BMC Cancer

Research article

Association between an 8q24 locus and the risk of colorectal cancer in Japanese

Keitaro Matsuo*[1,6], Takeshi Suzuki[7], Hidemi Ito[1], Satoyo Hosono[1], Takakazu Kawase[1], Miki Watanabe[1], Kohei Shitara[3], Koji Komori[4], Yukihide Kanemitsu[4], Takashi Hirai[4], Yasushi Yatabe[5], Hideo Tanaka[1,6] and Kazuo Tajima[2]

Addresses: [1]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan, [2]Director, Aichi Cancer Center Research Institute, Nagoya, Japan, [3]Department of Medical Oncology, Aichi Cancer Center Hospital, Nagoya, Japan, [4]Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan, [5]Department of Pathology and Molecular Diagnosis, Aichi Cancer Center Hospital, Nagoya, Japan, [6]Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan and [7]Department of Medical Oncology and Immunology, Nagoya City University Graduate School of Medical Science, Nagoya, Japan

E-mail: Keitaro Matsuo* - kmatsuo@aichi-cc.jp; Takeshi Suzuki - takesuzu@med.nagoya-cu.ac.jp; Hidemi Ito - hidemi@aichi-cc.jp; Satoyo Hosono - shosono@aichi-cc.jp; Takakazu Kawase - t_kawase@aichi-cc.jp; Miki Watanabe - mwatanabe@aichi-cc.jp; Kohei Shitara - kshitara@aichi-cc.jp; Koji Komori - komori@aichi-cc.jp; Yukihide Kanemitsu - kanemitsu@aichi-cc.jp; Takashi Hirai - thirai@aichi-cc.jp; Yasushi Yatabe - yyatabe@aichi-cc.jp; Hideo Tanaka - hitanaka@aichi-cc.jp; Kazuo Tajima - ktajima@aichi-cc.jp
*Corresponding author

Published: 26 October 2009
BMC Cancer 2009, **9**:379 doi: 10.1186/1471-2407-9-379
Received: 8 January 2009
Accepted: 26 October 2009
This article is available from: http://www.biomedcentral.com/1471-2407/9/379

Abstract

Background: A genome-wide association study (GWAS), which assessed multiple ethnicities, reported an association between single nucleotide polymorphisms in the 8q24 region and colorectal cancer risk. Although the association with the identified loci was strong, information on its impact in combination with lifestyle factors is limited.

Methods: We conducted a case-control study in 481 patients with colorectal cancer (CRC) and 962 sex-age matched non-cancer controls. Data on lifestyle factors, including diet, were obtained by self-administered questionnaire. Two 8q24 loci, rs6983267 and rs10090154, were assessed by the TaqMan method. Associations were then assessed by multivariate logistic regression models that considered potential confounders.

Results: We found an increased risk of CRC with rs6983267 but not with rs10090154. An allelic OR was 1.22 (1.04-1.44, p for trend = 0.014), which remained significant after adjustment for confounders (OR = 1.25). No statistically significant interaction with potential confounding factors was observed.

Conclusion: The polymorphism rs6983267 showed a significant association with CRC in a Japanese population. Further investigation of the biological mechanism of this association is warranted.

Background

Colorectal cancer (CRC) remains major cancer worldwide [1]. Although numerous epidemiological and biological-studies have revealed risk/protective factors for CRC, present knowledge is still insufficient to allow the disease to be overcome, and the struggle to elucidate mechanisms is ongoing.

Recently, several a number of genome-wide association studies (GWAS) have revealed an association between variants on chromosome 8q24 and several sites of cancer, including CRC [2-11]. Each study showed that rs6983267 resides in 128.47-128.54 MB on Chromosome 8, denoted as 'region 3,' [7] and consistently associated with CRC [6,9,12]. This association was confirmed in a subsequent large-scale replication study in Caucasians [13-18]. Most of these CRC GWASs were conducted in Caucasian populations, however, and the data available for Asian populations is limited especially about possible gene-environment interaction [6,19].

The aim of the present case-control study was to clarify the impact of rs6983267 on CRC risk in a Japanese population. In addition, we explored the gene-environmental interaction between potential confounders and rs6983267.

Methods

Subjects

Cases were 481 patients who were histologically diagnosed with CRC (245 with colon cancer, 231 with rectum cancer) between January 2001 and November 2005 at Aichi Cancer Center Hospital (ACCH) and who had no prior history of cancer. Controls were first-visit outpatients at ACCH during the same periods who were confirmed to have no cancer or a prior history of neoplasm. Controls were randomly selected and matched for sex and age (± 4 years) with a 1:2 case-control ratio (n = 962). The subjects were selected from the database of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). The framework of HERPACC has been described elsewhere [20,21]. Briefly, all outpatients aged 20-79 years were asked at first visit to fill out a questionnaire regarding their lifestyle and provided 7 ml of blood. A trained interviewer checked the completion of each questionnaire. Approximately 95% of eligible subjects completed the questionnaire and 55% provided blood samples. Some 30% of first-visit outpatients were diagnosed at ACCH as having cancer. Under the assumption that the non-cancer population within HERPACC will visit ACCH if they develop cancer in the future, we defined non-cancer first-visit outpatients as those from among whom such cases may arise. Our previous study confirmed that the lifestyle patterns of first-visit outpatients matched the profile of a group randomly selected from the general population of Nagoya City, conferring external validity on the study [22]. Written informed consent was obtained from all subjects and the ethics committee of ACC approved the study.

