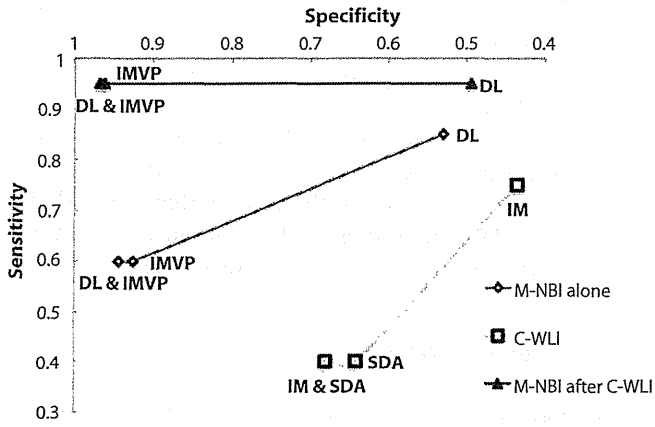

Figure 4. Comparison of the sensitivity and specificity of individual endoscopic findings in magnifying narrow-band imaging (M-NBI) alone, conventional white-light imaging (C-WLI), and M-NBI after C-WLI. Among all endoscopic findings, diagnostic performance improved significantly for C-WLI alone, followed by M-NBI alone, and then by M-NBI after C-WLI.

TABLE 4. Characteristics of correct and incorrect diagnosis by M-NBI

	Correct diagnosis (n = 330)	Incorrect diagnosis (n = 23)	*P*
Mean SDL size, mm	6	6	.67
SDL location (longitudinal)			
Upper third	61	5	
Middle third	82	7	.45
Lower third	187	11	
SDL location (circumferential)			
Anterior wall	57	4	.34
Lesser curvature	106	9	
Posterior wall	93	8	
Greater curvature	74	2	
Inspection time			
Average(s)	72	100	.15

M-NBI, Magnifying narrow-band imaging; *SDL*, small, depressed lesion.

DISCUSSION

In this study, we found that the endoscopic diagnostic performance increased for C-WLI, followed by M-NBI alone; M-NBI after C-WLI ultimately showed the best diagnostic performance for each diagnostic criterion (Fig. 4). Therefore, M-NBI should generally be performed after evaluation of C-WLI findings. The combination of DL and IMVP characterized by M-NBI after C-WLI contributed to the most reliable diagnosis for small, depressed gastric lesions and can be an ideal, simple, standard diagnostic strategy for small, depressed gastric lesions.

Based on the current results, we herein propose an efficient endoscopic diagnostic strategy for small, depressed gastric lesions, as indicated in Figure 5. In the M-NBI after C-WLI technique, both a DL and an IMVP had a high negative predictive value (99% and 99%, respectively), indicating that both criteria were sufficiently sensitive for exclusion of noncancerous lesions. A DL is technically easier to identify than an IMVP.[15] Therefore, the identification of a DL should be the first step in the diagnosis of gastric cancer because the absence of a DL alone allows for the exclusion of a noncancerous lesion. If a DL is absent, a noncancerous lesion can be diagnosed without any additional findings. Next, the presence of an IMVP is evaluated within a DL. If both a DL and IMVP are present, cancer is strongly suggested because an IMVP is sufficiently specific in the diagnosis of cancer (ie, it has a high positive predictive value) and an additional procedure with curative intent is indicated. If an IMVP is absent, the lesion can be diagnosed as a noncancerous lesion without a target biopsy sample because the negative predictive value of an IMVP is also very high. This strategy will provide a high level of accurate diagnosis for small, depressed gastric lesions. In particular, because the negative predictive value of both a DL and an IMVP is very high in M-NBI after C-WLI, the benefits of this strategy include reductions in the risk of hemorrhage, number of biopsy specimens for pathologic analysis, procedure time, and medical expenses, especially when a noncancerous lesion is diagnosed.

Analysis of lesions incorrectly diagnosed by M-NBI revealed reasons for misdiagnosis. The reasons for misdiagnosis included both technical and cognitive factors; thus, training should involve both aspects. Technical errors were mainly caused by difficulty in observation of a DL and an IMVP at maximum magnification. Attachment of a rubber cap is very helpful for capturing in-focus magnified images, but we failed to obtain sufficient images in some cases. To improve the performance of these techniques, 1 of the authors has published a book with a DVD[16] explaining the techniques necessary to perform M-NBI at maximum magnification. The role of videos in transferring information relating to endoscopic technique will be more important than that of text from now on. Cognitive factors included the lack of interpretative skill on the part of the endoscopist. In their review of the images, the 2 reviewers revised the diagnosis in 7 cases, indicating a false-positive rate of 4.5% and false-negative rate of 22.5%. These numbers could have improved with better mastery of interpretative skill. An e-learning system was developed to improve interpretative skill using M-NBI, and a multicenter study, entitled "Learning curve with an e-learning system on magnifying narrow-band imaging in endoscopic diagnosis of gastric lesions: A randomized

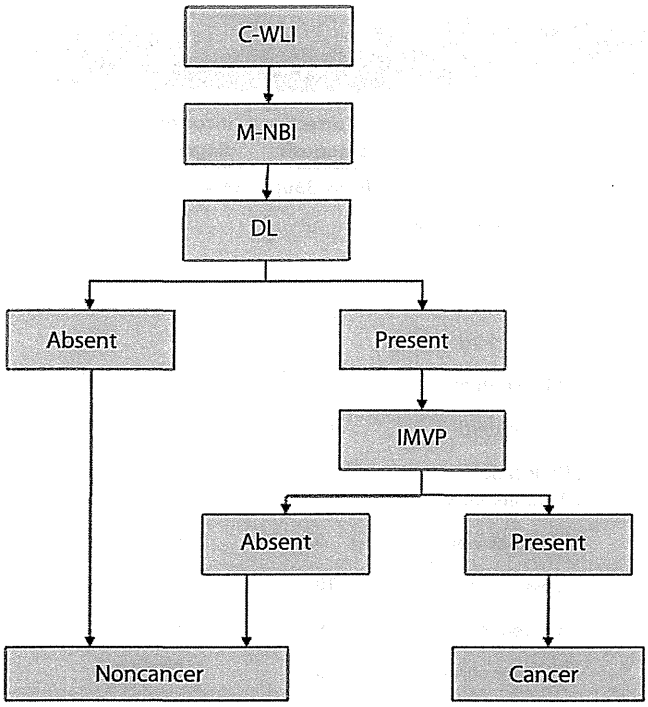

Figure 5. Strategy of using simplified criteria to make an endoscopic diagnosis of small, depressed lesions using magnifying narrowband imaging (M-NBI). When a small, depressed gastric lesion is detected by conventional white-light imaging (C-WLI), the presence or absence of a demarcation line (DL) should be the first step in diagnosing gastric cancer. If the DL is absent, a noncancerous lesion can be diagnosed. If the DL is present, the presence of an irregular microvascular pattern (IMVP) should be used for diagnosis. If an IMVP is absent, a noncancerous lesion can be diagnosed without a target biopsy sample and/or EMR/ESD. Finally, if both a DL and an IMVP are present, cancer is strongly suggested, and additional procedures are indicated.

study" (UMIN-CTR 000008569), was begun to examine the system's usefulness. After the review of recorded images by the 2 experts, there were limits of diagnosing lesions with M-NBI in 4 cases. The lesions did not fulfill the endoscopic cancerous or noncancerous diagnostic criteria of a DL and an IMVP according to M-NBI. For these lesions, development of other diagnostic equipment or another method is required to further improve the diagnostic performance.

This study has limitations. The study samples were limited to $\leq$10– mm depressed lesions. A diagnosis based on the microvascular pattern and a DL, but not the microsurface pattern, is not universally applicable to all macroscopic types of lesions. However, all small, depressed lesions in this study had a microvascular pattern that could be visualized, allowing the lesions to be diagnosed. Thus, the current authors are prospectively studying (UMIN-CTR 000004045) the ability of M-NBI to diagnose all macroscopic types of lesions using the vessel-plus-surface classification system[7] put forth by Yao et al without size or macroscopic type limitation. The vessel-plus-surface classification system uses the microsurface pattern, microvascular pattern, and DL as indices.

In conclusion, the current study suggests that although M-NBI alone provided good diagnostic performance, it is important to conduct a C-WLI evaluation before M-NBI diagnosis. When using M-NBI, identification of a DL is the first step in the diagnosis of cancer, and the subsequent identification of an IMVP is useful for excluding noncancerous lesions among the lesions that were identified to have a DL. Training in both techniques and knowledge is important to improve M-NBI diagnosis.

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Original Article

Coffee Consumption and Risk of Colorectal Cancer: The Japan Collaborative Cohort Study

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ABSTRACT

Background: Epidemiologic studies have reported coffee consumption to be associated with various health conditions. The purpose of this study was to examine the relationship of coffee consumption with colorectal cancer incidence in a large-scale prospective cohort study in Japan.

