Meat, milk, saturated fatty acids, the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene and colorectal cancer risk in Japanese

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The *peroxisome proliferator-activated receptor gamma* (*PPARγ*) gene plays important roles in energy homeostasis. To examine interactions between consumption of foods and fatty acids and the *Pro12Ala* and *C161T* (*His447His*) polymorphisms for colorectal cancer, we performed two case-control studies in Japanese. In study 1, there were 128 colorectal cancer cases and 238 non-cancer controls, and in study 2 there were 257 cases and 771 (age- and sex-matched) non-cancer controls. Assessment of food and nutrients consumption in study 1 was via a nine-item questionnaire, while in study 2 assessment of consumption was according to a more detailed semiquantitative food frequency questionnaire. Consumption of foods and fatty acids was divided into low, moderate and high groups. The overall frequency of the *Ala* allele was <4%, and the frequencies of the *Pro/Pro* + *C/C* and *Pro/Pro* + (*C/T* + *T/T*) genotypes were 70–73% and 20–26%, respectively. Compared with subjects with low meat intake and the *Pro/Pro* + *C/C* genotype, those with high meat consumption and the same genotype had a stronger increased risk in study 1 [OR, 2.88; 95% CI, 1.14–7.30; *P* for trend = 0.02], but a positive association with processed meat consumption was greatest in those with the *Pro/Pro* + (*C/T* + *T/T*) genotype (*P* for trend = 0.05) in study 2. Likewise, high consumption of saturated fatty acids and milk appeared to confer marginal increased risk and stronger decreased risk, respectively, in those with the *Pro/Pro* and *Pro/Pro* + *C/C* genotypes (OR, 1.35 and 0.65; 95% CI, 0.93–1.96 and 0.43–1.00; *P* for trend = 0.10 and 0.06). Further large-scale studies are needed to determine colorectal cancer risk according to relationships between the *PPARγ* gene polymorphisms and dietary intakes of meat, processed meat, milk and saturated fatty acids in Japanese with very low frequency of the *Ala* allele. (*Cancer Sci* 2006; 97: 1226–1235)

Peroxisome proliferator-activated receptor gamma is a member of a nuclear hormone receptor superfamily, predominantly expressed in adipose tissue, colon and the immune system,[1,2] and found to be altered in colorectal tumors as compared with non-tumor tissues.[3] PPARγ exists as two distinct γ1 and γ2 isoforms derived from different transcription start sites (in exon 1 and exon B, respectively) and alternative splicing,[4,5] PPARγ2 thereby containing 28 additional amino acids at its NH_2 terminus. PPARγ plays important roles in adipogenesis, energy homeostasis with regard to fat/lipid and glucose metabolism, and regulation of expression of adipocyte-specific genes.[6,7] The γ2 type has fivefold the activity of the γ1 type.[8,9] PPARγ has been suggested to act as a tumor suppressor gene, and PPARγ ligands therefore have been thought to be helpful for chemoprevention.[10]

The traditional diet in Japan has been remarkably changed due to adoption of a westernized diet, featuring high consumption of milk, meat, and animal fat/SFA.[11] Under the influence of a high fat diet, PPARγ plays critical roles in adipocyte hypertrophy and development of obesity, insulin resistance, and type 2 diabetes, reflecting a thrifty gene nature.[12–14] The incidence rate for colon cancer among Japanese in Japan has been steadily increasing, and the rate among Japanese immigrants to the USA (Japanese Americans) is the same or rather higher than that among Caucasian Americans.[15] We previously found that the incidence rates of colon cancer have a positive correlation with prevalence rates of type 2 diabetes among both Japanese in Japan and Japanese Americans.[11]

The *Pro*/*Pro* genotype of the *Pro12Ala* polymorphism at exon B has been demonstrated to be positively associated with the development of type 2 diabetes in Japanese Americans,[16] probably due to an interaction with a high fat diet, while the *Ala* allele has a negative association.[17] The *Ala* allele has been suggested to decrease colorectal cancer risk, and a significantly increased risk has been demonstrated to relate to a westernized diet among Americans with the *Pro*/*Pro* genotype.[19] The *Ala* allele has only approximately 40% of the activity of the *Pro* allele,[9] and is found at much lower frequency in Japanese than in Americans and Europeans (<5% and about 15%, respectively).[17–20,25] Regarding possible risk reduction for colorectal cancer, the *Ala* allele had inconsistent associations with energy balance and inflammation,[21,22] but Japanese may have high susceptibility for this disease due to very low frequency of the *Ala* allele. In contrast, the *T* allele of the *C161T* (*His447His*) polymorphism at exon 6 has been described as resulting in a common silent substitution of histidine residue, but recent reports have indicated links with leptin levels (in obese subjects) or coronary artery disease.[23,24] The polymorphism may be functional in linkage to others in the promoter regions, although this has not been yet confirmed. The frequency of the *T* allele in Europeans is approximately 15–20%,[23–26] while that in Japanese has not yet been reported. In the present study, we focused on the two polymorphisms in association with dietary foods rich in fat and fatty acids.

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Abbreviations: BMI, body mass index; CI, confidence interval; IGF, insulin-like growth factor; MUFA, monounsaturated fatty acids; OR, odds ratio; PCR-CTPP; polymerase, chain reaction with confronting two-pair primers; PPARγ, peroxisome proliferator-activated receptor gamma; SFA, saturated fatty acids; SQFFQ, semiquantitative food frequency questionnaire; PUFA, polyunsaturated fatty acids.

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The hypothesis for the present study was that these gene polymorphisms might be linked to the elevated risk of colorectal cancer occurring with westernization of dietary habits, including increased meat and fat intake, in Japanese. Using two hospital-based case-control studies (studies 1 and 2), we examined gene–environment interactions between consumption of the selected 15 foods rich in fat, along with fatty acids, and the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene regarding the risk of colorectal cancer.

Materials and Methods

Population. The designs of case-control studies 1 and 2 are described in detail elsewhere.[27,28] In brief, the subjects in study 1 were recruited from patients who visited the outpatient services at Aichi Cancer Center Hospital between March 1999 and July 2000. Eligible subjects were defined as individuals aged 40–79 years. Seven cases (aged ≤ 39 years) were excluded who might have had hereditary ailments such as familial adenomatous polyposis or were more likely to be affected by host-related than environmental factors.[28] Two controls (aged ≤ 39 years) were also excluded. In total, 128 patients (50% with colon and 50% with rectal cancers), comprised of both incident and prevalent cases, were included. Those with prevalent cancer (50.4% of total cases) had been histologically diagnosed within 5 years (except for one case) prior to study entry. The controls were 238 outpatients confirmed to be cancer-free at Aichi Cancer Center Hospital.

For study 1, one trained interviewer collected and checked information on physical characteristics including height and weight, food consumption, lifestyle and medical histories from a self-administrated questionnaire, and we performed 7 mL blood sampling from a peripheral vein. The rate for donating blood samples was about 60% for both cases and controls. We excluded two cases with missing information on variables in the questionnaire.