Determination of the 8q24 loci genotype

DNA of each subject was extracted from the buffy coat fraction with a Blood Mini Kit (Qiagen K.K., Tokyo, Japan) and assessed using the polymerase chain reaction (PCR) TaqMan method [23] with the 7500 Fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The probes used were specifically designed for rs6983267 and rs10090154 in 8q24. rs10090154 in the 8q24 'region 1' [7] was chosen because it showed a significant association for a Japanese population in Hawaii [6]. The quality of genotyping was assessed by duplicate analysis of 5% of random samples, with an agreement rate of 100%.

Exposure data

Cumulative smoking dose was evaluated as pack-years, the product of the number of packs consumed per day and years of smoking. Smoking habit was classified into the three categories of never, pack-years < 20 (low-moderate) and ≥ 20 pack years (heavy). Consumption of types of alcoholic beverages (Japanese *sake*, beer, *shochu*, whiskey and wine) per occasion was determined with reference to the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent (one unit sake = 23 g ethanol) [24]. Daily ethanol consumption was estimated as the product of the frequency of alcohol beverage and average ethanol consumption occasion, and drinking habit was classified into the four categories of non-drinker, low (< 5 g/day), moderate (< 23 g/day) and heavy (≥ 23 g/day). Consumption of folate was determined using a semi-quantitative food frequency questionnaire (SQFFQ) as described in detail elsewhere [25]. Briefly, the SQFFQ consisted of 47 single food items with frequencies in the eight categories of 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. Average daily intake of nutrients was estimated by multiplying the food intake (in grams) or serving size by the nutrient content per 100 grams of food as listed in the Standard Tables of Food Composition in Japan, 5th edition. Consumption of supplemental folate was not considered in total consumption because the questionnaire for multi-vitamins was not quantitative. Energy-adjusted intake of nutrients was calculated by the residual method [26]. The SQFFQ was validated by reference to a 3-day weighted dietary record as a standard, which showed the reproducibility and validity to be acceptable [27,28]. The de-attenuated correlation coefficients for energy-adjusted intakes of folate were 0.36 in men and 0.38 in women. Body mass index (BMI) was calculated as the self-reported weight (kilograms) divided by the square of self-reported height (meters). A family history of CRC in first-degree relatives was based on self-reporting, as described elsewhere [29]. The questionnaire also covered the regularity of physical exercise: subjects were asked to report the frequency and intensity of recreational exercise, with average daily exercise hours in any intensity calculated and categorized into the three levels of none, and < 0.5 and ≥ 0.5 hours/day.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) for assessment of the impact of each 8q24 locus, included in the model as an ordinal score (1 to 3), were calculated using multivariable conditional logistic regression models. We explored two models: model 1 was a crude model; model 2 included age and sex plus potential confounders as indicator variables. Confounders considered in model 2 were smoking status (never, former, current moderate, and heavy), drinking habit (non, low, moderate, and heavy), folate consumption by tertile (T1-3), BMI (< 22.5, 22.5 - 24.9, 25.0-27.4 and ≥ 27.5 kg/m^2), family history of colorectal cancer (yes or no), and regular exercise (none, < 0.5 hour/day, and ≥ 0.5 hour/day). Interactions between rs6983267 assuming linear effect of allele and potential confounders similarly assuming linear effect were assessed in multivariable unconditional logistic regression models to avoid the dropping of subjects in conditional logistic regression models. To assess possible discrepancies between expected and observed haplotypes, accordance with the Hardy-Weinberg equilibrium (HWE) was checked for controls with the χ^2 test. Statistical analyses were performed using STATA version 10 (Stata, College Station, TX), with *P*-values < 0.05 considered statistically significant.

Results

Table 1 shows baseline characteristics of the 481 CRC cases, with an average age of 60 years, and the 962