Methods: We used data from the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study). Here, we analyzed a total of 58 221 persons (23 607 men, 34 614 women) followed from 1988 to the end of 2009. During 738 669 person-years of follow-up for the analysis of colorectal cancer risk with coffee consumption at baseline, we identified 687 cases of colon cancer (355 males and 332 females) and 314 cases of rectal cancer (202 males and 112 females). We used the Cox proportional-hazard regression model to estimate hazard ratio (HR).

Results: Compared to those who consumed less than 1 cup of coffee per day, men who consumed 2–3 cups of coffee per day had an HR of 1.26 (95% confidence interval [CI] 0.93–1.70), and men who consumed more than 4 cups of coffee per day had an HR of 1.79 (95% CI 1.01–3.18). A statistically significant increase in the risk of colon cancer was associated with increasing coffee consumption among men (P for trend = 0.03). On the other hand, coffee consumption in women was not associated with incident risk of colon cancer. Coffee consumption was also not associated with rectal cancer incidence in men or women.

Conclusions: This large-scale population-based cohort study showed that coffee consumption increases the risk of colon cancer among Japanese men.

Key words: coffee; colorectal cancer; incidence; prospective study; the Japan Collaborative Cohort Study

INTRODUCTION

Colorectal cancer is already one of the most common cancers in Western countries and is rapidly increasing in incidence across Asia.[1] This increase is considered to be associated with changes in environmental factors such as dietary habits and lifestyle. Therefore, primary prevention of colorectal cancer worldwide is a considerable public health concern.[2]

Coffee is one of the most widely consumed beverages in the world. Recent national data from Japan have revealed that the average per capita coffee consumption is about 127.1 g per day (Japan Ministry of Health Labour and Welfare, 2010). Therefore, even small effects of coffee on individuals could have a large effect on general public health. A number of epidemiologic studies have investigated the relationship between coffee consumption and colorectal cancer, but findings regarding the effect of coffee on the incidence of this cancer have been inconsistent.[3–13] A meta-analysis by Je et al reported no association between coffee consumption and colon cancer risk, whereas another meta-analysis by Li et al reported a significantly increased risk.[14,15] Further, most previous studies have been conducted in Western countries.[3–10] The three major cohort studies from Japan on the relationship between coffee consumption and the risk of

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colorectal cancer[11–13] have also reported inconsistent results: the Japan Public Health Center (JPHC) Cohort Study and Takayama Cohort Study suggested that coffee consumption may lower the risk of colon cancer in women,[11] whereas the Miyagi Cohort Study concluded that consumption was not associated with risk of colorectal cancer in either sex.[12,13]

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study) is a large-scale population-based cohort study. The study has provided many findings on cancer risk associated with lifestyle and living conditions in the Japanese population but has not reported results on the association between coffee consumption and colorectal cancer incidence.[16]

The aim of this study was to examine the relationship of coffee consumption with colon and rectal cancer incidence in a large-scale prospective cohort study in Japan.

METHODS

Study subjects and data collection

Details of the study concept and design of the JACC Study have been described elsewhere.[16] Briefly, the JACC Study was started between 1988 and 1990 and enrolled subjects living in 45 areas in Japan. A total of 110 585 Japanese subjects (46 395 men and 64 190 women) aged 40–79 at baseline were followed to the end of 2009.

We analyzed colorectal cancer risk with coffee consumption at baseline using data from a baseline survey. Of 110 585 participants at baseline, subjects for the present analysis were restricted to 65 042 participants who lived in the 24 areas in which information on cancer incidence was available. We excluded subjects in the study whose baseline questionnaire did not include a section on coffee consumption, who skipped questions about coffee consumption, or who had a history of colorectal cancer. After exclusion, 58 221 subjects (23 607 men, 34 614 women) remained for the final analysis.

Information about coffee consumption and other lifestyle factors was obtained using a self-administered questionnaire. Subjects were grouped into four categories according to daily coffee intake at baseline, namely: less than 1 cup, 1 cup, 2–3 cups, or 4 or more cups a day. These four categories were determined by reference to a previous study.[17] The question regarding coffee consumption was previously assessed by a validation study, which reported a strong agreement with 12-day weighted dietary records (Spearman correlation: 0.81).[18]

Follow-up

Subjects were followed from the baseline survey until 2009. Individuals who moved away from the study area were treated as study dropouts, because deaths after such moves could not be confirmed in our follow-up system. The occurrence of cancer was confirmed from population-based cancer registries or by reviewing the records of local major hospitals. We defined colon cancer as C18 and rectal cancer as C20 according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (http://www.who.int/classifications/icd/en/). The study protocol was approved by the Ethical Board of Nagoya University School of Medicine.

Analysis

For the analysis of the association between colorectal cancer risk and coffee consumption, hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox's proportional hazards model adjusted for 5-year age groups by gender. In multivariate analyses, we further adjusted for several factors known to be associated with colorectal cancer and/or coffee consumption, including smoking status (current smoker of more than 20 cigarettes/day, current smoker of at least 1 cigarette/day but no more than 20 cigarettes/day, former smoker, or never smoker), daily walking duration (walking more than 30 min per day or not), education (attended school up to 15–18 years old, or >18 years old), body mass index (BMI; <18.5 kg/m^2, 18.5 kg/m^2 to 25 kg/m^2, or >25.0 kg/m^2), alcohol intake (daily drinker, less than daily drinker, former drinker, nondrinker), family cancer history (yes or no), and meat consumption (high consumption of beef and pork, middle consumption, or low consumption). Data for the above factors were self-reported. For all covariates, missing values were treated as an additional category of variable and were included in the model. The linear trend in incident risk was assessed by treating the number of cups of coffee intake per day as an ordinary variable. All analyses were performed using the SAS statistical package, version 9.3 (SAS Institute, Cary, NC, USA).

In addition, we performed stratified analyses by smoking status (never smoker or current smoker), BMI (<18.5 kg/m, 18.5 kg/m^2 to 25 kg/m^2, or >25.0 kg/m^2), meat consumption (high consumption of beef and pork, first tertile; middle consumption, second tertile; and low consumption, third tertile), age (40–59 years or 60–69 years), and drinking status (drinker or never drinker).

RESULTS

Baseline characteristics of the study cohort by coffee consumption are presented in Table 1. Subjects with high coffee consumption were younger, better educated, and more likely to be smokers, alcohol drinkers, and to regularly eat beef or pork. Similar trends were obtained for men and women. However, unlike in men, alcohol drinking in women increased with an increase in coffee consumption, albeit the proportion of drinkers was relatively low.

During 738 669 person-years of follow-up, we identified 687 cases of colon cancer (355 males and 332 females) and 314 cases of rectal cancer (202 males and 112 females). Table 2 shows the HRs and 95% CIs for colorectal cancer incidence by coffee consumption. A statistically significant

Table 1. Characteristics of subjects for analysis of coffee consumption

Characteristics at baseline survey		Men					Women				
		<1 cup/day	1 cup/day	2–3 cups/day	≥4 cups/day	*P*	<1 cup/day	1 cup/day	2–3 cups/day	≥4 cups/day	*P*
Number of subjects		15569	3172	4116	750		23345	5844	4932	493	
Age, years; mean (standard deviation)		59.0 (10.0)	57.3 (10.4)	54.0 (9.9)	51.4 (9.6)	<0.0001	60.0 (9.4)	57.2 (10.0)	53.8 (9.5)	49.9 (8.5)	<0.0001
Smoking habit	Current (%)	47.7	51.6	65.8	80.9		3.5	5.4	10.3	32.5	
	Former (%)	28.9	28.4	20.1	12.9	<0.0001	1.3	1.8	2.2	2.7	<0.0001
	Never (%)	23.3	20.1	14.0	6.3		95.2	92.8	87.5	64.9	
Drinking habit	Current (%)	76.1	76.5	73.8	62.6		19.7	30.0	35.0	38.0	
	Former (%)	6.7	5.5	5.1	6.9	<0.0001	1.7	1.9	2.0	4.8	<0.0001
	Never (%)	17.2	18.0	21.1	30.5		78.6	68.1	63.0	57.3	
Education:	High (%)	14.9	19.9	21.1	26.1	<0.0001	8.4	10.3	12.3	15.4	<0.0001
Family history of colorectal cancer:	Yes (%)	2.1	2.5	2.3	2.7	0.27	2.6	2.7	2.3	2.0	0.46
Body mass index:	<18.5 kg/m^2 (%)	5.6	4.9	4.8	6.4	0.24	6.7	5.8	5.4	7.6	<0.0001
	≥25 kg/m^2 (%)	18.3	18.0	18.6	19.4		23.1	20.4	20.7	15.2	
Walking time:	≥30 min/day (%)	58.9	64.7	64.9	64.8	<0.0001	59.7	68.0	67.7	66.9	<0.0001
Regular consumption of meat:	Low (%)	39.3	35.1	33.0	29.6		39.3	32.6	29.2	32.1	
	Middle (%)	35.0	38.5	42.8	41.0	<0.0001	34.3	41.3	41.0	31.2	<0.0001
	High (%)	25.7	26.4	24.2	29.5		26.4	26.1	29.7	36.8	

Table 2. Hazard ratio and 95% confidence interval for colorectal cancer incidence for analysis of coffee consumption

