter JD. Colorectal cancer incidence in Asian migrants to the United States and their descendants. Cancer Causes Control. 2000;11:403-11.
6. Marchand LL. Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans. J Natl Cancer Inst Monogr. 1999;(26):101-5.
7. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, physical activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 2007.
8. Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int J Cancer. 2006;119:2657-64.
9. Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer. 2002;98:241-56.
10. Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol Biomarkers Prev. 2001;10:439-46.
11. Sato Y, Nakaya N, Kuriyama S, Nishino Y, Tsubono Y, Tsuji I. Meat consumption and risk of colorectal cancer in Japan: the Miyagi Cohort Study. Eur J Cancer Prev. 2006; 15:211-8.
12. Kojima M, Wakai K, Tamakoshi K, Tokudome S, Toyoshima H, Watanabe Y et al. Diet and colorectal cancer mortality: results from the Japan Collaborative Cohort Study. Nutr Cancer. 2004;50:23-32.
13. Oba S, Shimizu N, Nagata C, Shimizu H, Kametani M, Takeyama N, Ohnuma T, Matsushita S. The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: a prospective study in Japan. Cancer Lett. 2006;244:260-7.
14. 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. Nutr Cancer. 2009;61: 194-205.
15. Khan MM, Goto R, Kobayashi K, Suzumura S, Nagata Y, Sonoda T, Sakauchi F, Washio M, Mori M. Dietary habits and cancer mortality among middle aged and older Japanese living in hokkaido, Japan by cancer site and sex. Asian Pac J Cancer Prev. 2004;5:58-65.
16. Benno Y, Suzuki K, Suzuki K, Narisawa K, Bruce WR, Mitsuoka T. Comparison of the fecal microflora in rural Japanese and urban Canadians. Microbiol Immunol. 1986; 30:521-32.
17. Saunders BP, Masaki T, Sawada T, Halligan S, Phillips RK, Muto T, Williams CB. A peroperative comparison of Western and Oriental colonic anatomy and mesenteric attachments. Int J Colorectal Dis. 1995;10:216-21.
18. Bingham SA. High-meat diets and cancer risk. Proc Nutr Soc. 1999;58:243-8.
19. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mutagen. 2004;44:44-55.
20. Dich J, Jarvinen R, Knekt P, Penttila PL. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. Food Addit Contam. 1996; 13:541-52.
21. 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 Res. 1994;54: 2390-7.
22. Wu K, Giovannucci E, Byrne C, Platz EA, Fuchs C, Willett WC, Sinha R. Meat mutagens and risk of distal colon adenoma in a cohort of U.S. men. Cancer Epidemiol Biomarkers Prev. 2006;15:1120-5.
23. 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. Int J Cancer. 2005;113:829-34.
24. Tsugane S, Sobue T. Baseline survey of JPHC study--design and participation rate. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol. 2001;11:S24-9.
25. World, Health, and Organization. International Classification of Diseases for Oncology, 3rd ed. Geneva, Switzerland: World Health Organization;2000.
26. Tsubono Y, Takamori S, Kobayashi M, Takahashi T, Iwase Y, Iitoi Y, Akabane M, Yamaguchi M, Tsugane S. A data-

based approach for designing a semiquantitative food frequency questionnaire for a population-based prospective study in Japan. J Epidemiol. 1996;6:45-53.
27. Resource Council. Science and Technology Agency. The Govenrment of Japan. Standard Tables of Food Composition in Japan, the fifth revised edition. Tokyo: Printing Bureau, Ministry of Finance; 2002.
28. Sasaki S, Kobayashi M, Tsugane S. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I: comparison with dietary records for food groups. J Epidemiol. 2003;13:S57-63.
29. Ishihara J, Sobue T, Yamamoto S, Yoshimi I, Sasaki S, Kobayashi M et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC Study Cohort II: study design, participant profile and results in comparison with Cohort I. J Epidemiol. 2003;13:S134-47.
30. Sasaki S, Ishihara J, Tsugane S. Reproducibility of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess food and nutrient intake. J Epidemiol. 2003;13:S115-24.
31. Willett WC. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998.
32. 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. Int J Cancer. 1999;80:852-6.
33. Takachi R, Inoue M, Shimazu T, Sasazuki S, Ishihara J, Sawada N et al. Consumption of sodium and salted foods in relation to cancer and cardiovascular disease: the Japan Public Health Center-based Prospective Study. Am J Clin Nutr. 2010;91:456-64.
34. Nagengast FM, Grubben MJ, van Munster IP. Role of bile acids in colorectal carcinogenesis. Eur J Cancer. 1995;31A: 1067-70.
35. Enck P, Zimmermann K, Rusch K, Schwiertz A, Klosterhalfen S, Frick JS. The effects of ageing on the colonic bacterial microflora in adults. Z Gastroenterol. 2009;47:653-8.
36. Woodmansey EJ. Intestinal bacteria and ageing. J Appl Microbiol. 2007;102:1178-86.
37. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002;101:403-8.
38. McMichael AJ, Potter JD. Diet and colon cancer: integration of the descriptive, analytic, and metabolic epidemiology. Natl Cancer Inst Monogr. 1985;69:223-8.
39. McMichael AJ, Potter JD. Host factors in carcinogenesis: certain bile-acid metabolic profiles that selectively increase the risk of proximal colon cancer. J Natl Cancer Inst. 1985; 75:185-91.
40. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst. 2005;97:906-16.
41. Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, Rodriguez C, Sinha R, Calle EE. Meat consumption and risk of colorectal cancer. JAMA. 2005;293: 172-82.
42. Rohrmann S, Hermann S, Linseisen J. Heterocyclic aromatic amine intake increases colorectal adenoma risk: findings from a prospective European cohort study. Am J Clin Nutr. 2009;89:1418-24.
43. McCashland TM, Brand R, Lyden E, de Garmo P. Gender differences in colorectal polyps and tumors. Am J Gastroenterol. 2001;96:882-6.
44. 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. N Engl J Med. 1990;323:1664-72.
45. Tiemersma EW, Kampman E, Bueno de Mesquita HB, Bunschoten A, van Schothorst EM, Kok FJ, Kromhout D. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. Cancer Causes Control. 2002;13:383-93.
46. English DR, MacInnis RJ, Hodge AM, Hopper JL, Haydon AM, Giles GG. Red meat, chicken, and fish consumption and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2004;13:1509-14.
47. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med. 2007;4:e325.
48. Kabat GC, Miller AB, Jain M, Rohan TE. A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. Br J Cancer. 2007;97:118-22.
49. Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, Colditz GA. Comparison of risk factors for colon and rectal cancer. Int J Cancer. 2004;108:433-42.