Table 1: Characteristics of cases and controls

Variables	Cases		Controls		p-values
Total	481		962		
Sex					1.00
Male	300	62.4%	600	62.4%	
Female	181	37.6%	362	37.6%	
Age (years)					0.803
< 40	20	4.2%	39	4.1%	
40-49	50	10.4%	105	10.9%	
50-59	169	35.1%	328	34.1%	
60-69	164	34.1%	353	36.7%	
70-	78	16.2%	137	14.2%	
Mean age (SD)	60 (10.2)		60 (9.86)		
Site of Cancer					
Colon	245	50.9%			
Rectum	236	49.1%			
Smoking					0.102
None	215	44.7%	493	51.3%	
Low-moderate (< 20 pack-years)	59	12.3%	116	12.1%	
Heavy (≥ 20 pack-years)	203	42.2%	345	35.9%	
Unknown	4	0.8%	8	0.8%	
Drinking					0.695
None	190	39.5%	383	39.8%	
Low (< 5 g ethanl/day)	64	13.3%	125	13.0%	
Moderate (5≤ and < 23 g ethanol/day)	85	17.7%	196	20.4%	
High (≥ 23 g ethanol/day)	135	28.1%	243	25.3%	
Unknown	7	1.5%	15	1.6%	
Daily folate consumption					0.857
T1 (≤ 262.0 μg/day)	153	31.8%	286	29.7%	
T2 (≤ 346.6 μg/day)	158	32.9%	318	33.1%	
T3 (> 346.6 μg/day)	162	33.7%	341	35.5%	
Unknown	8	1.7%	17	1.8%	
Body-Mass Index (BMI) kg/m^2					0.923
< 22.5	206	42.8%	397	41.3%	
22.5 ≤ and < 25	157	32.6%	314	32.6%	
25 ≤ and < 27.5	73	15.2%	162	16.8%	
≥ 27.5	41	8.5%	79	8.2%	
Unknown	4	0.8%	10	1.0%	
Family history of colorectal cancer in the first degree relatives					0.014
No	453	94.2%	932	96.9%	
Yes	28	5.8%	30	3.1%	
Average recreational exercise					0.329
None	192	39.9%	349	36.3%	
< 0.5 hour/day	194	40.3%	398	41.4%	
0.5 ≤ hour/day	95	19.8%	215	22.4%	

controls matched for sex and age. Males accounted for 62.4% of subjects. Apart from a family history of CRC in a first-degree relative, potential confounders showed no clear difference between cases and controls. A family history of CRC was significantly more frequent among CRC cases.

Genotype distributions for 8q24 rs6983267 and rs10090154 are shown in Table 2. Among controls, both genotypes were accordant with the HWE. The minor allele frequency for rs6983267 was 0.338 (G-allele). The age- and sex-adjusted in the allelic model showed an OR of 1.22 (1.04-1.44, p = 0.0144) and the confounder-adjusted model an OR of 1.25 (1.06-1.48, p = 0.0071). Genotypic model showed a significant association only with rs6983267 GG genotype (OR = 1.64, 1.15-2.35, p = 0.0063). In contrast, rs10090154 showed no association with CRC risk. Table 3 shows stratified analyses conducted to explore possible interactions between potential confounders although point estimates for ORs were not static; no significant interactions were seen between the factors examined and rs6983267. The lack of association in those with a positive family history was of interest vis a vis the significant association in those without it, albeit that the number of subjects with a family history was limited.

Discussion

In this study, we found that the G allele in rs6983267 was associated with a significantly increased risk of CRC in a Japanese population. This finding is consistent with those from previous GWASs [6,9,11] and a pooled analysis [12], as reviewed in Table 4, which reported the consistency of this association with CRC and colorectal adenoma in populations with European ancestry. The only previous study of rs6983267 in a population with Asian ethnicity (Japanese-American) was that by Haiman et al [6], and to our knowledge the present study is the first indication in Japanese living in Japan. Tenesa et al. reported significant association with rs7014346 in 8q24, which is in high linkage disequilibrium with rs6983267, in Japanese population [19], supporting significant association between the rs6983267 in CRC in Japanese. Recent advances in genetic analysis have enabled a comprehensive approach to identifying disease susceptibility loci. The consistency of findings in this and the previous studies warrants the usefulness of the GWAS approach across ethnicities. We also evaluated potential interactions between common background factors and rs6983267, but found no significant interaction between them. Berndt et al. also reported a lack of interaction between rs6983267 and age, sex, smoking, family history of CRC and cancer site [12]. The consistency of this finding indicates that rs6983267 is associated with CRC risk independently of common risk factors.

Rs6983267 was originally identified using a non-hypothesis-based approach, and evidence has suggested a possible biological mechanism behind this observed association. The rs6983267 polymorphism resides 15 kb upstream of a processed pseudogene (*POU5F1P1*) of the POU-domain factor gene, *POU5F1*, which encodes transcription factor OCT4, with 97.5% shared identity [30]. OCT4, a transcript of *POU5F1*, plays a role in maintaining stem cell pluripotency, self-renewal and chromatin structure in stem cells [31], and promotes tumor growth in a dose-dependent manner [32]. A conserved POU5F1-binding site I at the 5′ promoter

Table 2: Genotypes distribution of 8q24 polymorphisms and odds ratios for the minor alleles and genotypes

					Allelic model						Genotype model					
					Model 1 *1			Model 2 *2			Heterozygote			Minor homozygote		
8q24 locus		Genotype			OR	95% CI	p-value	OR[a]	95% CI	p-value	OR[a]	95% CI	p-value	OR[a]	95% CI	p-value
rs6983267 (Minor allele: G, MAF*1 in controls = 0.338)																
	TT	TG	GG	UK*4												
case/	181/	222/	73/107	3/1	1.22	1.04-1.44	0.0144	1.25	1.06-1.48	0.0071	1.19	0.93-1.52	0.1665	1.64	1.15-2.35	0.0063
control	418	436														
rs10090154 (Minor allele: T, MAF in controls = 0.153)																
	CC	CT	TT	UK												
case/	355/	112/	11/23	3/3	0.90	0.72-1.12	0.3443	0.87	0.69-1.09	0.2140	0.83	0.63-1.08	0.1690	0.90	0.43-1.89	0.7854
control	689	247														