Coffee consumption	Men					Women				
	Number of cases	Age adjusted HR (95% confidence interval)	*P* value	Multivariable adjusted HR[a] (95% confidence interval)	*P* value	Number of cases	Age adjusted HR (95% confidence interval)	*P* value	Multivariable adjusted HR[a] (95% confidence interval)	*P* value
For colon cancer incidence										
<1 cup/day	240	1.00		1.00		254	1.00		1.00	
1 cup/day	44	1.08 (0.79–1.50)	0.63	1.06 (0.76–1.47)	0.73	46	1.03 (0.75–1.42)	0.84	1.00 (0.72–1.37)	0.98
2–3 cups/day	58	1.26 (0.94–1.69)	0.12	1.26 (0.93–1.70)	0.13	27	0.90 (0.60–1.34)	0.59	0.86 (0.57–1.30)	0.49
≥4 cups/day	13	1.72 (0.99–3.02)	0.06	1.79 (1.01–3.18)	0.05	5	2.16 (0.88–5.30)	0.09	2.02 (0.81–5.03)	0.13
P for trend			0.02		0.03			0.77		0.96
For rectum cancer incidence										
<1 cup/day	139	1.00		1.00		82	1.00		1.00	
1 cup/day	28	1.19 (0.79–1.78)	0.41	1.19 (0.79–1.80)	0.40	13	0.85 (0.47–1.53)	0.58	0.88 (0.48–1.59)	0.67
2–3 cups/day	30	1.08 (0.72–1.62)	0.71	1.12 (0.75–1.70)	0.58	17	1.45 (0.84–2.49)	0.18	1.55 (0.89–2.69)	0.12
≥4 cups/day	5	1.06 (0.43–2.60)	0.90	1.19 (0.48–2.95)	0.71	0	0.00 (—)	0.97	0.00 (—)	0.98
P for trend			0.71		0.53			0.52		0.37
For colorectal cancer incidence										
<1 cup/day	379	1.00		1.00		336	1.00		1.00	
1 cup/day	72	1.12 (0.87–1.44)	0.38	1.11 (0.86–1.43)	0.44	59	0.99 (0.75–1.30)	0.91	0.97 (0.73–1.28)	0.82
2–3 cups/day	88	1.19 (0.94–1.51)	0.14	1.21 (0.95–1.54)	0.12	44	1.05 (0.76–1.45)	0.77	1.04 (0.75–1.44)	0.81
≥4 cups/day	18	1.47 (0.91–2.37)	0.12	1.57 (0.97–2.55)	0.07	5	1.46 (0.60–3.56)	0.41	1.42 (0.57–3.50)	0.45
P for trend			0.04		0.03			0.55		0.61

HR, hazard ratio.
[a]Hazard ratio was adjusted for age, smoking, drinking, family history of colorectal cancer, education, body mass index, walking time, and regular meat consumption, and distict.

increase in the risk of colon cancer with increasing levels of coffee consumption was seen among men (*P* for trend = 0.03). Compared with men who consumed less than 1 cup of coffee per day, men who consumed 2–3 cups per day showed an unadjusted HR of 1.26 (95% CI 0.94–1.69) and an HR of 1.26 (95% CI 0.93–1.70) after multivariable adjustment for potential confounding factors, and men who consumed more than 4 cups of coffee per day showed an unadjusted HR of 1.72 (95% CI 0.99–3.02) and an HR of 1.79 (95% CI 1.01–3.18) after multivariable adjustment for potential confounding factors. In contrast, coffee consumption was not associated with incident risk of colorectal cancer in women. Compared with women who consumed less than 1 cup of coffee per day, women who consumed 2–3 cups of coffee per day showed an unadjusted HR of 0.90 (95% CI 0.60–1.34) and a HR of 0.86 (95% CI 0.57–1.30) after

Table 3. Hazard ratio for colorectal cancer incidence for analysis of coffee consumption, stratified by sex and smoking status

Coffee consumption	Men						Women					
	Current			Never			Current			Never		
	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value
For colon cancer incidence												
<1 cup/day	109	1.00		42	1.00		4	1.00		222	1.00	
1 cup/day	21	1.08	0.77	7	1.18	0.68	1	1.03	0.98	37	1.03	0.88
2–3 cups/day	34	1.21	0.36	10	1.85	0.09	5	4.71	0.04	17	1.46	0.23
≥4 cups/day	9	1.68	0.15	2	5.58	0.02	2	6.06	0.09	3	1.69	0.19
P for trend			0.13			0.01			0.02			0.60
For rectum cancer incidence												
<1 cup/day	59	1.00		29	1.00		2	1.00		70	1.00	
1 cup/day	14	1.36	0.31	9	2.13	0.05	0			10	0.84	0.62
2–3 cups/day	19	1.19	0.52	4	1.11	0.85	2	1.44	0.75	15	1.74	0.06
≥4 cups/day	2	0.67	0.59	2	6.31	0.01	0			0		
P for trend			0.91			0.12						0.20
For colorectal cancer incidence												
<1 cup/day	168	1.00		71	1.00		6	1.00		292	1.00	
1 cup/day	35	1.17	0.41	16	1.57	0.11	1	0.61	0.66	47	0.98	0.91
2–3 cups/day	53	1.21	0.26	14	1.52	0.16	7	3.65	0.04	32	1.00	0.99
≥4 cups/day	11	1.32	0.38	4	5.92	0.00	2	2.87	0.24	3	1.46	0.52
P for trend			0.20			0.01			0.04			0.80

HR, hazard ratio.
[a]Hazard ratio was adjusted for age, drinking, family history of colorectal cancer, education, body mass index, walking time, and regular meat consumption.

multivariable adjustment for potential confounding factors; women who consumed more than 4 cups of coffee per day showed an unadjusted HR of 2.16 (95% CI 0.88–5.30) and a HR of 2.02 (95% CI 0.81–5.03) after multivariable adjustment for potential confounding factors. Coffee consumption was not associated with increased risk of rectal cancer in men or women.

To control for the potential impact of subclinical symptoms of colorectal cancer at the baseline survey, we repeated the analysis by excluding cases occurring within two years after the baseline survey. Results after exclusion still showed a significant increase in the risk of colon cancer associated with increasing levels of coffee consumption among men (*P* for trend = 0.037): compared to men who consumed less than 1 cup of coffee per day, men who consumed 2–3 cups had an adjusted HR of 1.24 (95% CI 0.90–1.71), and those who consumed more than 4 cups had an adjusted HR of 1.82 (95% CI 1.00–3.32). On the other hand, coffee consumption in women was not associated with increased risk of colorectal cancer (data not shown).

Although we adjusted for lifestyle risk factors known to be associated with coffee consumption, such as smoking status, drinking status, BMI, meat consumption, and age, it remains possible that these factors could perturb the incident risk of colon and rectal cancer. To clarify the effect of these potential confounders, we performed additional analyses stratified by smoking status, drinking status, BMI, meat consumption, and age (Tables 3–7).

Table 3 shows HRs for incident colorectal cancer associated with coffee consumption in subjects stratified by smoking status. Participants were classified dichotomously as current smokers or never smokers. Former smokers were excluded due to the small number of participants in this category. Table 4 shows HRs for incident colorectal cancer associated with coffee consumption in participants stratified by drinking status (current drinker or never drinker). We excluded former drinkers due to the small number of participants in this category. Table 5 shows HRs for incident colorectal cancer associated with coffee consumption in subjects stratified by BMI (<18.5 kg/m^2, 18.5 kg/m^2 to 25 kg/m^2, or >25.0 kg/m^2); Table 6 shows HRs by tertile of meat consumption; and Table 7 shows HRs by age. Consistent with the main findings in Table 2, these stratified analyses showed that coffee consumption was associated with an increased risk of colon cancer among men.

DISCUSSION

In our analysis of a large population-based prospective study, we found that coffee consumption increased the risk of colon cancer among Japanese men. In contrast to these results, coffee consumption by women was not associated with incident risk of colon cancer. We used the data from a large-scale cohort study in Japan. Data for the analysis of coffee drinking frequency at baseline included 687 cases of colon cancer and 317 cases of rectal cancer.

Table 4. Hazard ratio for colorectal cancer incidence for analysis of coffee consumption, stratified by sex and drinking status

Coffee consumption	Men						Women					
	Drinker			Non-drinker			Drinker			Non-drinker		
	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value
For colon cancer incidence												
<1 cup/day	190	1.02		27	1.00		42	1.00		193	1.00	
1 cup/day	33	1.28	0.93	6	1.01	0.98	7	0.63	0.27	30	1.03	0.88
2–3 cups/day	44	1.74	0.16	10	1.21	0.63	7	0.77	0.53	16	1.46	0.23
≥4 cups/day	8	1.68	0.13	2	0.86	0.84	2	2.68	0.20	2	1.69	0.19
P for trend			0.06			0.85			0.98			0.78
For rectum cancer incidence												
<1 cup/day	107	1.00		17	1.00		9	1.00		63	1.00	
1 cup/day	25	1.43	0.11	3	0.80	0.72	2	0.83	0.82	9	0.86	0.69
2–3 cups/day	22	1.11	0.66	5	1.16	0.78	3	1.39	0.64	13	1.74	0.08
≥4 cups/day	3	1.08	0.89	2	2.37	0.28	0			0	0.00	0.99
P for trend			0.91			0.12			0.83			0.25
For colorectal cancer incidence												
<1 cup/day	297	1.00		44	1.00		51	1.00		256	1.00	
1 cup/day	58	1.16	0.30	9	0.95	0.89	9	0.66	0.26	39	0.96	0.83
2–3 cups/day	66	1.22	0.17	15	1.19	0.58	10	0.89	0.74	29	1.10	0.64
≥4 cups/day	11	1.49	0.20	4	1.33	0.61	2	1.99	0.37	2	1.04	0.95
P for trend			0.08			0.49			0.91			0.69

HR, hazard ratio.
[a]Hazard ratio was adjusted for age, smoking, family history of colorectal cancer, education, body mass index, walking time, and regular meat consumption.