In study 2, subjects aged 20–79 years were enrolled in the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center Research.[27] The 257 cases were histologically diagnosed as having colorectal cancers (48.8% in the colon and 51.2% in the rectum) between January 2001 and August 2004 at Aichi Cancer Center Hospital. The controls were first-visit outpatients who visited Aichi Cancer Center Hospital during the same period, and were confirmed to have no cancer and also no prior history of cancer. Of these 771 were randomly selected and matched for age (±5 years, range 23–79 years) and sex to cases with a 1:3 case-control ratio.

Briefly, for study 2 all first-visit outpatients were asked to complete a self-administrated questionnaire regarding their lifestyle as well as to provide 7 mL of blood. Several trained interviewers systematically collected and checked information from the questionnaire. Approximately 95% of eligible subjects completed the questionnaire and 60% provide blood samples.

All subjects in both studies 1 and 2 were Japanese, and lived in and around Aichi Prefecture, central Japan. Our previous study demonstrated that it is feasible to use non-cancer outpatients at Aichi Cancer Center Hospital as controls in epidemiological studies because their general lifestyles are accordant with those of general population randomly selected from the electoral roll in Nagoya City, Aichi Prefecture.[29] All subjects were provided with an explanatory document and gave their written informed consent for participation in this study. The two studies were approved by the Ethics Committee of the Aichi Cancer Center.

Questionnaire. In study 1, assessment of food and beverage consumption was according to a nine-item frequency questionnaire. The five food items were whole meat (including beef, pork and poultry), fish, raw vegetables, fruit and tofu, and the three beverage items were Japanese tea, coffee and milk. The final item was preference for salt. Consumption of each food was divided into low (less than 1 time/week), moderate (1–3 times/week) and high (4 or more times/week). Consumption of each beverage was divided into rarely, sometimes and daily.

Lifestyle factors were also classified into three groups. For habitual exercise for more than 15 min that was other than work, these were: low (less than 1 time/week), moderate (1–2 times/week) and high (3 or more times/week). For alcohol drinking, these were: low (less than 1 time/week), moderate (1–4 times/week) and high (5 or more times/week). For smoking status, the groups were: current, former, and never. We defined former smokers as those who quit smoking at least 2 years before the study.

We asked the cases to provide information about their lifestyle before the onset of disease, and the controls before the study enrollment.

In study 2, assessment of various foods and nutrients consumption was according to a SQFFQ. The questionnaire asked about habitual dietary intake during the previous 1 year for 47 foods/food groups with division of frequency into eight categories: never or seldom, 1–3 times/month; 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day and ≥3 times/day. The methods for developing the SQFFQ and computing the average daily consumption of energy and selected nutrients have been described elsewhere.[30,31] Compared with 3-day weighed diet records, the SQFFQ has been demonstrated to have an acceptable relative validity for assessing dietary intake of most nutrients, and the Spearman's rank correlation coefficients for fatty acids were from 0.13 (for n-6 PUFA) to 0.52 (SFA) for men, and from 0.16 (PUFA) to 0.34 (SFA) for women, respectively.[32] Therefore, it appeared appropriate to use the SQFFQ for the study subjects, as well as for the general population.

Briefly, fat and fatty acids were computed by multiplying the food intake (in grams) and the content (per gram) of fat or each fatty acid in foods as listed in the Standard Tables of Food Consumption and the Follow-up of the Standard Tables of Food Consumption. Some dietary fatty acid intakes based on the SQFFQ had been demonstrated to have significant correlations with the corresponding fatty acid concentrations in plasma as the biomarkers.[33]

Regarding associations with the risk of colorectal cancer, we focused on the following 15 foods/food groups rich in fat or various groups of fatty acids: beef and pork, liver, processed meat, fish, tuna, small fish, poultry, egg, milk, yogurt, mayonnaise, margarine, butter, fried foods and deep-fried foods. For these categorical variables, the levels of consumption were divided into three groups (low, moderate or high) based on distributions for each food/food group in controls. With respect to nutrient intakes, each fatty acid intake (g/day) in 47 foods/food groups was summed up as SFA, MUFA, n-6 PUFA and n-3 PUFA. Fat, meat and fish intakes (g/day) were also calculated. These continuous variables were adjusted for total energy intake of each person and tertile cut-points (g/1000 kcal) in controls were used to designate low (lowest tertile), moderate (middle tertile), and high (highest tertile).

Lifestyle factors were classified into three groups, as in study 1.

Laboratory analysis. In both studies, DNA of each subject was extracted from buff-coated fractions with a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), and a BioRobot EZ1 and EZ1 DNA Blood 350 μL Kit (Qiagen, Tokyo, Japan), respectively. Primers were designed to detect both the *Pro12Ala* and *C161T* polymorphisms at once, and the genotyping of these polymorphisms was carried out by PCR-CTPP.[34] The sequences of primers were as follows: F1, 5′-AGC AGT GAA CAT TAT TAT GAC ACA ACT TT-3′ (sense) and R1, 5′-AGT TAA GGA ATC GCT TTC TGC-3′ (antisense) for the *G* (*Ala*) allele; and F2, 5′-TCT GGG AGA TTC TCC TAT TGA CC-3′ (sense) and R2, 5′-GCA TTA AAA TAC TGG AGT GTA CAC ATG-3′ (antisense) for the *C* (*Pro*) allele on the *Pro12Ala* polymorphism (dbSNP

ID: rs1801282). Then, F1, 5′-CCT GGA GCT CCA GCT GAA-3′ (sense) and R1, 5′-CAC CTG CAG TAG CAC A-3′ (antisense) for the *T* allele; and F2, 5′-CAG ACA GAT TGT CAC GGA ACA C-3′ (sense) and R2, 5′-TTT CCC TCA GAA TAG TGC AAC TG-3′ (antisense) for the *C* allele on the *C161T* polymorphism (dbSNP ID: rs3856806).

Genomic DNA (30–100 ng) was assessed in 25 μL of reaction mixture, with 0.15 mM dTNPs, 25 pmol of each primer, 1.0 units of AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA), 2.5 μL 10 × PCR buffer including 15 mL $MgCl_2$. Conditions were 5 min of initial denaturation at 95°C, followed by 35 cycles of 60 s at 95°C, 60 s at 64°C, and 60 s at 72°C, and 5 min final extension at 72°C. PCR products were visualized on 3% agarose gels with ethidium bromide staining and genotypes were defined with reference to allele-specific bands and a common band as follows: 303 and 203 bp for the *G* and *C* alleles and 463 bp on the *Pro12Ala* polymorphism, and 118 and 170 bp for the *T* and *C* alleles and 248 bp on the *C161T* polymorphism, respectively. One case in study 1 and two cases and two controls in study 2 failed to be amplified for both polymorphisms.

The primers for PCR-CTPP were also checked with reference to the gene sequence of products of PCR for the *Pro12Ala* polymorphism (General Elute Minus EtBr Spin Columns; Sigma, St Louis, MO, USA) and PCR restriction fragment length polymorphism for the *C161T* polymorphism (PmlI, New England Biolabs, USA),[24] respectively.

Data analysis. Body mass index (kg/m^2) was calculated by height (m) and weight (kg). Student's *t*-test and chi-squared test were performed for each variable and $P < 0.05$ was considered statistical significant. OR and 95% CI were calculated, using unconditional and conditional logistic regression models in studies 1 and 2, respectively, after adjustment for BMI (continuous), habitual exercise, drinking and smoking habits to control for the effects of potential environmental confounding factors. In study 2, the variable regarding group number matched for age and sex to each case was adjusted in the latter model. Tests for trends in consumption of foods/food groups and fatty acids were conducted by assigning the mean frequencies for categorical variables and the median values for continuous variables in controls.