*1 Crude conditional logistic regression model.
*2 Adjusted for age as continuous variable, drinking (non, low, moderate, heavy, and unknown), smoking (non, moderate, heavy, unknwon), BMI (< 22.5, < 25, < 27.5, 27.5-, unknown), folate in tertile (T1, T2, T3, and unknown), total energy intake, family history of colorectal cancer, average recreational exercise (none, < 0.5 hour/day, 0.5-hour/day) in conditional logisitic regression.
*3 MAF indicates minor allele frequency.
*4 UK indicates the subjects whose genotyping was unsuccessful.

Table 3: Stratified analysis according to potential confounding factors for 8q24 rs6983267 genotype

			rs6983267 allelic model						
			*Model 1*1*			*Model*2*			
Exposure	**Number of controls with each genotype (TT/TG/GG)**	**Number of cases with each genotype (TT/TG/GG)**	**OR*1**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**Interaction P**
Sex									0.181
Male	**122/133/43**	**259/270/70**	1.11	0.91-1.37	0.295	1.14	0.93-1.41	0.212	
Female	**61/89/30**	**159/166/37**	1.44	1.10-1.88	0.007	1.34	1.01-1.78	0.040	
Smoking									0.401
None	**73/106/35**	**206/232/55**	1.34	1.05-1.70	0.018	1.29	1.01-1.65	0.042	
Low-moderate (< 20 pack-years)	**19/31/9**	**52/52/12**	1.51	0.93-2.43	0.094	1.49	0.89-2.49	0.130	
Heavy (≥ 20 pack-years)	**88/84/29**	**158/147/39**	1.11	0.86-1.43	0.423	1.12	0.86-1.44	0.407	
Drinking									0.437
None	**69/92/30**	**166/179/38**	1.36	1.04-1.76	0.023	1.35	1.03-1.77	0.028	
Low (< 5 g ethanl/day)	**23/29/12**	**51/58/16**	1.25	0.81-1.93	0.308	1.44	0.91-2.28	0.124	
Moderate (5≤ and < 23 g ethanol/day)	**34/41/9**	**85/83/27**	0.98	0.67-1.43	0.918	0.99	0.67-1.46	0.941	
High (≥ 23 g ethanol/day)	**55/59/19**	**112/108/23**	1.22	0.89-1.67	0.217	1.22	0.88-1.69	0.231	
Daily folate consumption									0.694
T1 (≤ 262.0 μg/day)	**54/73/25**	**112/137/37**	1.14	0.85-1.53	0.375	1.22	0.90-1.66	0.197	
T2 (≤ 346.6 μg/day)	**59/75/22**	**104/141/36**	1.21	0.91-1.61	0.191	1.25	0.93-1.67	0.141	
T3 (> 346.6 μg/day)	**66/72/24**	**157/152/32**	1.24	0.94-1.64	0.135	1.30	0.97-1.73	0.080	
Body-Mass Index (BMI) kg/m^2									0.678
< 22.5	**73/100/32**	**177/176/44**	1.34	1.05-1.72	0.020	1.43	1.10-1.85	0.007	
22.5 ≤ and < 25	**69/69/19**	**128/148/37**	0.93	0.70-1.25	0.663	0.93	0.69-1.25	0.637	
25 ≤ and < 27.5	**23/34/16**	**74/69/19**	1.58	1.06-2.36	0.024	1.67	1.09-2.55	0.018	
≥ 27.5	**18/17/5**	**31/41/7**	0.98	0.54-1.79	0.944	1.06	0.54-2.10	0.863	
Family history of colorectal cancer in the first degree relatives									0.765
No	**169/212/69**	**404/422/105**	1.24	1.05-1.46	0.011	1.26	1.06-1.48	0.008	
Yes	**14/10/4**	**14/14/2**	1.09	0.50-2.38	0.833	0.84	0.29-2.43	0.741	
Average recreational exercise									0.109
None	**77/90/24**	**161/140/48**	1.10	0.85-1.42	0.462	1.13	0.87-1.47	0.356	
< 0.5 hour/day	**73/89/31**	**158/199/40**	1.20	0.93-1.56	0.163	1.20	0.92-1.57	0.174	
0.5 ≤ hour/day	**33/43/18**	**99/97/19**	1.59	1.11-2.28	0.012	1.70	1.15-2.50	0.008	

*1 Odds ratios were adjusted for age and sex in unconditional logistic regression models. Conditional logistic models were not applied because keeping matching in stratficiation gave unstabel estimation.

*2 Odds ratio adjusted for age, sex and all variables in this examination except variable used for stratification.

*3 Interaction term between rs6983267 genotype in score and stratifiying factor in socre was added in model 2.