Table 5. Hazard ratio for colorectal cancer incidence for analysis of coffee consumption, stratified by sex and BMI status

Coffee consumption	Men									Women								
	BMI < 18.5			18.5 ≤ BMI < 25.0			25.0 ≤ BMI			BMI < 18.5			18.5 ≤ BMI < 25.0			25.0 ≤ BMI		
	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value
For colon cancer incidence																		
<1 cup/day	6	1.00		177	1.00		48	1.00		16	1.00		163			57	1.00	
1 cup/day	3	3.34	0.10	33	1.06	0.75	8	1.04	0.93	2	0.73	0.68	31	0.97	0.86	11	1.25	0.52
2–3 cups/day	6	5.06	0.01	38	1.14	0.48	10	1.05	0.89	2	0.89	0.88	14	0.64	0.12	8	1.35	0.45
≥4 cups/day	2	5.88	0.05	8	1.64	0.18	3	1.92	0.29	0			4	2.12	0.15	0		
P for trend			0.01			0.20			0.48			0.69			0.49			0.59
For rectum cancer incidence																		
<1 cup/day	6	1.00		99			25	1.00		5	1.00		52	1.00		16	1.00	
1 cup/day	1	1.49	0.72	23	1.43	0.13	2	0.42	0.25	1	1.42	0.77	11	0.82	1.08	1	0.49	0.50
2–3 cups/day	1	0.76	0.81	25	1.43	0.13	3	0.46	0.22	1	1.83	0.60	11	0.31	1.42	5	3.34	0.04
≥4 cups/day	0			5	1.94	0.16	0			0			0			0		
P for trend			0.74			0.06			0.11			0.62			0.61			0.08
For colorectal cancer incidence																		
<1 cup/day	12	1.00		276	1.00		73	1.00		21	1.00		215	1.00		73	1.00	
1 cup/day	4	2.40	0.14	56	1.19	0.24	10	0.82	0.56	3	0.80	0.72	42	0.99	0.97	12	1.11	0.74
2–3 cups/day	7	2.86	0.04	63	1.25	0.13	13	0.82	0.54	3	1.07	0.92	25	0.84	0.42	13	1.79	0.07
≥4 cups/day	2	3.64	0.12	13	1.74	0.06	3	1.13	0.84	0			4	1.51	0.42	0		
P for trend			0.03			0.03			0.73			0.87			0.75			0.16

BMI, body mass index; HR, hazard ratio.
[a]Hazard ratio was adjusted for age, smoking, drinking, family history of colorectal cancer, education, walking time, and regular meat consumption.

The reason for the different results between sexes is unclear but might be at least partially attributable to residual confounding effects in men. Most subjects who consumed a large amount of coffee were smokers, and the proportion of smokers among men was higher than that among women (Table 1). Although we adjusted for lifestyle risk factors, such as smoking, drinking, and meat consumption, the effects of these lifestyle risk factors might not have been completely removed due to their strong associations with coffee consumption. One way to account for the influence of these factors is to analyze the groups after stratification by smoking status, BMI, meat consumption, and drinking status

Table 6. Hazard ratio for colorectal cancer incidence for analysis of coffee consumption, stratified by sex and meat consumption status

Coffee consumption	Men									Women								
	Low meat consumption			Middle meat consumption			High meat consumption			Low meat consumption			Middle consumption			High meat consumption		
	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value
For colon cancer incidence																		
<1 cup/day	75	1.00		69	1.00		35	1.00		70	1.00		67			50	1.00	
1 cup/day	14	1.12	0.71	12	0.77	0.41	12	1.64	0.15	10	1.01	0.97	16	1.00	0.99	11	0.99	0.98
2–3 cups/day	17	1.25	0.42	18	0.91	0.72	13	2.01	0.04	5	0.82	0.67	7	0.63	0.25	11	1.17	0.65
≥4 cups/day	2	1.03	0.97	5	1.53	0.37	4	2.98	0.05	3	7.19	0.00	1	1.76	0.58	1	0.74	0.77
P for trend			0.51			0.79			0.01			0.25			0.41			0.82
For rectum cancer incidence																		
<1 cup/day	46	1.00		40	1.00		24	1.00		29	1.00		17	1.00		12	1.00	
1 cup/day	6	0.79	0.60	10	1.221	0.58	8	1.90	0.12	6	1.239	0.64	5	1.119	0.83	2	0.71	0.66
2–3 cups/day	7	0.88	0.77	12	1.122	0.74	8	1.94	0.13	3	0.872	0.83	7	1.905	0.18	2	0.89	0.89
≥4 cups/day	3	2.51	0.14	1	0.599	0.62	1	1.25	0.83	0			0			0		
P for trend			0.54			0.99			0.22			0.68			0.31			0.61
For colorectal cancer incidence																		
<1 cup/day	121	1.00		109	1.00		59	1.00		99	1.00		84	1.00		62	1.00	
1 cup/day	20	1.00	0.99	22	0.93	0.77	20	1.78	0.03	16	1.08	0.78	21	1.02	0.92	13	0.96	0.89
2–3 cups/day	24	1.12	0.62	30	0.98	0.94	21	1.98	0.01	8	0.85	0.66	14	0.94	0.83	13	1.14	0.69
≥4 cups/day	5	1.59	0.32	6	1.21	0.65	5	2.36	0.08	3	3.89	0.03	1	1.12	0.91	1	0.58	0.60
P for trend			0.36			0.84			0.01			0.49			0.89			0.96

HR, hazard ratio.
[a]Hazard ratio was adjusted for age, smoking, drinking, family history of colorectal cancer, education, body mass index, and walking time.

Table 7. Hazard ratio for colorectal cancer incidence for analysis of coffee consumption, stratified by sex and age

Coffee consumption	Men						Women					
	40–59 years			60–79 years			40–59 years			60–79 years		
	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value	Number of cases	HR[a]	*P* value
For colon cancer incidence												
<1 cup/day	103	1.00		137	1.00		75	1.00		179	1.00	
1 cup/day	22	0.91	0.70	22	1.11	0.65	20	0.91	0.72	26	0.91	0.64
2–3 cups/day	31	0.86	0.46	27	1.52	0.05	14	0.63	0.12	13	0.81	0.48
≥4 cups/day	9	1.24	0.55	4	1.73	0.28	3	0.98	0.98	2	2.80	0.15
P for trend			0.89			0.03			0.21			0.84
For rectum cancer incidence												
<1 cup/day	52	1.00		87	1.00		30	1.00		52	1.00	
1 cup/day	18	1.57	0.10	10	0.82	0.55	7	0.93	0.87	6	0.74	0.49
2–3 cups/day	21	1.27	0.37	9	0.81	0.54	11	1.51	0.26	6	1.38	0.46
≥4 cups/day	4	1.30	0.62	1	0.73	0.75	0	0.00	0.99	0	0.00	0.99
P for trend			0.41			0.47			0.62			0.68
For colorectal cancer incidence												
<1 cup/day	155	1.00		224	1.00		105	1.00		231	1.00	
1 cup/day	40	1.13	0.50	32	1.00	1.00	27	0.92	0.69	32	0.87	0.47
2–3 cups/day	52	0.99	0.95	36	1.24	0.24	25	0.86	0.51	19	0.94	0.78
≥4 cups/day	13	1.27	0.42	5	1.36	0.50	3	0.76	0.64	2	2.25	0.26
P for trend			0.70			0.19			0.44			0.98

HR, hazard ratio.
[a]Hazard ratio was adjusted for smoking, drinking, family history of colorectal cancer, education, body mass index, walking time, and regular meat consumption.

(Tables 3–7). Consistent with the findings from the primary analysis (Table 2), stratified results showed that coffee consumption was associated with an increased risk of colon cancer among men.