Accordance with the Hardy–Weinberg equilibrium, indicating an absence of discrepancies between genotype and allele frequencies, was checked for controls with the chi-squared test and the exact *P*-value. Because of very low *Ala* allele frequencies of the *Pro12Ala* polymorphism, we modeled genotype as a dichotomous variable (the *Ala* allele carriers *vs* non-carriers). Genotypes of the *C161T* polymorphism were also defined as a dichotomous variable (the *T* allele carriers *vs* non-carriers). Regarding the combined genotypes, the *Pro*/*Pro* + *C*/*C* or *Pro*/*Pro* + (*C*/*T* + *T*/*T*) genotypes were estimated for risk of colorectal cancer according to consumption of foods/food groups and fatty acids, excluding the other two genotypes (included in the *Ala* allele) with frequencies less than 6%. Moreover, haplotype and diplotype frequencies of the two polymorphisms in study subjects were estimated with the program PHASE version 2.1,[35] and then former frequencies in hypothetical general population in both studies were calculated according to a special option of the software. We evaluated the linkage disequilibrium (both D′ and r^2 values) between the two polymorphisms with Haploview software version 3.2.[36] The dipolotypes of the two polymorphisms were also estimated for colorectal cancer risk.

We assessed the joint interaction between the genetic factors and dietary factors by individuals at high risk as a possible common referent point (the genotype of *Pro*/*Pro* or *C*/*C* or *Pro*/*Pro* + *C*/*C* as a genetic factor and low consumption of foods/food groups or fatty acids as a dietary factor). Tests for trend were calculated by stratification of each genotype and groups of combined polymorphisms. Tests for multiplicative interaction were performed including multiplicative variables in the logistic model and performing likelihood ratio tests. Except for the linkage disequilibrium between the two polymorphisms, all statistical analyses were assessed with the Stata statistical package version 8.0 (Stata, College Station, TX, USA).

Results

In study 1, the mean age of cases was older than that of controls, especially in women (Table 1). The rate of high frequency for habitual exercise was lower in cases than in controls ($P < 0.05$), but that for drinking habit was higher in cases ($P < 0.05$). Sex ratios, BMI, food consumption and rates for smoking habit did not differ between the cases and controls. In study 2, background characteristics did not differ between cases and controls, except for a family history of this cancer ($P < 0.05$).

Table 1. Background characteristics of colorectal cancer cases and controls in studies 1 and 2

Characteristic	Study 1		Study 2	
	Cases	Controls	Cases	Controls
Number (*n*)	128	238	257	771
Age (years), mean ± SD	59.9 ± 8.9*	56.9 ± 7.8	59.0 ± 10.2	58.8 ± 10.3
Men/Women	74/54	116/122	162/95	486/285
BMI (kg/m^2), mean ± SD	22.8 ± 3.1	22.3 ± 3.2	23.1 ± 2.9	23.1 ± 3.1
Drinking habit (%)				
Low (<1 time/week)	44.5*	56.7	50.2	50.1
Moderate (1–4 times/week)	13.3	18.9	13.6	16.0
High (≥5 times/week)	42.2	24.4	36.2	34.0
Smoking habit (%)				
Non-smokers	46.1	58.4	41.2	44.2
Ex-smokers	26.6	18.1	24.9	24.4
Smokers	27.3	23.5	33.9	31.4
Habitual exercise (%)				
Low (<1 time/week)	65.6*	52.1	52.1	54.3
Moderate (1–2 times/week)	14.8	19.3	19.1	18.4
High (≥3 times/week)	19.5	28.6	28.8	27.2
Past or current history of diabetes (%)	0	2.9	9.0	8.6
Family history of colorectal cancer (%)	9.4	10.1	12.6*	7.9

*$P < 0.05$ for Student's *t*-test or chi-squared test between cases and controls.

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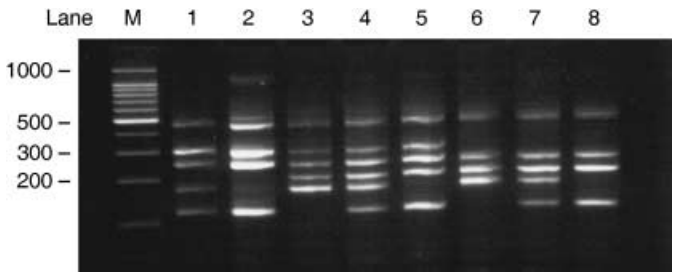

Fig. 1. Representative results for the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene by the PCR-CTPP (polymerase chain reaction with confronting two-pair primers) method. DNA fragments stained with ethidium bromide are shown. Lane M, markers; lane 1, *Ala/Ala + C/T*; lane 2, *Ala/Ala + T/T*; lane 3, *Pro/Ala + C/C*; lane 4, *Pro/Ala + C/T*; lane 5, *Pro/Ala + T/T*; lane 6, *Pro/Pro + C/C*; lane 7, *Pro/Pro + C/T*; and lane 8, *Pro/Pro + T/T*.

In study 1, high meat consumption significantly increased the risk of colorectal cancer, whereas a tendency for decrease was noted with high milk intake.[28] In study 2, high milk consumption decreased the risk (P for trend <0.05), but intake of beef and pork, processed meat, poultry and meat consumption (g/1000 kcal) demonstrated no association. Similarly there were no links between the two polymorphisms and consumption of fat and each fatty acid (data not shown). In both studies, moreover, consumption of fish, green-yellow vegetables, other vegetables and fruit appeared to demonstrate no impact.

Figure 1 shows representative results of the *Pro12Ala* and *C161T* genotyping by PCR-CTPP. No differences in the genotype frequencies of the two polymorphisms were evident between cases and controls in either study (Table 2). The frequency of the *Ala* allele (2.6%) among controls in study 2 diverged from the Hardy–Weinberg equilibrium ($P < 0.0005$), but that of controls in study 1 (3.6%) did not because of absence of the *Ala/Ala* genotype ($P = 0.57$). The allele and genotype frequencies of the *Pro12Ala* polymorphism were very similar to values previously observed for Japanese populations (<5%).[17,18] Compared with the *Pro/Pro* genotype, significantly decreased risks of colorectal cancer for the *Pro/Ala* and *Pro/Ala + Ala/Ala* genotypes were not found in study 1 and 2 (OR, 0.61 and 0.76; 95% CI, 0.23–1.58 and 0.36–1.60). With the combined data from the two studies, likewise, the *Pro/Ala + Ala/Ala* genotype had no significant inverse association with risk (OR adjusted for age and sex, 0.71; 95% CI, 0.39–1.30). The frequencies of the *C* allele among controls in both studies (13.9 and 16.1%) were in accordance with the Hardy–Weinberg equilibrium ($P = 0.82$ and 0.76). Compared with the *C/C* genotype, cancer risk with the *C/T + T/T* genotype was also not modified. Regarding combinations between the two polymorphisms, the *Pro/Pro + C/C* and *Pro/Pro + (C/T + T/T)* genotypes comprised more than 90% of the subjects in the two studies.