*4 Subjects were exlucded from analysis because of lack of information, smoking (4 cases and 8 controls), drinking (7 cases and 15 controls), folate (8 cases and 17 controls), and BMI (3 cases and 10 controls).

region of the WNT-signaling gene, *FZD5*, has been reported [33]. Tomlinson et al. reported the expression of either POU5F1 or POU5F1P1 in cell lines and primary CRCs [9], while Suo et al. similarly reported the expression of these genes in cancer cell lines and cancer tissues [30]. Given that OCT4 pseudogenes in mice are reported to mediate stem cell regulatory function [34], it is possible to hypothesize that OCT4 pseudogenes, including *POU5F1P1*, might play a role in stem cell proliferation. However, no difference in expression according to rs6983267 status was observed [9]. Berndt discussed the potential contribution of *MYC*, which is located > 300 KB distant to rs6983267[12]. Recently, Pomerantz et al. reported rs6983267 displays a difference in binding of transcription factor 7-like 2 (TCF7L2) leading to a different physical interaction with *MYC* [35]; however, Tuupanen et al. failed to find clear association between rs6983267 genotype and *MYC* expression. There still remains controversy between *MYC* and rs6983267 requiring further studies. Moreover, Tuupanen et al. reported rs6983267 affects a binding site for the Wnt-regulated transcription factor (TCF4), with the risk allele G showing stronger binding *in vivo* and *in vitro*. Overall, these findings indicate that the possible

Table 4: Review of results of 8q24 rs6983267 for colorectal cancer in allelic model

Author	Year	Case/Control	Country	Study subjects	Per allele OR (95%CI)	Adjustment
Haiman et al.	2007	**1,807/5,511**	**USA**	**Pooled**	**1.25 (1.12-1.38)**	Sex
		217/1,049		African American	1.37 (0.98-1.91)	Sex
		381/1,197		Japanese American	1.13 (0.96-1.34)	Sex
		61/347		Native Hawaiian	1.59 (1.02-2.47)	Sex
		251/1,007		Latinos	1.26 (1.02-1.55)	Sex
		214/973		European Americans	1.28 (1.03-1.58)	Sex
Tomlinson et al.	2007	**7,954/6,206**	**UK**	**CRC pooled**	**1.21 (1.15-1.27)**	Crude
		620/960		Panel A CRC White UK residents	1.38 (1.19-1.59)	Crude
		4,361/3,752		Panel B White UK residents	1.19 (1.12-1.26)	Crude
		1,901/1,079		Panel C	1.21 (1.09-1.35)	Crude
		1,072/415		Panel D European Ancestry	1.13 (0.96-1.33)	Crude
		1,425/2,255		**Adenoma pooled**	**1.22 (1.10-1.34)**	Crude
		407/1,027		Panel A Adenoma White UK residents	1.53 (1.29-1.81)	Crude
		607/765		Panel E	1.05 (0.90-1.23)	Crude
		411/463		Panel F	1.13 (0.93-1.37)	Crude
Poynter et al.	2007	**1,339/2,191**	**USA**	**Population – based**	1.11 (0.96-1.29)	Age and sex
Tuupanen et al.	2008	996/1,012	**Finland**	**Population-based**	1.22 (1.08-1.38)	Crude
Berndt et al.	2008	**3,134/4,454**	**USA**	**Colorectal neoplasms pooled**	**1.16 (1.07-1.25)**	Age, sex, and study
		547/1,656		PLCO	1.17 (1.01-1.35)	Age and sex
		1,174/1,293		PLCO adenoma	1.24 (1.11-1.39)	Age and sex
		364/363		PLCO II	0.93 (0.75-1.16)	Age and sex
		544/542		NHS	1.10 (0.93-1.30)	Age and sex
		505/600		Minnesota	1.21 (1.01-1.44)	Age and sex
Ghousaini et al.	2008	**2,299/2,284**	**UK**	**Cases from prospective study at East Anglia**	**1.27 (1.16-1.37)**	Crude
Lie et al.	2008	**561/721**	USA	Colon cancer cases from SEER Kentucky	**1.69 (1.19-2.40)**	Age, sex, BMI, and NSAID use.
				Cuacasian	1.61 (1.36-2.30)	Age, sex, BMI, and NSAID use.
Schafmayer	2009	**2,713/2,718**	Germany	Colorectal cancer cases with German ancestry	**1.22 (1.13-1.31)**	Crude
Curtin et al.	2009	**1,069/1,040**	**USA/UK**	**Colorectal cancer cases from USA/UK**	**1.17 (1.03-1.32)**	Crude
Our study		**481/962**	**Japan**	**HERPACC II participatns (Japanese)**	**1.22 (1.04-1.44)**	Age and sex
					1.26 (1.06-1.48)	Age, sex, drinking, smoking, BMI, folate consumption, energy, physical exercise, and family history of CRC

biological mechanism behind the effect of rs6983267 polymorphism on CRC carcinogenesis requires further study.