Although many studies in various populations have examined the association between coffee consumption and colorectal cancer, epidemiologic evidence for an effect has been inconsistent. In fact, a 2007 report by the World Cancer Research Fund and the American Institute for Cancer Research determined that no firm conclusions on this association could be reached because of inconsistent epidemiologic evidence. Several case-control studies have

reported null associations between coffee consumption and colorectal cancer risk.[19–23] In contrast, other case-control studies reported a modest but statistically significant decrease in colon cancer risk,[24–26] whereas one study reported a statistically significant increase in risk among men.[27] Epidemiologic evidence from cohort studies has been inconsistent.[3–13] While most previous cohort studies were conducted in Western countries, several cohort studies in Asia have been conducted, including several major cohort studies on the risk of colorectal cancer from Japan.[11–13] However, results from these studies were also inconsistent. The JPHC Cohort Study and the Takayama Cohort Study suggested that coffee consumption may lower the risk of colon cancer in women,[11,13] whereas the Miyagi Cohort Study concluded that consumption is not associated with risk of colorectal cancer in either sex.[12] Elsewhere in Asia, the Singapore Chinese Health Study found a null association between coffee intake and risk of colorectal cancer overall but also found that consumption may protect against smoking-related advanced colon cancer.[28]

A few recent meta-analyses of prospective studies have shown rather wide discrepancies in findings. Je et al confirmed that coffee drinking is not associated with colorectal cancer risk.[14] On the contrary, Yu et al analyzed results from 15 worldwide cohorts and reported that consumption had a significant inverse association with colorectal cancer risk.[29] Further, Li et al reported that coffee consumption significantly decreased the risks of colorectal cancer and colon cancer, especially in Europe and for females.[15] In a pooled analysis of prospective cohort studies, Zhang et al found no association between coffee consumption and colon cancer.[30] Contrary to our expectation, our results showed that coffee consumption increased the risk of colon cancer among men. This observation may be due to the complex biological effects of coffee.

The biological mechanisms of coffee's effects have been examined in many studies.[31–33] Roasted coffee is a complex mixture of more than a thousand chemicals. Coffee intake may increase colonic motility, thereby decreasing the exposure of epithelial cells to potential carcinogens in the colon.[34] Also, coffee consumption may reduce the synthesis and secretion of bile acids, which are known to be potential promoters of carcinogenesis.[35] Thus, these complex compounds in coffee with their various effects may explain the lack of association or the inconsistent results observed to date.

Our study has several limitations. First, data were collected at the baseline survey only, and the consumption of coffee was assessed by self-report. Thus, some measurement error at baseline was inevitable. Second, we did not collect details of coffee consumption, such as the use of caffeinated or decaffeinated coffee, and the method of coffee preparation (eg filtered or boiled). Although coffee preparation and consumption habits may change considerably over time and vary seasonally and geographically, our present and previous published studies lack these considerations. These limitations may have biased or produced inconsistencies in the epidemiologic evidence for the effect of coffee on the incidence of colorectal cancer, and further detailed cohort studies which take account of these points are required.

In conclusion, a large-scale population-based cohort study showed that coffee consumption increases the risk of colon cancer among Japanese men. In contrast to these results, coffee consumption by women is not associated with the incident risk of colorectal cancer.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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Polymorphisms of genes involved in lipid metabolism and risk of chronic kidney disease in Japanese - cross-sectional data from the J-MICC study

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Abstract

Background: Chronic kidney disease (CKD) is known to be one of the causes of cardiovascular disease and end-stage renal disease. Among the several treatable risk factors of CKD, that of dyslipidemia is relatively controversial. To clarify the association of polymorphisms in genes involved in lipid metabolism with the risk of CKD in the Japanese population, we used cross-sectional data from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study.

Methods: A total of 3,268 men and women, aged 35–69 years, were selected from J-MICC Study participants for inclusion in this study. Twenty-eight candidate single nucleotide polymorphisms (SNPs) were selected in 17 genes associated with the risk of lipid metabolism disorders, and genotyping of the subjects was conducted using the multiplex PCR-based invader assay. The prevalence of CKD was determined for stages 3–5 (defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2).

Results: Logistic regression analysis revealed that SNPs *APOA5* T – 1131C (rs662799), *APOA5* T1259C (rs2266788), *TOMM40* A/G (rs157580), and *CETP* TaqIB (rs708272) were significantly associated with CKD risk in those individuals genotyped, with age- and sex-adjusted odds ratios (ORs) per minor allele (and 95% confidence intervals (CIs)) of OR 1.22 (95% CI: 1.06–1.39), 1.19 (1.03–1.37), 1.27 (1.12–1.45), and 0.81 (0.71–0.92), respectively. Analysis of the gene–environment interaction revealed that body mass index (BMI) was a significant effect modifier for *APOA5* T – 1131C (rs662799) and a marginally significant effect modifier for *APOA5* T/C (rs2266788), with the interaction between BMI ≥30 and individuals with at least one minor allele of each genotype of OR 10.43 (95% CI: 1.29–84.19) and 3.36 (0.87–13.01), respectively.

Conclusions: Four polymorphisms in *APOA5*, *TOMM40*, and *CETP* were shown to be significantly associated with CKD risk, and a significant interaction between the two *APOA5* SNPs and BMI on CKD risk was also demonstrated. This suggests the future possibility of personalized risk estimation for this life-limiting disease.

Keywords: Lipid metabolism, Chronic kidney disease, Single nucleotide polymorphism

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Background

Chronic kidney disease (CKD) is emerging as a major public health and financial burden worldwide, and the number of affected patients is increasing in East Asian countries such as Japan, where more than 10 million people currently have CKD stage ≥3. CKD is also known to be a cause of cardiovascular disease (CVD) and end-stage renal disease, so prevention of this potentially life-limiting disease is becoming a pressing issue [1]. CKD has a number of treatable risk factors, such as diabetes mellitus, hypertension, glomerular nephritis, and dyslipidemia [2]. Of these, the effect of dyslipidemia on human CKD risk is relatively controversial, although substantial evidence from animal models is supportive of this association [3,4], suggesting that dyslipidemia plays an important role in the development and progression of CKD. Additionally, data from 4,483 healthy men participating in the Physician's Health Study showed that elevated total cholesterol, high non-high-density lipoprotein (HDL) cholesterol, a high ratio of total cholesterol to HDL cholesterol, and low HDL cholesterol in particular were significantly associated with an increased risk of developing renal dysfunction with an initial creatinine level <1.5 mg/dl [5]. Some other reports also suggested that blood lipids modify the decline in renal function as well as hypertension [6,7].

In 2005, we launched the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, a large genome cohort study to confirm and detect gene–environment interactions in lifestyle-related diseases, particularly cancer [8,9]. Considering the potentially important roles of lipid metabolisms in the etiology of CKD, we hypothesized that genetic polymorphisms modulating lipid metabolizing pathways would also affect CKD risk in humans. Accordingly, to clarify the association of polymorphisms in genes involved in lipid metabolism with CKD risk, we examined this in Japanese subjects using the cross-sectional data of the J-MICC Study.

Results

Subject characteristics and allele frequencies of genes involved in lipid metabolism

Subject characteristics are summarized by CKD status in Table 1. The mean age ± standard deviation was 56.6 ± 8.6 years, and 48.6% of all subjects were men. Subjects with CKD accounted for 17.3% (564/3,268) of the entire study population. Estimated glomerular filtration rate (eGFR), age, systolic blood pressure, total cholesterol, uric acid, use of anti-hypertensive or lipid-lowering drugs, history of CVD or cerebrovascular disease, and current smokers were all significantly different between subjects with and without CKD.

The genotype frequencies included in the analyses were in accordance with Hardy–Weinberg equilibrium (HWE), except for the following: apolipoprotein A5 gene (*APOA5*) G553T (Cys185Gly, rs2075291) (*T* allele = 0.066, $\chi^2 = 4.791$, $P = 0.029$), apolipoprotein E gene (*APOE*) T471C (Cys112Arg, rs429358) (*C* allele = 0.099, $\chi^2 = 6.833$, $P = 0.009$), *APOE* T – 219G (rs405509) (*G* allele = 0.307, $\chi^2 = 8.666$, $P = 0.003$), and the translocase of outer mitochondrial membrane 40 homolog gene (*TOMM40*) A/G (rs157580) (*G* allele = 0.473, $\chi^2 = 6.626$, $P = 0.010$). The genotype call rate was more than 99.6% for all individuals with serum creatinine (SCr) data ($n = 3,326$).

Polymorphisms involved in lipid metabolism and risk of CKD

The potential confounders tested did not fulfill the significance criteria of change in estimate (CIE) >0.1 (10%), so we adopted the odds ratios (ORs) adjusted only for age and sex. Logistic regression analysis revealed that *APOA5* T – 1131C (rs662799), *APOA5* T1259C (rs2266788), *TOMM40* A/G (rs157580), and cholesterol ester transfer protein gene (*CETP*) TaqIB (rs708272) were significantly associated with the risk of CKD, with age- and sex-adjusted ORs (aORs) and 95% confidence intervals (95% CIs) of aOR 1.22 (95% CI: 1.06–1.39), 1.19 (1.03–1.37), 1.27 (1.12–1.45), and 0.81 (0.71–0.92), respectively (Table 2). Because the two *APOA5* SNPs (rs662799 and rs2266788) are reported to be closely linked, we also conducted haplotype analysis for these loci. This confirmed that the two SNPs were tightly linked, with linkage disequilibrium (LD) coefficients $D' = 0.99$, $r^2 = 0.72$, while the *C-C* haplotype was found to be significantly associated with an increased risk of CKD (aOR 1.18 (95% CI: 1.02–1.36)) (Additional file 1: Table S1). We also conducted the analysis of serum lipid levels according to these genotypes found to be significant, as it may provide important information about the possible underlying mechanisms for the associations found (Additional file 2: Table S2).