Table 3 shows the estimated haplotype and diplotype frequencies of the *Pro12Ala* and *C161T* polymorphisms in colorectal cancer cases and controls. In studies 1 and 2, linkage disequilibrium for the two polymorphisms among controls was observed (D′ values = 0.76 and 0.77; 95% CI, 0.50–0.90 and 0.59–0.87, respectively) and the r^2 values indicate that the allelic association is not strong ($r^2 = 0.14$ and 0.08, respectively). The estimated haplotype frequencies in studies 1 and 2 demonstrated no

Table 2. Distribution of genotypes on the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene in colorectal cancer cases and controls

Genotype	Cases *n* (%)	Controls *n* (%)	OR (95% CI) [age + sex adjusted]†	OR (95% CI) [other variables]‡
Study 1				
Pro12Ala				
Pro/Pro	120 (93.8)	221 (92.9)	1.00 (ref)	1.00 (ref)
Pro/Ala	7 (5.5)	17 (7.1)	0.70 (0.28–1.77)	0.61 (0.23–1.58)
Unknown	1 (0.8)	0 (0)		
C161T				
C/C	92 (71.9)	177 (74.4)	1.00 (ref)	1.00 (ref)
C/T + T/T	35 (27.3)	61 (25.6)	1.08 (0.66–1.78)	1.07 (0.64–1.80)
Unknown	1 (0.8)	0 (0)		
Combination				
Pro/Pro + C/C	92 (72.4)	174 (73.1)	1.00 (ref)	1.00 (ref)
Pro/Pro + (C/T + T/T)	28 (22.0)	47 (19.7)	1.12 (0.65–1.94)	1.17 (0.66–2.07)
Pro/Ala + C/C	0 (0)	3 (1.3)	NE	NE
Pro/Ala + (C/T + T/T)	7 (5.5)	14 (5.9)	0.89 (0.34–2.31)	0.74 (0.28–2.00)
Study 2				
Pro12Ala				
Pro/Pro	248 (96.5)	732 (94.9)	1.00 (ref)	1.00 (ref)
Pro/Ala + Ala/Ala	9 (3.5)	37 (4.4)	0.73 (0.35–1.53)	0.76 (0.36–1.60)
Unknown	0 (0)	2 (0.3)		
C161T				
C/C	184 (71.6)	543 (70.4)	1.00 (ref)	1.00 (ref)
C/T + T/T	73 (28.4)	226 (29.3)	0.94 (0.69–1.29)	0.96 (0.70–1.32)
Unknown	0 (0)	2 (0.3)		
Combination				
Pro/Pro + C/C	182 (70.8)	537 (69.6)	1.00 (ref)	1.00 (ref)
Pro/Pro + (C/T + T/T)	66 (25.7)	195 (25.3)	0.99 (0.71–1.37)	1.00 (0.72–1.39)
(Pro/Ala + Ala/Ala) + C/C	2 (0.8)	6 (0.8)	0.99 (0.20–4.96)	0.99 (0.20–5.00)
(Pro/Ala + Ala/Ala) + (C/T + T/T)	7 (2.7)	31 (4.0)	0.67 (0.29–1.55)	0.71 (0.31–1.65)

†In study 1 adjusted for age and sex, in study 2 adjusted for a series of group numbers matched age and sex to each case. ‡In study 1 adjusted for age, sex, BMI, habitual exercise, drinking and smoking habits, and family history of colorectal cancer; in study 2, adjusted for BMI, habitual exercise, drinking and smoking habits, family history of colorectal cancer along with a series of group numbers matched age and sex to each case. NE, not estimated because case was absent in this category; Unknown, genotyping failure.

Table 3. Estimated haplotype and diplotype frequencies for the *Pro12Ala and C161T* polymorphisms of the *PPARγ* gene in colorectal cancer cases and controls

Frequency	Cases[§]	Controls	OR (95% CI) [age + sex adjusted][†]	OR (95% CI) [other variables]
Study 1				
Haplotype (%) [mean ± SE]				
Pro-C[¶]	85.39 ± 0.20	86.46 ± 0.14		
Pro-T	11.85 ± 0.20	8.92 ± 0.14		
Ala-C	0.04 ± 0.20	0.73 ± 0.14		
Ala-T	2.72 ± 0.20	3.89 ± 0.14		
Diplotype [n (%)]				
Pro-C/Pro-C	92 (72.4)	174 (73.1)	1.00 (ref)	1.00 (ref)
Pro-C/Pro-T	26 (20.5)	42 (17.7)	1.15 (0.66–2.03)	1.19 (0.65–2.15)
Pro-T/Pro-T	2 (1.6)	5 (2.1)	0.81 (0.15–4.33)	0.99 (0.18–5.36)
Pro-C/Ala-C	0 (0)	3 (1.3)	NE	NE
Pro-C/Ala-T	7 (5.5)	14 (5.9)	0.89 (0.34–2.31)	0.74 (0.28–2.00)
Study 2				
Haplotype (%) [mean ± SE]				
Pro-C[¶]	83.75 ± 0.13	83.41 ± 0.08		
Pro-T	14.11 ± 0.13	13.99 ± 0.08		
Ala-C	0.68 ± 0.13	0.53 ± 0.08		
Ala-T	1.46 ± 0.13	2.07 ± 0.08		
Diplotype [n (%)]				
Pro-C/Pro-C	182 (70.8)	538 (70.0)	1.00 (ref)	1.00 (ref)
Pro-C/Pro-T	60 (23.3)	180 (23.4)	0.97 (0.69–1.36)	0.99 (0.70–1.39)
Pro-T/Pro-T	6 (2.3)	15 (2.0)	1.16 (0.44–3.03)	1.17 (0.44–3.06)
Pro-C/Ala-C	2 (0.8)	6 (0.8)	0.99 (0.20–4.96)	0.99 (0.20–5.00)
Pro-C/Ala-T	5 (1.9)	23 (3.0)	0.66 (0.25–1.75)	0.70 (0.26–1.88)
Others	2 (0.8)	7 (0.9)	0.82 (0.17–3.99)	0.87 (0.18–4.26)

[†]In study 1 adjusted for age and sex, in study 2 adjusted for a series of group numbers matched age and sex to each case. [‡]In study 1 adjusted for age, sex, BMI, habitual exercise, drinking and smoking habits, and family history of colorectal cancer; in study 2, adjusted for BMI, habitual exercise, drinking and smoking habits, family history of colorectal cancer along with a series of group numbers matched age and sex to each case. [§]One colorectal cancer case in study 1 and two controls in study 2 with unknown *PPARγ* genotypes were excluded from analyses. [¶]Not significant for chi squared test between colorectal cancer cases and controls. NE, not estimated because case was absent in this category.

differences between cases and controls ($P = 0.43$ and 0.68, respectively). We estimated haplotype frequencies in a hypothetical general population according to the optional function of the software, and also found no differences to those of study controls (data not shown), with very low frequencies of the *Ala* allele and the *Pro*/*Ala* heterozygosity. The two most common haplotypes were found to account for more than 95% of the study populations. No differences in the estimated diplotype frequencies of the two polymorphisms were evident between cases and controls, and the *Pro-C*/*Pro-C* and *Pro-C*/*Pro-T* diplotypes also comprised more than 90% of the subjects in the two studies. Compared with the *Pro-C*/*Pro-C* diplotype, cancer risks with any of other diplotypes were not modified.