We did not observe any association with rs10090154 (OR = 0.90) on the contrary to the results from Multiethnic cohort study [6]. The point estimate for minor allele in the previous study was 1.41 (95%CI: 1.14-1.75). Following case-control study for Japanese American in Hawaii showed lack of association (OR = 1.07, 95%CI: 0.78-1.48)[6]. Inconsistency across studies might come from the finding in the original GWAS was by chance although threshold in statistical significance was high enough. Or, statistical power in following studies including ours was not good enough. By all means, more evidence is needed to clarify significance of the locus.

Several potential limitations of the present study require consideration. First, use of hospital-based control in this study for potential cause of selection bias. We used non-cancer patients at our hospital as controls, given the likelihood that our cases arose within this population base. Moreover, we previously showed that individuals selected randomly from our control population were similar to the general population in terms of baseline characteristics [22]. Given the similarity in minor allele frequency between our controls and that in the HapMap database for Japanese, it is reasonable to assume the external validity of our study results to the general

population. Second, as with other case-control studies, this study may have suffered from information bias: although the questionnaires were completed before the diagnosis in our hospital, some patients referred from other institutions might have known their diagnosis. Lack of interaction needs careful interpretation because confounders assessed in this study showed no association with CRC risk by themselves.

Conclusion

Our present investigation showed that rs6983267 in 8q24 is an independent risk factor of CRC in a Japanese population. Further studies to clarify the biological mechanisms of this association are warranted.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MW carried out the molecular genetic studies. JY carried out the immunoassays. MT participated in the sequence alignment. TS, TK, HT, and KT participated in the design of the study and helped to draft the manuscript. KS, KK, YK, TH and YY participated in the enrollment and conduct of the study. KM conceived of the study, participated in its design and statistical analyses. All authors read and approved the final manuscript.

Acknowledgements

The authors are grateful to the many doctors, nurses, and technical and administration staff of Aichi Cancer Center Hospital for the daily administration of the HERPACC study.

This study was supported by a Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan, for Cancer Research from the Ministry of Health, Labour and Welfare of Japan, and for the Third Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

Financial disclosure: The authors declare that they have nothing to disclose regarding financial issues.

References

1. Parkin DM, Whelan SL, Ferlay J, Teppo L and Thomas DB: **Cancer Incidence in Five Continents.**IARC Sci Publ. Lyon; 2003.
2. Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Cazier JB, Sainz J, Jakobsdottir M, Kostic J, Magnusdottir DN, Ghosh S, Agnarsson K, Birgisdottir B, Le Roux L, Olafsdottir A, Blondal T, Andresdottir M, Gretarsdottir OS, Bergthorsson JT, Gudbjartsson D, Gylfason A, Thorleifsson G, Manolescu A, Kristjansson K, Geirsson G, Isaksson H, Douglas J, Johansson JE, Balter K, Wiklund F, Montie JE, Yu X, Suarez BK, Ober C, Cooney KA, Gronberg H, Catalona WJ, Einarsson GV, Barkardottir RB, Gulcher JR, Kong A, Thorsteinsdottir U and Stefansson K: **A common variant associated with prostate cancer in European and African populations.** *Nat Genet* 2006, **38:**652–8.
3. Freedman ML, Haiman CA, Patterson N, McDonald GJ, Tandon A, Waliszewska A, Penney K, Steen RG, Ardlie K, John EM, Oakley-Girvan I, Whittemore AS, Cooney KA, Ingles SA, Altshuler D, Henderson BE and Reich D: **Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men.** *Proc Natl Acad Sci USA* 2006, **103:**14068–73.
4. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schurmann P, Dork T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, Ouweland van den A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X, Mannermaa A, Kosma VM, Kataja V, Hartikainen J and Day NE, *et al*: **Genome-wide association study identifies novel breast cancer susceptibility loci.** *Nature* 2007, **447:**1087–93.
5. Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeney LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A and Stefansson K: **Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24.** *Nat Genet* 2007, **39:**631–7.
6. Haiman CA, Le Marchand L, Yamamato J, Stram DO, Sheng X, Kolonel LN, Wu AH, Reich D and Henderson BE: **A common genetic risk factor for colorectal and prostate cancer.** *Nat Genet* 2007, **39:**954–6.
7. Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE and Reich D: **Multiple regions within 8q24 independently affect risk for prostate cancer.** *Nat Genet* 2007, **39:**638–44.
8. Schumacher FR, Feigelson HS, Cox DG, Haiman CA, Albanes D, Buring J, Calle EE, Chanock SJ, Colditz GA, Diver WR, Dunning AM, Freedman ML, Gaziano JM, Giovannucci E, Hankinson SE, Hayes RB, Henderson BE, Hoover RN, Kaaks R, Key T, Kolonel LN, Kraft P, Le Marchand L, Ma J, Pike MC, Riboli E, Stampfer MJ, Stram DO, Thomas G, Thun MJ, Travis R, Virtamo J, Andriole G, Gelmann E, Willett WC and Hunter DJ: **A common 8q24 variant in prostate and breast cancer from a large nested case-control study.** *Cancer Res* 2007, **67:**2951–6.
9. Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, Penegar S, Chandler I, Gorman M, Wood W, Barclay E, Lubbe S, Martin L, Sellick G, Jaeger E, Hubner R, Wild R, Rowan A, Fielding S, Howarth K, Silver A, Atkin W, Muir K, Logan R, Kerr D, Johnstone E, Sieber O, Gray R, Thomas H, Peto J, Cazier JB and Houlston R: **A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21.** *Nat Genet* 2007, **39:**984–8.
10. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N, Wang Z, Welch R, Staats BJ, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cancel-Tassin G, Cussenot O, Valeri A, Andriole GL, Gelmann EP, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hunter DJ, Chanock SJ and Thomas G: **Genome-wide association study of prostate cancer identifies a second risk locus at 8q24.** *Nat Genet* 2007, **39:**645–9.
11. Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, Prendergast J, Olschwang S, Chiang T, Crowdy E, Ferretti V, Laflamme P, Sundararajan S, Roumy S, Olivier JF,