To detect the lifestyle factors involved in gene–environment interactions with the genes significantly associated with CKD risk, we evaluated the interaction term for effect measure modification using the Breslow–Day (B–D) test of homogeneity with $\alpha = 0.05$. Body mass index (BMI) was extracted as the only covariate that significantly contributed to the outcome prediction. Gene–environment interactions were then assessed by the logistic model incorporating a multiplicative interaction term, which revealed that BMI was a significant effect modifier for *APOA5* T – 1131C (rs662799) and a marginally significant effect modifier of *APOA5* T1259C (rs2266788). The interaction between high BMI (≥30) and individuals with at least one minor allele of each SNP was OR 10.43 (95% CI: 1.29–84.19) and 3.36 (0.87–13.01), respectively. Stratified analyses of the CKD risk for the *APOA5* T – 1131C (rs662799) SNP by BMI resulted in a strikingly higher OR for individuals with at least one *C* allele of *APOA5* T – 1131C (rs662799) when only those

Table 1 Comparison of characteristics between subjects with and without CKD (n = 3,268)

	CKD (+) (n = 564)	CKD (-) (n = 2,704)	*P*
CKD stage 3 (eGFR < 60 ml/min/1.73m^2)	561 (99.5%)	-	
Stage 4 (eGFR < 30)	0 (0%)	-	-
Stage 5 (eGFR < 15)	3 (0.5%)	-	
eGFR (ml/min/1.73m^2)	53.7 ± 6.0	78.3 ± 12.5	< 0.001
Age (years)	60.4 ± 7.2	55.9 ± 8.7	< 0.001
Male	260 (46.1%)	1,327 (49.1%)	0.198
Body mass index	23.5 ± 3.1	23.4 ± 3.3	0.413
Systolic blood pressure (mm Hg)	130.1 ± 19.8	128.1 ± 19.4	0.029
Diastolic blood pressure (mm Hg)	78.8 ± 12.4	78.6 ± 11.9	0.746
Use of anti-hypertensive drugs	149 (26.4%)	486 (18.0%)	< 0.001
Fasting plasma glucose (mg/dL)	98.8 ± 22.1	100.0 ± 20.8	0.291
HbA1c (%)	5.22 ± 0.70	5.22 ± 0.66	0.942
Use of glucose-lowering drugs	31 (5.5%)	110 (4.1%)	0.129
Total cholesterol (mg/dL)	218.2 ± 34.0	211.1 ± 33.9	< 0.001
HDL cholesterol (mg/dL)	62.0 ± 15.9	63.3 ± 16.3	0.071
Triglyceride (mg/dL)*	106 (76.5-151)	104 (74-154)	0.992
Use of lipid-lowering drugs	70 (12.4%)	225 (8.3%)	0.002
Uric acid (mg/dL)	5.53 ± 1.47	5.10 ± 1.33	< 0.001
History of cardiovascular diseases	34 (6.0%)	80 (3.0%)	<0.001
History of cerebrovascular diseases	29 (5.1%)	52 (1.9%)	< 0.001
Current smokers	69 (12.2%)	484 (17.9%)	0.001
Current drinkers	295 (52.3%)	1,510 (55.8%)	0.138

Values are expressed as means ± standard deviation, n (%), or median (interquartile range). CKD: chronic kidney disease. CKD was defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2.
*Non-fasting.

individuals with BMI ≥30 were included (OR 12.39 (95% CI: 1.55–99.09); Table 3). Additionally, we conducted an exhaustive investigation of the gene–environment interactions for all SNPs tested, which revealed several statistically significant interactions in addition to the one described above (Additional file 3: Table S3).

Discussion

The present study examined the associations between polymorphisms in genes involved in lipid metabolism and the risk of CKD in the Japanese population, and identified a total of four SNPs (two in *APOA5* (T – 1131C [rs662799] and T1259C [rs2266788]), *TOMM40* A/G (rs157580), and *CETP* TaqIB (G > A) (rs708272)) that were significantly associated with CKD risk. While the functional implications of some of these polymorphisms are well-known, others have not yet been sufficiently clarified.

The *APOA5* SNP T – 1131C (rs662799) is located in the *APOA5* promoter region and is thought to modulate gene expression, with minor allele carriers found to have high triglyceride levels. It is also reported to be in strong LD with the *APOA5* SNP rs2266788 [10,11]. There exist a considerable number of reports supporting these effects of *APOA5* SNPs on blood triglyceride levels [10,12,13], and given the potentially important roles of blood triglyceride concentrations in the development of human CKD [13,14], the modulation of blood triglyceride levels due to these *APOA5* SNPs may contribute to the genesis of CKD in humans. The result of our haplotype analysis of *APOA5* SNPs (rs662799 and rs2266788) suggested the potentially substantial role of rs662779. As this rs662779 (T-1131C) SNP in *APOA5* is located in the promoter region of the *APOA5* gene, it is speculated to modulate the expression of APOA5, although further biological investigations will be required to confirm it.

TOMM40 is located within 15 kb of *APOE*, and this is reflected by the strong LD of *TOMM40* polymorphisms, including SNP rs157580, with the *ε4* allele. *TOMM40* polymorphisms are therefore very interesting targets to study in association with human disorders such as Alzheimer's disease [15]. A recent genome-wide association study revealed that the *TOMM40* SNP rs157580 was significantly associated with low triglyceride levels [16], although another study reported a significant association

Table 2 Polymorphisms in lipid metabolizing genes and risk of CKD

Polymorphism	Genotype	CKD (+) (n = 564)	CKD (-) (n = 2,704)	Per allele aOR (95% CI)*	*P*
APOA1 Ala61Thr (G219A)	*G/G*	506 (89.7%)	2,423 (89.6%)		
(rs12718465)	*A/G*	56 (9.9%)	268 (9.9%)	0.98 (0.74-1.31)	0.901
	A/A	2 (0.4%)	13 (0.5%)		
APOA5 G553T (Cys185Gly)	*G/G*	483 (85.6%)	2,375 (87.8%)		
(rs2075291)	*G/T*	79 (14.0%)	309 (11.4%)	1.12 (0.87-1.43)	0.378
	T/T	2 (0.4%)	20 (0.7%)		
APOA5 T-1131C	*T/T*	221 (39.2%)	1,197 (44.3%)		
(rs662799)	*C/T*	258 (45.7%)	1,183 (43.8%)	1.22 (1.06-1.39)	*0.004*
	C/C	85 (15.1%)	324 (12.0%)		
APOA5 C/A	*C/C*	210 (37.2%)	1,028 (38.0%)		
(rs6589567)	*C/A*	264 (46.8%)	1,241 (45.9%)	1.03 (0.90-1.17)	0.684
	A/A	90 (16.0%)	435 (16.1%)		
APOA5 T1259C	*T/T*	282 (50.0%)	1,437 (53.1%)		
(rs2266788)	*T/C*	224 (39.7%)	1,059 (39.2%)	1.19 (1.03-1.37)	*0.019*
	C/C	58 (10.3%)	208 (7.7%)		
APOB A/G	*A/A*	308 (54.6%)	1,421 (52.6%)		
(rs673548)	*A/G*	210 (37.2%)	1,056 (39.1%)	0.95 (0.82-1.10)	0.490
	G/G	46 (8.2%)	227 (8.4%)		
APOE Arg158Cys (C609T)	*C/C*	523 (92.7%)	2,472 (91.4%)		
(rs7412)	*C/T*	40 (7.1%)	226 (8.4%)	0.82 (0.58-1.15)	0.255
	T/T	1 (0.2%)	6 (0.2%)		
APOE Cys112Arg (T471C)	*T/T*	469 (83.2%)	2,200 (81.4%)		
(rs429358)	*T/C*	88 (15.6%)	466 (17.2%)	0.91 (0.73-1.13)	0.388
	C/C	7 (1.2%)	38 (1.4%)		
APOE T-219G	*T/T*	290 (51.4%)	1,317 (48.7%)		
(rs405509)	*T/G*	228 (40.4%)	1,090 (40.3%)	0.90 (0.78-1.03)	0.124
	G/G	46 (8.2%)	297 (11.0%)		
APOE cluster A/G	*A/A*	452 (80.1%)	2,155 (79.7%)		
(rs4420638)	*A/G*	104 (18.4%)	509 (18.8%)	0.97 (0.79-1.20)	0.798
	G/G	8 (1.4%)	40 (1.5%)		
TOMM40 A/G	*A/A*	118 (20.9%)	825 (30.5%)		
(rs157580)	*A/G*	299 (53.0%)	1,257 (46.5%)	1.27 (1.12-1.45)	*<0.001*
	G/G	147 (26.1%)	622 (23.0%)		
HMGCR G/A	*G/G*	163 (28.9%)	736 (27.2%)		
(rs3846662)	*G/A*	278 (49.3%)	1,326 (49.0%)	0.91 (0.80-1.04)	0.162
	A/A	123 (21.8%)	642 (23.7%)		
LPL G/A	*G/G*	366 (64.9%)	1,797 (66.5%)		
(rs331)	*G/A*	177 (31.4%)	816 (30.2%)	1.04 (0.88-1.22)	0.681
	A/A	21 (3.7%)	91 (3.4%)		
LPL G1791C (Ser474Stop)	*G/G*	423 (75.0%)	2,083 (77.0%)		
(rs328)	*G/C*	131 (23.2%)	579 (21.4%)	1.07 (0.89-1.30)	0.466
	C/C	10 (1.8%)	42 (1.6%)		