In study 1, the significant positive associations between meat consumption and the risk of colorectal cancer were stronger in subjects with the *Pro*/*Pro* genotype (OR, 1.94 and 2.01; 95% CI, 1.06–3.58 and 0.91–4.45, for moderate and high consumption; $P = 0.05$ for trend) (Table 4). As with the *C*/*C* genotype, the *Pro*/*Pro* + *C*/*C* genotype with moderate and high consumption of meat was associated with significantly increased risk (OR, 2.68 and 2.88; 95% CI, 1.26–5.66 and 1.14–7.30, respectively; $P = 0.02$ for trend). Interactions between meat consumption and the two polymorphisms, however, were not significant. Milk consumption and the two polymorphisms tended to interact to decrease the risk, but this also did not reach statistical significance.

In study 2, the positive association between processed meat consumption and the risk of colorectal cancer appeared stronger in subjects with the *Pro*/*Pro* + (*C*/*T* + *T*/*T*) genotype than those with the *Pro*/*Pro* + *C*/*C* genotype ($P = 0.05$ and 0.83 for trend, respectively), but the interaction was not significant ($P = 0.15$ for interaction) (Table 4). The cancer risk had no association with the two polymorphisms and consumption of beef and pork, poultry, fish and fried foods, as well as butter and margarine (data not shown). Consumption (g/1000 kcal) of meat and fish demonstrated no links with the cancer risk (data not shown).

As with the *C*/*C* genotype, high milk consumption conferred a significantly decreased risk of colorectal cancer in subjects with the *Pro*/*Pro* + *C*/*C* genotype (OR, 0.65; 95% CI, 0.43–1.00; $P = 0.06$ for trend), but any interaction was not statistically significant. Moderate and high consumption of egg had a marginal inverse association in those with the *Pro*/*Pro* + *C*/*C* genotype (OR, 0.71 and 0.64; 95% CI, 0.48–1.04 and 0.40–1.03, respectively; $P < 0.05$ for trend). Moderate and high consumption of deep-fried foods also marginally decreased the risk in those with the *Pro*/*Pro* + *C*/*C* genotype (OR, 0.72 and 0.63; 95% CI, 0.49–1.06 and 0.39–1.04; $P = 0.06$ for trend), but the interaction was not statistically significant. Consumption of yogurt and mayonnaise modified the risk, but with no dose-dependence.

In subjects with the *Pro*/*Pro* genotype, high SFA intake marginally increased risk of colorectal cancer (OR, 1.35; 95% CI, 0.93–1.96; $P = 0.10$ for trend), but the interaction was not statistically significant (Table 5). No suggestive evidence of modification by any of other fatty acids or fat, including the ratio of n-6 to n-3 PUFA (data not shown), was obtained. Additionally, on analysis of interactions between variables, multivariate ORs and tests of interactions did not change materially after controlling for consideration of green-yellow and other vegetables, one at a time or simultaneously.

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Table 4. Odds ratios and 95% confidence intervals for colorectal cancer according to the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene, with reference to consumption of foods or food groups

Foods/food groups	Number of cases/controls[†,¶]			OR (95% CI)[‡]			*P* for
	Low	Moderate	High	Low	Moderate	High	trend
Study 1							
Meat[§] (times/week)	<1	1–4	≥5				
Pro12Ala, Pro/Pro	20/56	79/130	21/35	1.00 (ref)	1.94 (1.06–3.58)*	2.01 (0.91–4.45)	0.05
Pro/Ala	1/3	4/12	2/2	0.56 (0.05–6.24)	0.93 (0.26–3.34)	2.48 (0.27–22.82)	0.45
						P for interaction = 0.64	
Pro/Pro + C/C[¶]	12/43	62/104	18/27	1.00 (ref)	2.68 (1.26–5.66)	2.88 (1.14–7.30)	0.02
Pro/Pro + (C/T + T/T)[¶]	8/13	17/26	3/8	2.86 (0.91–9.00)	2.49 (0.98–6.33)	2.56 (0.50–13.23)	0.95
						P for interaction = 0.21	
Milk (frequency)	Rarely	Sometimes	Daily				
Pro12Ala, Pro/Pro	34/41	35/72	51/107	1.00 (ref)	0.61 (0.32–1.16)	0.63 (0.34–1.16)	0.17
Pro/Ala	2/1	4/5	1/11	1.47 (0.11–19.47)	1.08 (0.25–4.75)	0.08 (0.01–0.69)	0.08
						P for interaction = 0.08	
Pro/Pro + C/C[¶]	28/34	25/51	39/89	1.00 (ref)	0.58 (0.27–1.22)	0.58 (0.29–1.17)	0.23
Pro/Pro + (C/T + T/T)[¶]	6/7	10/21	12/18	1.06 (0.29–3.91)	0.72 (0.28–1.86)	0.83 (0.32–2.16)	0.86
						P for interaction = 0.67	
Study 2							
Beef and pork (times/week)	<1	1–2	≥3				
Pro12Ala, Pro/Pro	62/192	118/343	64/194	1.00 (ref)	1.07 (0.75–1.53)	1.04 (0.69–1.58)	0.77
Pro/Ala + Ala/Ala	1/9	5/17	3/11	0.32 (0.04–2.60)	1.06 (0.37–3.00)	0.89 (0.24–3.32)	0.76
						P for interaction = 0.55	
Pro/Pro + C/C[¶]	48/142	84/258	48/136	1.00 (ref)	0.99 (0.65–1.50)	1.11 (0.69–1.79)	0.51
Pro/Pro + (C/T + T/T)[¶]	14/50	34/85	16/58	0.88 (0.44–1.75)	1.19 (0.71–2.01)	0.86 (0.44–1.66)	0.84
						P for interaction = 0.69	
Processed meat (times/week)	<1	1–2	≥3				
Pro12Ala, Pro/Pro	129/421	76/190	38/115	1.00 (ref)	1.34 (0.96–1.88)	1.09 (0.71–1.67)	0.35
Pro/Ala + Ala/Ala	3/16	5/13	1/8	0.65 (0.19–2.28)	1.36 (0.47–3.90)	0.43 (0.05–3.51)	0.80
						P for interaction = 0.81	
Pro/Pro + C/C[¶]	101/311	53/136	25/86	1.00 (ref)	1.26 (0.84–1.88)	0.93 (0.55–1.54)	0.83
Pro/Pro + (C/T + T/T)[¶]	28/110	23/54	13/29	0.80 (0.50–1.29)	1.33 (0.77–2.28)	1.48 (0.73–3.02)	0.05
						P for interaction = 0.15	
Poultry (times/week)	<1	1–2	≥3				
Pro12Ala, Pro/Pro	106/275	98/329	33/114	1.00 (ref)	0.79 (0.57–1.09)	0.78 (0.49–1.23)	0.17
Pro/Ala + Ala/Ala	1/10	5/16	3/10	0.29 (0.04–2.28)	0.88 (0.31–2.49)	0.83 (0.22–3.07)	0.49
						P for interaction = 0.25	
Pro/Pro + C/C[¶]	75/201	77/248	23/78	1.00 (ref)	0.87 (0.60–1.27)	0.84 (0.48–1.46)	0.58
Pro/Pro + (C/T + T/T)[¶]	31/74	21/81	10/36	1.15 (0.70–1.90)	0.70 (0.40–1.23)	0.83 (0.39–1.78)	0.13
						P for interaction = 0.46	
Fish (times/week)	≤2	3–4	≥5				
Pro12Ala, Pro/Pro	97/277	92/284	56/168	1.00 (ref)	0.93 (0.66–1.29)	0.91 (0.61–1.34)	0.55
Pro/Ala + Ala/Ala	4/17	4/12	1/8	0.69 (0.23–2.10)	1.09 (0.34–3.47)	0.34 (0.04–2.81)	0.26
						P for interaction = 0.49	
Pro/Pro + C/C[¶]	72/200	64/212	45/122	1.00 (ref)	0.84 (0.57–1.24)	0.98 (0.62–1.53)	0.79
Pro/Pro + (C/T + T/T)[¶]	25/77	28/72	11/46	0.92 (0.54–1.56)	1.10 (0.65–1.84)	0.61 (0.29–1.25)	0.59
						P for interaction = 0.75	
Egg (times/week or day)	≤2/week	3–6/ week	≥1/d				
Pro12Ala, Pro/Pro	111/282	86/284	47/156	1.00 (ref)	0.77 (0.55–1.08)	0.75 (0.50–1.11)	0.09
Pro/Ala + Ala/Ala	2/14	1/17	5/6	0.41 (0.09–1.85)	0.15 (0.02–1.12)	2.15 (0.64–7.22)	0.36
						P for interaction = 0.07	
Pro/Pro + C/C[¶]	83/195	65/219	32/116	1.00 (ref)	0.71 (0.48–1.04)	0.64 (0.40–1.03)	<0.05
Pro/Pro + (C/T + T/T)[¶]	28/87	21/65	15/40	0.77 (0.47–1.28)	0.77 (0.44–1.35)	0.84 (0.44–1.63)	0.85
						P for interaction = 0.20	
Milk (times/week or day)	<1/week	1–6/week	≥1/d				
Pro12Ala, Pro/Pro	89/233	84/229	68/265	1.00 (ref)	0.98 (0.69–1.40)	0.67 (0.46–0.97)*	0.03
Pro/Ala + Ala/Ala	6/13	1/13	2/11	1.31 (0.48–3.55)	0.22 (0.03–1.74)	0.48 (0.10–2.23)	0.15
						P for interaction = 0.39	
Pro/Pro + C/C[¶]	68/175	60/165	50/194	1.00 (ref)	0.96 (0.64–1.45)	0.65 (0.43–1.00)*	0.06
Pro/Pro + (C/T + T/T)[¶]	21/58	24/64	18/71	0.94 (0.53–1.67)	0.98 (0.56–1.71)	0.66 (0.36–1.19)	0.62
						P for interaction = 0.88	
Yoghurt (times/week or day)	<1/week	1–6/week	≥1/d				
Pro12Ala, Pro/Pro	123/412	84/206	36/104	1.00 (ref)	1.40 (1.00–1.95)*	1.14 (0.73–1.77)	0.26