Robidoux F, Sladek R, Montpetit A, Campbell P, Bezieau S, O'Shea AM, Zogopoulos G, Cotterchio M, Newcomb P, McLaughlin J, Younghusband B, Green R, Green J, Porteous ME, Campbell H, Blanche H, Sahbatou M, Tubacher E, Bonaiti-Pellie C, Buecher B, Riboli E, Kury S, Chanock SJ, Potter J, Thomas G, Gallinger S, Hudson TJ and Dunlop MG: **Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24.** *Nat Genet* 2007, **39:**989–94.

12. Berndt SI, Potter JD, Hazra A, Yeager M, Thomas G, Makar KW, Welch R, Cross AJ, Huang WY, Schoen RE, Giovannucci E, Chan AT, Chanock SJ, Peters U, Hunter DJ and Hayes RB: **Pooled analysis of genetic variation at chromosome 8q24 and colorectal neoplasia risk.** *Hum Mol Genet* 2008, **17:**2665–72.
13. Ghoussaini M, Song H, Koessler T, Al Olama AA, Kote-Jarai Z, Driver KE, Pooley KA, Ramus SJ, Kjaer SK, Hogdall E, DiCioccio RA, Whittemore AS, Gayther SA, Giles GG, Guy M, Edwards SM, Morrison J, Donovan JL, Hamdy FC, Dearnaley DP, Ardern-Jones AT, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Brown PM, Hopper JL, Neal DE, Pharoah PD, Ponder BA, Eeles RA, Easton DF and Dunning AM: **Multiple loci with different cancer specificities within the 8q24 gene desert.** *J Natl Cancer Inst* 2008, **100:**962–6.
14. Schafmayer C, Buch S, Volzke H, von Schonfels W, Egberts JH, Schniewind B, Brosch M, Ruether A, Franke A, Mathiak M, Sipos B, Henopp T, Catalcali J, Hellmig S, ElSharawy A, Katalinic A, Lerch MM, John U, Folsch UR, Fandrich F, Kalthoff H, Schreiber S, Krawczak M, Tepel J and Hampe J: **Investigation of the colorectal cancer susceptibility region on chromosome 8q24.21 in a large German case-control sample.** *Int J Cancer* 2009, **124:**75–80.
15. Curtin K, Lin WY, George R, Katory M, Shorto J, Cannon-Albright LA, Bishop DT, Cox A and Camp NJ: **Meta association of colorectal cancer confirms risk alleles at 8q24 and 18q21.** *Cancer Epidemiol Biomarkers Prev* 2009, **18:**616–21.
16. Li L, Plummer SJ, Thompson CL, Merkulova A, Acheson LS, Tucker TC and Casey G: **A common 8q24 variant and the risk of colon cancer: a population-based case-control study.** *Cancer Epidemiol Biomarkers Prev* 2008, **17:**339–42.
17. Poynter JN, Figueiredo JC, Conti DV, Kennedy K, Gallinger S, Siegmund KD, Casey G, Thibodeau SN, Jenkins MA, Hopper JL, Byrnes GB, Baron JA, Goode EL, Tiirikainen M, Lindor N, Grove J, Newcomb P, Jass J, Young J, Potter JD, Haile RW, Duggan DJ and Le Marchand L: **Variants on 9p24 and 8q24 are associated with risk of colorectal cancer: results from the Colon Cancer Family Registry.** *Cancer Res* 2007, **67:**11128–32.
18. Tuupanen S, Niittymaki I, Nousiainen K, Vanharanta S, Mecklin JP, Nuorva K, Jarvinen H, Hautaniemi S, Karhu A and Aaltonen LA: **Allelic imbalance at rs6983267 suggests selection of the risk allele in somatic colorectal tumor evolution.** *Cancer Res* 2008, **68:**14–7.
19. Tenesa A, Farrington SM, Prendergast JG, Porteous ME, Walker M, Haq N, Barnetson RA, Theodoratou E, Cetnarskyj R, Cartwright N, Semple C, Clark AJ, Reid FJ, Smith LA, Kavoussanakis K, Koessler T, Pharoah PD, Buch S, Schafmayer C, Tepel J, Schreiber S, Volzke H, Schmidt CO, Hampe J, Chang-Claude J, Hoffmeister M, Brenner H, Wilkening S, Canzian F, Capella G, Moreno V, Deary IJ, Starr JM, Tomlinson IP, Kemp Z, Howarth K, Carvajal-Carmona L, Webb E, Broderick P, Vijayakrishnan J, Houlston RS, Rennert G, Ballinger D, Rozek L, Gruber SB, Matsuda K, Kidokoro T, Nakamura Y, Zanke BW, Greenwood CM, Rangrej J, Kustra R, Montpetit A, Hudson TJ, Gallinger S, Campbell H and Dunlop MG: **Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21.** *Nat Genet* 2008, **40:**631–7.