Table 2 Polymorphisms in lipid metabolizing genes and risk of CKD *(Continued)*

NR1H3 G/A	*G/G*	301 (53.4%)	1,512 (55.9%)		
(rs7120118)	*G/A*	224 (39.7%)	1,027 (38.0%)	1.12 (0.97-1.31)	0.125
	A/A	39 (6.9%)	165 (6.1%)		
NR1H3 A/G	*A/A*	299 (53.0%)	1,524 (56.4%)		
(rs2167079)	*A/G*	227 (40.2%)	1,017 (37.6%)	1.14 (0.99-1.33)	0.078
	G/G	38 (6.7%)	163 (6.0%)		
MTNR1B A/G	*A/A*	250 (44.3%)	1,245 (46.0%)		
(rs1447352)	*A/G*	247 (43.8%)	1,176 (43.5%)	1.06 (0.92-1.22)	0.392
	G/G	67 (11.9%)	283 (10.5%)		
FADS2 C/T	*C/C*	227 (40.2%)	979 (36.2%)		
(rs174570)	*C/T*	250 (44.3%)	1,266 (46.8%)	0.91 (0.80-1.04)	0.167
	T/T	87 (15.4%)	459 (17.0%)		
KCNJ11 A1577G (Ile337Val)	*A/A*	240 (42.6%)	1,068 (39.5%)		
(rs5215)	*A/G*	241 (42.7%)	1,274 (47.1%)	0.95 (0.83-1.09)	0.485
	G/G	83 (14.7%)	362 (13.4%)		
TMEM57 A/G	*A/A*	256 (45.4%)	1,186 (43.9%)		
(rs10903129)	*A/G*	254 (45.0%)	1,234 (45.6%)	0.90 (0.78-1.04)	0.168
	G/G	54 (9.6%)	284 (10.5%)		
DOCK7 A/C	*A/A*	347 (61.5%)	1,542 (57.0%)		
(rs1167998)	*A/C*	188 (33.3%)	996 (36.8%)	0.86 (0.74-1.01)	0.061
	C/C	29 (5.1%)	166 (6.1%)		
CELSR2 C/T	*C/C*	516 (91.5%)	2,413 (89.2%)		
(rs4970834)	*C/T*	46 (8.2%)	280 (10.4%)	0.79 (0.58-1.07)	0.133
	T/T	2 (0.4%)	11 (0.4%)		
LIPC Val95Met (G340A)	*G/G*	342 (60.6%)	1,616 (59.8%)		
(rs6078)	*G/A*	193 (34.2%)	945 (34.9%)	0.96 (0.82-1.13)	0.643
	A/A	29 (5.1%)	143 (5.3%)		
LIPC T-514C	*T/T*	132 (23.4%)	731 (27.0%)		
(rs1800588)	*T/C*	290 (51.4%)	1,353 (50.0%)	1.10 (0.97-1.26)	0.143
	C/C	142 (25.2%)	620 (22.9%)		
CETP TaqIB (G > A)	*G/G*	234 (41.5%)	934 (34.5%)		
(rs708272)	*G/A*	251 (44.5%)	1,315 (48.6%)	0.81 (0.71-0.92)	*0.002*
	A/A	79 (14.0%)	455 (16.8%)		
CETP G/T	*G/G*	372 (66.0%)	1,692 (62.6%)		
(rs3764261)	*G/T*	170 (30.1%)	872 (32.2%)	0.86 (0.73-1.01)	0.068
	T/T	22 (3.9%)	140 (5.2%)		
CETP Ile405Val (G > A)	*G/G*	147 (26.1%)	753 (27.8%)		
(rs5882)	*G/A*	277 (49.1%)	1,312 (48.5%)	1.06 (0.93-1.20)	0.403
	A/A	140 (24.8%)	639 (23.6%)		
CETP A-629C	*A/A*	162 (28.7%)	825 (30.5%)		
(rs1800775)	*A/C*	279 (49.5%)	1,366 (50.5%)	1.10 (0.96-1.25)	0.174
	C/C	123 (21.8%)	513 (19.0%)		

*aOR: adjusted odds ratio (adjusted for age and sex); 95% CI: 95% confidence interval; CKD: chronic kidney disease.

Table 3 Stratified analyses for the CKD risk associated with *APOA5* polymorphisms by BMI levels

Proportion of minor heterozygous plus homozygous (among subjects with all genotypes)		CKD (+) (n = 564)		CKD (-) (n = 2,704)		OR*	95% CI*	$P_{interaction}$ #
Genotype	BMI	N	(%)	N	(%)			
APOA5 T-1131C								
(rs662799)								
	≥ 30	15/16	(93.8)	54/95	(56.8)	12.39	1.55-99.09	
	< 30	328/548	(59.9)	1,453/2,609	(55.7)	1.21	0.999-1.47	0.028
APOA5 T1259C								
(rs2266788)								
	≥ 30	13/16	(81.3)	50/95	(52.6)	3.68	0.97-13.93	
	< 30	269/548	(49.1)	1,217/2,609	(46.6)	1.14	0.95-1.38	0.079

*aOR: adjusted odds ratio (adjusted for age and sex); 95% CI: 95% confidence interval.
#OR for interaction =10.43 (95% CI: 1.29-84.19) for *APOA5* rs662799, and 3.36 (95% CI: 0.87-13.01) for *APOA5* rs2266788.

between the *TOMM40* rs157580 minor (*G*) allele and increased levels of triglycerides in the Chinese population [17]. Given that population-specific effects appear to exist between different ethnicities in East Asian countries for the same polymorphism [18], our present finding may provide valuable information for future genetic investigations and help prevent publication bias [19].

The *CETP* TaqI B polymorphism was previously shown to be associated with an effect on HDL cholesterol concentrations [20], as well as subsequent CVD risk [21], which is thought to result from LD between this SNP and an as yet unknown functional mutation in the regulatory region of *CETP* [22]. The functional roles of this *CETP* SNP in the regulation of human blood cholesterol levels have been well established by a number of previous studies [23,24]. Taking into considerations the important roles of blood cholesterol levels in the risk of renal dysfunction, this *CETP* SNP is considered to be involved in the CKD development through the modulation of blood cholesterol concentrations.

To date, only a few associations between SNPs in lipid metabolizing genes and CKD risk have been reported, with recent studies reporting a role for apolipoprotein L1 variants in the risk and progression of CKD in African American populations [25,26], and associations of the apolipoprotein A1 gene (*APOA1*) and *APOA5* with CKD risk. Associations between the four SNPs and CKD risk have not previously been reported, so this study provides novel evidence for the effect of genetic variations in these genes involved in lipid metabolism and CKD risk. Moreover, a previous significant association observed between *APOE* rs405509 and CKD risk was not replicated in the present study. The associations of some, but not all, of the SNPs in our study with CKD followed a similar trend to that previously reported for CVD [27,28], which might be expected given that CKD is considered to be a form of CVD. The differences could reflect the existence of etiologies specific to each vascular disease, and different gene–environment or gene–gene interactions between races/ethnicities. Nevertheless, the present research appears to confirm the previously reported findings of the possible influence of lipid disorders on the risk of CKD in humans [3,22].

SNPs shown to be marginally significant in the present study, liver X receptor-alpha gene (*NRIH3*) rs2167079, dedicator of cytokinesis 7 gene (*DOCK7*) rs1167998, and *CETP* rs70827, suggest a possible involvement of these genes in CKD development. NRIH3 inhibits cholesterol absorption, while CETP mediates the exchange of lipids between lipoproteins, resulting in the net transfer of cholesteryl ester from HDL cholesterol to other lipoproteins. Considering the important roles of these genes in lipid metabolism and subsequent CKD onset, the involvement of their functional polymorphisms in CKD risk seems biologically plausible. However, their marginal significance could have been detected as a result of a type I error, which necessitates further investigation with sufficiently larger sample sizes. The remaining SNPs found not to be associated with CKD risk may not play a major role in CKD development, thus discouraging us from their further investigation.

One of the most marked as well as important findings of the present study is the significant interaction between the *APOA5* SNP and BMI on CKD risk, considering its possible future application in the personalized prevention of CKD. BMI can be regarded as a convenient proxy for energy intake and consumption, as demonstrated by the association between dietary fat and obesity [29], which was represented in a study of the interaction between a polymorphism in the *nitric oxide synthase 3 (NOS3)* gene and BMI on the risk of type 2 diabetes [30] and in other studies [18]. *APOA5* polymorphisms have previously been reported to be associated with elevated serum triglyceride levels by several studies [10,12,13], and an interaction between the *APOA5* polymorphism and BMI on high serum triglyceride levels was reported in the East Asian

population [18]. Taking these findings into consideration, we speculate that the synergistic effect of obesity and dyslipidemia caused by *APOA5* polymorphisms may confer the increased risk of CKD.