Table 4. *Continued*

Foods/food groups	Number of cases/controls[†,¶]			OR (95% CI)[‡]			*P* for trend
	Low	Moderate	High	Low	Moderate	High	
Pro/Ala + Ala/Ala	5/24	2/8	2/5	0.74 (0.28–2.00)	0.90 (0.19–4.31)	1.45 (0.27–7.67)	0.67
						P for interaction = 0.70	
Pro/Pro + C/C[¶]	89/306	62/146	28/79	1.00 (ref)	1.49 (1.01–2.20)*	1.16 (0.70–1.93)	0.27
Pro/Pro + (C/T + T/T)[¶]	34/106	22/60	8/25	1.08 (0.68–1.71)	1.29 (0.75–2.24)	1.14 (0.49–2.64)	0.62
						P for interaction = 0.74	
Mayonnaise (times/week)	<1	1–2	≥3				
Pro12Ala, Pro/Pro	109/312	74/257	59/157	1.00 (ref)	0.84 (0.59–1.18)	1.12 (0.76–1.64)	0.59
Pro/Ala + Ala/Ala	2/13	4/13	3/10	0.47 (0.10–2.12)	1.02 (0.32–3.23)	0.90 (0.24–3.34)	0.90
						P for interaction = 0.62	
Pro/Pro + C/C[¶]	83/215	50/194	45/124	1.00 (ref)	0.68 (0.46–1.03)	1.02 (0.66–1.59)	0.83
Pro/Pro + (C/T + T/T)	26/97	24/63	14/33	0.71 (0.43–1.18)	1.03 (0.60–1.79)	1.12 (0.57–2.22)	0.17
						P for interaction = 0.23	
Fried foods (times/week)	<1	1–2	>3				
Pro12Ala, Pro/Pro	72/193	105/304	65/229	1.00 (ref)	0.94 (0.65–1.34)	0.77 (0.51–1.16)	0.24
Pro/Ala + Ala/Ala	1/12	1/15	7/10	0.23 (0.03–1.82)	0.20 (0.03–1.56)	2.04 (0.74–5.63)	0.28
						P for interaction = 0.01	
Pro/Pro + C/C[¶]	53/146	79/215	46/172	1.00 (ref)	1.04 (0.68–1.58)	0.78 (0.48–1.25)	0.35
Pro/Pro + (C/T + T/T)[¶]	19/47	26/89	19/57	1.15 (0.61–2.15)	0.84 (0.48–1.45)	0.93 (0.50–1.74)	0.52
						P for interaction = 0.86	
Deep-fried foods (times/week)	<1	1–2	≥3				
Pro12Ala, Pro/Pro	96/240	105/329	43/160	1.00 (ref)	0.81 (0.59–1.13)	0.68 (0.44–1.03)	0.08
Pro/Ala + Ala/Ala	2/12	4/19	6/3	0.45 (0.10–2.05)	0.59 (0.19–1.77)	1.33 (0.32–5.49)	0.93
						P for interaction = 0.17	
Pro/Pro + C/C[¶]	72/166	77/253	31/116	1.00 (ref)	0.72 (0.49–1.06)	0.63 (0.39–1.04)	0.06
Pro/Pro + (C/T + T/T)[¶]	24/74	28/76	12/44	0.76 (0.44–1.31)	0.85 (0.50–1.44)	0.66 (0.32–1.33)	0.57
						P for interaction = 0.43	

[†]One colorectal cancer case in study 1 and two controls in study 2 with unknown *PPARγ* genotypes were excluded from analyses. [‡]In study 1 were adjusted for age, sex, BMI, habitual exercise, drinking and smoking habits, and family history of colorectal cancer. In study 2, adjusted for BMI, habitual exercise, drinking and smoking habits, family history of colorectal cancer along with a series of group numbers matched age and sex to each case. [§]Meat included beef, pork, processed meat and poultry. [¶]The genotypes were combined with the two polymorphisms of the *PPARγ* gene, but subjects with the *Ala* allele were excluded in the combined genotypes due to a low frequency of the allele. *$P < 0.05$.