20. Tajima K, Hirose K, Inoue M, Takezaki T, Hamajima N and Kuroishi T: **A Model of Practical Cancer Prevention for Out-patients Visiting a Hospital: the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC).** *Asian Pac J Cancer Prev* 2000, **1:**35–47.
21. Hamajima N, Matsuo K, Saito T, Hirose K, Inoue M, Takezaki T, Kuroishi T and Tajima K: **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* 2001, **2:**99–107.
22. Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, Kuroishi T and Tominaga S: **Epidemiological features of first-visit outpatients in Japan: comparison with general population and variation by sex, age, and season.** *J Clin Epidemiol* 1997, **50:**69–77.
23. Livak KJ: **Allelic discrimination using fluorogenic probes and the 5' nuclease assay.** *Genet Anal* 1999, **14:**143–9.
24. Matsuo K, Wakai K, Hirose K, Ito H, Saito T and Tajima K: **Alcohol dehydrogenase 2 His47Arg polymorphism influences drinking habit independently of aldehyde dehydrogenase 2 Glu487Lys polymorphism: analysis of 2,299 Japanese subjects.** *Cancer Epidemiol Biomarkers Prev* 2006, **15:**1009–13.
25. Matsuo K, Ito H, Wakai K, Hirose K, Saito T, Suzuki T, Kato T, Hirai T, Kanemitsu Y, Hamajima H and Tajima K: **One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan.** *Carcinogenesis* 2005, **26:**2164–71.
26. Willett W and Stampfer MJ: **Total energy intake: implications for epidemiologic analyses.** *Am J Epidemiol* 1986, **124:**17–27.
27. Imaeda N, Goto C, Tokudome Y, Hirose K, Tajima K and Tokudome S: **Reproducibility of a short food frequency questionnaire for Japanese general population.** *J Epidemiol* 2007, **17:**100–7.
28. Tokudome Y, Goto C, Imaeda N, Hasegawa T, Kato R, Hirose K, Tajima K and Tokudome S: **Relative validity of a short food frequency questionnaire for assessing nutrient intake versus three-day weighed diet records in middle-aged Japanese.** *J Epidemiol* 2005, **15:**135–45.
29. Suzuki T, Matsuo K, Wakai K, Hiraki A, Hirose K, Sato S, Ueda R and Tajima K: **Effect of familial history and smoking on common cancer risks in Japan.** *Cancer* 2007, **109:**2116–23.
30. Suo G, Han J, Wang X, Zhang J, Zhao Y and Dai J: **Oct4 pseudogenes are transcribed in cancers.** *Biochem Biophys Res Commun* 2005, **337:**1047–51.
31. Campbell PA, Perez-Iratxeta C, Andrade-Navarro MA and Rudnicki MA: **Oct4 targets regulatory nodes to modulate stem cell function.** *PLoS ONE* 2007, **2:**e553.
32. Gidekel S, Pizov G, Bergman Y and Pikarsky E: **Oct-3/4 is a dose-dependent oncogenic fate determinant.** *Cancer Cell* 2003, **4:**361–70.
33. Katoh Y and Katoh M: **Conserved POU-binding site linked to SP1-binding site within FZD5 promoter: Transcriptional mechanisms of FZD5 in undifferentiated human ES cells, fetal liver/spleen, adult colon, pancreatic islet, and diffuse-type gastric cancer.** *Int J Oncol* 2007, **30:**751–5.
34. Lin H, Shabbir A, Molnar M and Lee T: **Stem cell regulatory function mediated by expression of a novel mouse Oct4 pseudogene.** *Biochem Biophys Res Commun* 2007, **355:**111–6.
35. Pomerantz MM, Ahmadiyeh N, Jia L, Herman P, Verzi MP, Doddapaneni H, Beckwith CA, Chan JA, Hills A, Davis M, Yao K, Kehoe SM, Lenz HJ, Haiman CA, Yan C, Henderson BE, Frenkel B, Barretina J, Bass A, Tabernero J, Baselga J, Regan MM, Manak JR, Shivdasani R, Coetzee GA and Freedman ML: **The 8q24 cancer risk variant rs6983267 shows long-range interaction with MYC in colorectal cancer.** *Nat Genet* 2009, **41:**882–4.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/9/379/prepub