Our additional exhaustive investigations of the gene-environment interactions using all polymorphisms tested revealed several statistically significant associations. Although we didn't take all these interactions into further considerations in the present study, these findings may suggest the way for our future investigations.

The present study has several potential limitations. Serum lipid levels, such as cholesterol and/or triglyceride, could be considered as covariates to be adjusted; however, they can also be regarded as causal intermediates that link the polymorphisms involved in lipid metabolism and CKD risk. Therefore, because adjusting for even a partially causal intermediate phenotype would incorrectly remove a true association and potentially bias the true association [31], we chose not to adjust for these variables in this study. Second, although the genotype frequencies of some of the SNPs investigated significantly deviated from HWE, the actual differences in the number of subjects for all genotypes compared with that expected from the equilibrium were small (up to 2.5%), or the deviation was caused by the relatively small frequency of the minor allele (<10%). Increasing the sample size may have resulted in more robust findings, but it was not easy to do this because of study design constraints. Third, we chose not to adopt the correction of multiple comparisons by Bonferroni procedures because this study was conducted under an exploratory context, and because such adjustments can be regarded as too conservative [32]. Fourth, all CKD cases were diagnosed from SCr data, which potentially differ from the actual GFR based on renal measurements, so could have diluted the effect of each genotype on CKD risk. Finally, albuminuria was not detected in the present study. Further investigations with improved study designs are therefore required.

Conclusions

The present study found that two *APOA5* polymorphisms (T – 1131C [rs662799] and T1259C [rs2266788]), as well as *TOMM40* A/G (rs157580) and *CETP* TaqIB (G > A) (rs708272) were significantly associated with CKD risk in the Japanese population. A significant interaction between the *APOA5* T – 1131C SNP and BMI on CKD risk was also demonstrated, indicating the future possibility of personalized risk estimation for this life-limiting disease.

Methods

Study subjects

Subjects were participants of the J-MICC Study, initially conducted in 10 areas of Japan, in which around 75,000 voluntarily enrolled participants aged 35–69 years provided blood samples, health check-up data, and lifestyle data through a questionnaire after providing their written informed consent [8].

In the present analysis, 4,519 randomly selected participants (about 500 subjects from each of the 10 areas) were analyzed for whom 108 selected polymorphisms had been genotyped [9]. Of these individuals, six were excluded because of ineligibility or withdrawal from the study. SCr had been measured in 3,326 respondents from eight of the 10 areas of Japan. Of these, 58 were excluded because of genotyping failure, and the remaining 3,268 were included in the analyses. Informed consent was obtained from all subjects and the study protocol was approved by the Institutional Review Board (IRB) of Nagoya University Graduate School of Medicine (IRB approval no. 253-6) and the affiliated Medical Universities.

Evaluation of lifestyle exposure

Lifestyle exposures were evaluated by a self-administered questionnaire that was checked by trained staff. The questionnaire included items on smoking status, alcohol consumption, and medical history. Smoking status was classified as current, former, or never, and the level of exposure was evaluated in pack-years. Former smokers were defined as people who had quit smoking for at least 1 year. Alcohol consumption for each type of beverage was determined by average intake frequency and quantity, then converted into the Japanese sake unit gou (180 ml), which is equivalent to 23 g of ethanol. The participants were categorized into non-habitual drinkers, habitual drinkers who drank less than 1 gou per day, and those who drank at least 1 gou per day; the latter two groups were coded as indicator variables. Intakes of energy and macronutrients were estimated based on responses to a food frequency questionnaire (FFQ), for which its reproducibility and validity to estimate nutrient intakes had been tested and confirmed [33-36]. Correlation coefficients between the FFQ and 3-day food records were 0.49 for energy, 0.61 for % energy from fat, and 0.86 for % energy from carbohydrate in men. The corresponding figures in women were 0.44, 0.48, and 0.66, respectively [35].

eGFR and definitions of CKD

SCr was measured in all participants using an enzymatic method. The eGFR of each participant was calculated based on SCr, age, and sex using the following Japanese eGFR equation proposed by the Japanese Society of Nephrology [37]: eGFR (ml/min/1.73 m^2) = $194 \times SCr$ $(mg/dl)^{-1.094} \times age^{-0.287}$ (×0.739 if female). The prevalence of CKD was determined for CKD stages 3–5 (defined as eGFR <60 ml/min/1.73 m^2).

Selection of SNPs

We selected 28 candidate SNPs in 17 genes based on the notion that they are well characterized and reported to be associated with the risk of lipid metabolism disorders using public databases such as PubMed and Online Mendelian Inheritance in Man. The selected SNPs were as follows: *APOA1* Ala61Thr (G219A) (rs12718465), *APOA5* G553T (Cys185Gly) (rs2075291), *APOA5* T – 1131C (rs662799), *APOA5* C/A (rs6589567), *APOA5* T1259C (rs2266788), apolipoprotein B gene (*APOB*) A/G (rs673548), *APOE* Arg158Cys (C609T) (rs7412), *APOE* Cys112Arg (T471C) (rs429358), *APOE* T – 219G (rs405509), *APOE cluster* A/G (rs4420638), *TOMM40* A/G (rs157580), 3-hydroxy-3-methylglutaryl-CoA reductase gene (*HMGCR*) G/A (rs3846662), lipoprotein lipase gene (*LPL*) G/A (rs331), *LPL* G1791C (Ser474Stop) (rs328), *NR1H3* G/A (rs7120118), *NR1H3* A/G (rs2167079), melatonin receptor 1B gene (*MTNR1B*) A/G (rs1447352), fatty acid desaturase 2 gene (*FADS2*) C/T (rs174570), potassium channel, subfamily J, member 11 gene (*KCNJ11*) A1577G (Ile337Val) (rs5215), macoilin gene (*TMEM57*) A/G (rs10903129), *DOCK7* A/C (rs1167998), cadherin gene (*CELSR2*) C/T (rs4970834), hepatic lipase gene (*LIPC*) Val95Met (G340A) (rs6078), *LIPC* T – 514C (rs1800588), *CETP* TaqIB (G > A) (rs708272), *CETP* G/T (rs3764261), *CETP* Ile405Val (G > A) (rs5882), and *CETP* A – 629C (rs1800775).

Genotyping

DNA was extracted from buffy coat using a BioRobot® M48 Workstation (QIAGEN, Tokyo, Japan), or from whole blood samples using an automatic nucleic acid isolation system (NA-3000, Kurabo Industries Ltd., Osaka, Japan). Genotyping was performed by the RIKEN institute (Wako, Japan) using the multiplex PCR-based invader assay (Third Wave Technologies, Madison, WI) as described previously [38].

Statistical analysis

Differences in the distribution of each characteristic variable between individuals with and without CKD were examined by the Student's *t*-test or χ^2 test. Accordance with HWE, indicating an absence of discrepancy between genotype and allele frequencies, was determined using the χ^2 test. Logistic regression analysis was performed to estimate age- and sex-adjusted ORs and 95% CIs for CKD by genotype. All other potential confounding variables, including BMI, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, glycated hemoglobin, total cholesterol, HDL cholesterol, triglycerides, uric acid, past history of cardiovascular or cerebrovascular diseases, use of anti-hypertensive, glucose-lowering, or lipid-lowering drugs, smoking status, and drinking habit were tested [39] to determine if they produced significant CIEs, as described previously [40,41].

We next evaluated the interaction term for effect measure modification using the B–D test of homogeneity with $\alpha = 0.05$ [42]. Using the variables extracted by the process above, gene–environment interactions were assessed by the logistic model, which included a multiplicative interaction term as well as variables for genotypes, environment factors, age, and sex. Age adjustments in the analyses were performed with ages regarded as continuous variables. Analyses by genotype based on per allele model were carried out with genotypes for each polymorphism coded as ordinal–categorical variables according to the number of minor alleles. Differences of serum lipid levels by genotype were analyzed with Kruskal-Wallis test. All *P* values were two-sided, and all calculations were performed using Stata® version 10 software (StataCorp, College Station, TX).

Additional files

Additional file 1: Table S1. Estimated haplotype frequencies of *APOA5* SNPs T-1131C (rs662799) and T1259C (rs2266788) and risk of CKD.

Additional file 2: Table S2. Lipid profiles according to genotypes of *APOA5* and *TOMM40*.

Additional file 3: Table S3. Exhaustive interaction analyses for the CKD risk between all tested polymorphisms and lifestyle factors.

Abbreviations

CKD: Chronic kidney disease; HDL-C: High-density lipoprotein cholesterol; SCr: Serum creatinine; APOA5: Apolipoprotein A5; CETP: Cholesteryl ester transfer protein; LPL: Lipoprotein lipase; TOMM40: Translocase of the outer mitochondrial membrane 40; OR: Odds ratio; CI: Confidence interval.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AH did the data analysis and drafted the manuscript. KW, NH and HT designed and supervised the study. MK conducted the genotyping of the entire study subjects. MN, S. Suma, TS, SH, MH, TCT, S. Suzuki, TSK, HM, KO, IW and HU all contributed to the collection of data. All authors read and approved the final manuscript.

Authors' information

We all agree that every author is the co-first author of the paper.

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