Discussion

We here established that the frequency of the *Ala* allele (as a possible preventive factor) on the *Pro12Ala* polymorphism is much lower in Japanese than in Americans and Europeans, consistent with previous studies of Japanese populations; the frequencies of the *Pro/Pro + C/C* and *Pro/Pro + (C/T + T/T)* genotypes were 70–73% and 20–25%, respectively. Meat consumption was found to have a significant association with colorectal cancer with the *Pro/Pro + C/C* genotype, whereas in the processed meat case it was with the *Pro/Pro + (C/T + T/T)* genotype. We found a marginal increase in the trend for risk according to SFA intake with the *Pro/Pro* genotype, but this did not attain statistical significance. In contrast, high milk consumption was associated with a more substantial decreased risk in those with the *Pro/Pro + C/C* genotype than in those with *Pro/Pro + (C/T + T/T)* genotype. High consumption of egg was also associated with decreased risks in those with the *Pro/Pro + C/C* genotype.

Compared with the traditional Japanese diet, meat consumption has now remarkably increased and the current diet is rich in animal fat/SFA,[11,37] possibly increasing the likelihood of colorectal cancer development.[38] Regarding meat consumption in the Japanese diet, the proportions of beef : pork : poultry : processed meat are 1.0 : 1.5–1.6 : 0.7–0.8 : 0.6 for both energy and fat intake, while ratios of meat to seafood are 1.3 and 2.2 for energy and fat intake, respectively.[37] For colorectal cancer risk, we observed increased risks according to dietary intake of meat in the *Pro/Pro + C/C* or *Pro/Pro + (C/T + T/T)* genotypes, but the risk was inconsistent with regard to relationships between combined genotypes and the type of meat. The discrepancy might be related to differences in food frequency questionnaires in the two studies. Meat was whole meat (including beef, pork, processed meat and poultry) in study 1, and separately defined as beef and pork, processed meat and poultry in study 2. The categories for the frequencies of meat consumption also differed. Amounts of SFA, per-oxidized fat and nitrates in meat might be linked to risk, and processed meat might exert influence through both effects of included fat on bile acid production and the presence of carcinogenic nitrosamines.[38]

In contrast, high consumption of fish and n-3 PUFA demonstrated no associations with either of the two polymorphisms regarding the risk of colorectal cancer. The activation of PPARγ by n-3 PUFA as one of the natural PPARγ ligands has been reported to induce apoptosis related to Bcl-2 and NF-κB in human colorectal cancer.[39] Activation of PPARγ by synthetic agents such as antidiabetic drugs has also been linked to anticancer effects.[40] Some agonists of PPARγ have been reported to suppress inflammatory processes and colorectal tumor progression,[41,42] but results from *in vivo* and *in vitro* studies with synthetic agonists have not also been consistent.[43–46] As PPARγ plays a central role in modulating the transcription of genes with promoters having a peroxisome proliferator response element,[47] the development of colorectal cancer may be associated with dose–responses of PPARγ to natural and/or synthetic ligands. However, there have been few reports about associations between PPARγ ligands and these gene polymorphisms.

According to data from the National Nutritional Survey, consumption of milk and egg has also dramatically increased

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Table 5. Odds ratios and 95% confidence intervals for colorectal cancer according to the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene, with reference to consumption of fat or individual fatty acids in study 2

Fat or fatty acids	Number of cases/controls[†,§]			OR (95% CI)[‡]			*P* for
	Low	Moderate	High	Low	Moderate	High	trend
Fat (g/1000 kcal)	≤25.60	25.61–31.28	≥31.29				
Pro12Ala, Pro/Pro	85/245	67/243	93/243	1.00 (ref)	0.83 (0.57–1.20)	1.18 (0.82–1.70)	0.35
Pro/Ala + Ala/Ala	1/10	2/14	6/13	0.32 (0.04–2.54)	0.44 (0.10–1.96)	1.55 (0.56–4.27)	0.51
						P for interaction = 0.15	
Pro/Pro + C/C[§]	64/177	44/186	72/173	1.00 (ref)	0.71 (0.46–1.11)	1.26 (0.83–1.92)	0.30
Pro/Pro + (C/T + T/T)[§]	21/68	22/57	21/70	0.88 (0.49–1.56)	1.13 (0.63–2.02)	0.89 (0.50–1.59)	0.99
						P for interaction = 0.58	
SFA (g/1000 kcal)	≤7.18	7.19–9.05	≥9.06				
Pro12Ala, Pro/Pro	76/247	73/243	96/241	1.00 (ref)	1.01 (0.70–1.46)	1.35 (0.93–1.96)	0.10
Pro/Ala + Ala/Ala	1/8	6/13	2/16	0.42 (0.05–3.42)	1.71 (0.62–4.71)	0.45 (0.10–2.02)	0.29
						P for interaction = 0.51	
Pro/Pro + C/C[§]	60/179	49/182	72/175	1.00 (ref)	0.84 (0.55–1.31)	1.30 (0.85–2.00)	0.25
Pro/Pro + (C/T + T/T)[§]	16/68	24/61	24/66	0.72 (0.38–1.34)	1.20 (0.68–2.08)	1.18 (0.67–2.10)	0.13
						P for interaction = 0.64	
MUFA (g/1000 kcal)	≤8.00	8.01–10.17	≥10.18				
Pro12Ala, Pro/Pro	82/245	70/243	93/243	1.00 (ref)	0.89 (0.61–1.29)	1.21 (0.84–1.75)	0.26
Pro/Ala + Ala/Ala	1/11	2/13	6/13	0.29 (0.04–2.31)	0.49 (0.11–2.24)	1.58 (0.57–4.34)	0.50
						P for interaction = 0.14	
Pro/Pro + C/C[§]	61/172	49/188	71/176	1.00 (ref)	0.77 (0.50–1.20)	1.22 (0.80–1.87)	0.35
Pro/Pro + (C/T + T/T)[§]	21/73	21/55	22/67	0.82 (0.46–1.47)	1.13 (0.62–2.01)	1.01 (0.56–1.81)	0.56
						P for interaction = 0.94	
n-6 PUFA (g/1000 kcal)	≤5.078	5.079–6.420	≥6.421				
Pro12Ala, Pro/Pro	83/242	73/246	89/242	1.00 (ref)	0.89 (0.61–1.28)	1.12 (0.78–1.60)	0.48
Pro/Ala + Ala/Ala	0/13	1/10	8/14	NE	0.30 (0.04–2.41)	1.84 (0.74–4.57)	0.25
						P for interaction: NE	
Pro/Pro + C/C[§]	63/171	46/182	72/182	1.00 (ref)	0.72 (0.46–1.12)	1.15 (0.76–1.74)	0.46
Pro/Pro + (C/T + T/T)[§]	20/71	27/64	17/60	0.80 (0.45–1.43)	1.18 (0.68–2.04)	0.82 (0.44–1.52)	0.85
						P for interaction = 0.72	
n-3 PUFA (g/1000 kcal)	≤1.055	1.056–1.330	≥1.331				
Pro12Ala, Pro/Pro	77/241	74/247	94/242	1.00 (ref)	0.93 (0.64–1.35)	1.22 (0.85–1.75)	0.23
Pro/Ala + Ala/Ala	1/14	4/8	4/15	0.23 (0.03–1.78)	1.83 (0.53–6.33)	0.88 (0.28–2.74)	0.37
						P for interaction = 0.50	
Pro/Pro + C/C[§]	53/168	55/176	73/191	1.00 (ref)	0.97 (0.62–1.50)	1.24 (0.82–1.89)	0.26
Pro/Pro + (C/T + T/T)[§]	24/73	19/71	21/51	1.04 (0.59–1.81)	0.89 (0.49–1.62)	1.26 (0.69–2.31)	0.39
						P for interaction = 0.91	
Cholesterol (g/1000 kcal)	≤124.05	124.06–175.40	≥175.41				
Pro12Ala, Pro/Pro	78/244	87/243	80/244	1.00 (ref)	1.13 (0.79–1.63)	1.01 (0.70–1.46)	0.97
Pro/Ala + Ala/Ala	3/11	1/14	5/12	0.92 (0.25–3.41)	0.25 (0.03–1.93)	1.32 (0.44–3.90)	0.24
						P for interaction = 0.52	
Pro/Pro + C/C[§]	59/164	64/192	58/180	1.00 (ref)	0.96 (0.63–1.46)	0.91 (0.59–1.40)	0.74
Pro/Pro + (C/T + T/T)[§]	19/80	23/51	22/64	0.69 (0.38–1.24)	1.29 (0.72–2.32)	0.94 (0.53–1.68)	0.40
						P for interaction = 0.37	

[†]One colorectal cancer case in study 1 and two controls in study 2 with unknown *PPARγ* genotypes were excluded from analyses. [‡]In study 1 adjusted for age, sex, BMI, habitual exercise, drinking and smoking habits, and family history of colorectal cancer. In study 2, adjusted for BMI, habitual exercise, drinking and smoking habits, family history of colorectal cancer along with a series of group number matched age and sex to each case. [§]The genotypes were combined with the two polymorphisms of the *PPARγ* gene, but subjects with the *Ala* allele were excluded in the combined genotypes due to a low frequency of the allele.
NE, not estimated because case was absent in this category.

over the last decades in Japan, whereas that of rice and potatoes has gradually decreased.[11,37] Intake of fish, beans, green-yellow vegetables and other vegetables has remained relatively constant. Consumption of milk, which is rich in short to middle-chain fatty acids, calcium, vitamin D and conjugated linoleic acid, was found in this study to decrease the risk of colorectal cancer and the effect was strongest with the *Pro/Pro + C/C* genotype. Risk of this cancer was modified by consumption of yogurt as a representative dairy product, but there was no dose-dependence, in contrast to earlier findings.[48] Effects may be associated with the ratio of circulating IGF-1 to IGF binding protein-3 and low fat milk has been demonstrated to particularly reduce cancer risk in individuals with high ratio of IGF-1 to IGF binding protein-3.[49] Our study subjects with high egg intake and the *Pro/Pro + C/C* genotype had a significantly decreased risk of this cancer incidence, but a recent cohort study in Japanese has reported a positive association between egg consumption and colorectal cancer death.[50] However, there have hitherto been no reports about gene–environment interactions regarding dietary intake of milk, dairy products and egg.

Consumption of meat, egg and milk has been reported to contribute about 40.2% of the dietary SFA intake in the Japanese diet (17.8%, 12.2%, 10.2% in that order).[51] We found a marginal positive association between high SFA intake and the incidence risk of colorectal cancer in the present study but our findings are in line with the *Pro12Ala* polymorphism liaising between fat/fatty acids and transcription,[52] and possibly contributing to colorectal tumor promotion through increased responsiveness to insulin and IGF-1.[53] We earlier reported a positive association between meat consumption and the *A52C* polymorphism of the *CD36* gene, one of the genes regulated by PPARγ and related to long-chain fatty acid translocation/oxidized low-density lipoprotein scavenging for fat/lipid metabolism.[28]

A previous study suggested that when the dietary ratio of PUFA to SFA is low, the levels of BMI and fasting plasma insulin are greater in carriers with the *Ala* allele than in the *Pro* homozygote for the *Pro12Ala* polymorphism, but when the ratio is high, the opposite may be the case.[54] Although there was no statistical significance due to low frequency of the *Ala* allele in Japanese, our findings implied that, in carriers with the *Ala* allele, moderate and high consumption of 'beef and pork' and poultry might confer a higher risk of colorectal cancer relative to low consumption. This may relate to roles of PPARγ as a thrifty gene. The *Ala* allele frequency in Japanese, at less than 5%, is much lower than in Americans and Europeans and the distribution of the *Pro12Ala* polymorphism was not in accordance with the Hardy–Weinberg equilibrium. However, the *C161T* polymorphism is a common silent substitution, and the *T* allele itself did not modify cancer risk in our subjects, in contrast to results from a case-control study in an Indian population,[55] who had both a very low incidence rate of colorectal cancer and a very low consumption of meat, fish and fat. Although the function of the *C161T* polymorphism could not be confirmed in the present study, we demonstrated positive interactions between meat intake and the combined polymorphism, namely the *Pro*/*Pro* + *C*/*C* and *Pro*/*Pro* + (*C*/*T* + *T*/*T*) genotypes, excluding carriers with the *Ala* allele due to very low frequency.

The sample size in study 1 was a small, and the age distribution differed between cases and controls. Cases were significantly older than controls, but the average age of the cases was the same as that in study 2. There were no differences in rates for excessive alcohol drinkers, heavy smokers and habitual exercisers between cases in the two studies. Compared with the ratio of rectal to colon cancer among Japanese,[15] the corresponding proportions were higher in both studies, but the reason for this is unclear. The risks of colorectal cancer may have been underestimated with data from prevalent cases because they might have partly modified their lifestyle after being diagnosed. Furthermore, the kinds of meat origin were not specified. In contrast, the sample size in study 2 was relatively large, all were incident cases, and controls were matched for age and sex to cases with a 1:3 case-control ratio. Consumption of each type of meat was specified according to the validated SQFFQ, and dietary intake of some fatty acids had significant correlations with the corresponding fatty acid concentrations in plasma as the biomarkers. The inverse association between the deep-fried food category and risk was unexpected and is difficult to explain.

Potential limitations of our studies, moreover, should be considered. One methodological issue is the selection of controls, and we used cancer-free outpatients at Aichi Cancer Center Hospital for this purpose because it is reasonable to assume our cases arose within this population base. Regarding general lifestyles, it is notable that our control population is similar to that of the general population randomly selected from the electoral roll in the same area.[39] Another potential source of bias is the medical background of controls. We have clarified that the majority do not have any specific medical conditions, and the remainder have benign tumors and/or non-neoplastic polyps, mastitis, digestive disease, in that order.[56,57] We therefore conclude that it is feasible to use non-cancer outpatients as references in the type epidemiological studies of Hospital-based Epidemiologic Research Program at Aichi Cancer Center Research.

In conclusion, our findings suggest significant positive associations between consumption of meat and processed meat and the *Pro12Ala* and *C161T* polymorphisms of the *PPARγ* gene regarding the risk of colorectal cancer. We also found that higher consumption of milk may reduce the risk in individuals with the *Pro*/*Pro* + *C*/*C* genotype. Because Japanese have a very low frequency of the *Ala* allele (as a possible preventive factor), further large-scale studies are needed to determine relationships between the *PPARγ* gene polymorphisms and dietary intake of meat (separately defined as beef, pork, processed meat and poultry), milk and SFA for the risk of colorectal cancer.

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