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Folate, alcohol, *ADH1B* and *ALDH2* and colorectal cancer risk

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Abstract

Objective: There is limited evidence on the interaction by *alcohol dehydrogenase* 2 (*ADH1B*) (rs1229984) and *aldehyde dehydrogenase* 2 (*ALDH2*) (rs671) regarding the associations of alcohol and a methyl diet (low folate and high alcohol intake) with cancer risk, partly because of rare polymorphisms in Western populations.
Design: In a case–control study, we estimated the ORs and 95 % CIs to evaluate the associations of *ADH1B* and *ALDH2* genotypes with colorectal cancer (CRC) and the joint association between methyl diets and *ADH1B* and *ALDH2* polymorphisms with CRC risk using logistic regression models.
Setting: A hospital-based case–control study.
Participants: In total, 1001 CRC cases and 899 cancer-free controls admitted to two university hospitals.
Results: We found that alcohol intake increased the risk of CRC; OR (95 % CI) was 2·02 (1·41, 2·87) for ≥60 g/d drinkers compared with non-drinkers ($P_{trend} < 0·001$). The associations for two polymorphisms with CRC were not statistically significant. However, we found a potential interaction of *ALDH2* with methyl diets and CRC. We observed a 9·08-fold (95 % CI 1·93, 42·60) higher risk of CRC for low-methyl diets compared with high-methyl diets among individuals with an *A* allele of *ALDH2*, but the association was not apparent among those with *ALDH2 GG* ($P_{interaction} = 0·02$).
Conclusions: Our data support the evidence that gene–methyl diet interactions may be involved in CRC risk in East Asian populations, showing that a low-methyl diet increased the risk of CRC among individuals with an *A* allele of *ALDH2*.

Keywords
Colorectal neoplasm
Folate
Alcohol
Aldehyde dehydrogenase 2
Alcohol dehydrogenase 2

Nutrients involved in one-carbon metabolism have been studied due to their active roles in DNA synthesis, repair and methylation. Folate has been long studied as a key nutrient in one-carbon metabolism. 5-Methyl-tetrahydrofolate (THF), a predominant cytoplasmic form of folate, provides one carbon to methylate homocysteine to methionine forming S-adenosylmethionine (SAM), a universal donor of methyl groups in DNA methylation. THF, a reduced form of folate, is converted to 5,10-methylene-THF by *serine hydroxymethyl transferase*[1–3]. Because of the important role of folate in DNA methylation and synthesis, folate has been suggested to be linked to carcinogenesis.

Alcohol intake increases the risk of colorectal cancer (CRC)[4], and the metabolism of alcohol differs according to the expression of two major ethanol-metabolising genes, alcohol dehydrogenase 2 (*ADH1B*) (rs1229984) and aldehyde dehydrogenase 2 (*ALDH2*) (rs671)[5,6]. Ethanol is oxidised by the *ADH1B* (rs1229984) gene into acetaldehyde, which leads to the cleavage of folate[7]. Acetaldehyde causes cytogenic abnormalities and produces reactive oxygen and nitrogen species (RONS) as well as DNA adducts. It also alters the structure and function of proteins, including enzymes involved in DNA repair and methylation and proteins involved in anti-oxidative defense, leading to DNA

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damage and inhibition of DNA methylation[5]. Acetaldehyde is further converted to acetate by the *ALDH2* (rs671) gene[8,9]. The effect of alcohol intake on CRC risk may vary between individuals according to *ADH1B* (rs1229984) and *ALDH2* (rs671) polymorphisms, and we also hypothesised that the association between methyl-related nutrients and CRC could be modified through alcohol metabolism.

Several epidemiological studies reported the potential link between methyl diet and CRC incidence. A US cohort study reported that a combination of high alcohol and a low intake of methionine and folate was associated with an increased CRC risk[10]. Another case–control study found a positive association between low or medium folate combined with high alcohol intake and CRC, and the association was stronger for men than for women[11], whereas the Iowa Women's Health Study found no association between high folate-low alcohol intake and the incidence of CRC risk[12].

One US cohort study has reported the association between a methyl-related diet and colorectal adenoma according to the aldehyde dehydrogenase 3 (*ADH1C*) and found an interaction by this genotype[13]. Because the proportion of A alleles in the *ADH1B* (rs1229984) and *ALDH2* (rs671) genes is extremely low in Western European populations[14] (online Supplemental Fig. S1), research on polymorphisms in these genes is not feasible in these populations. However, an A allele in *ADH1B* (rs1229984) is the wild type in East Asian populations, and an A allele in *ALDH2* (rs671) exists in East Asian populations. A few Asian studies have examined the association between *ADH1B* (rs1229984) and *ALDH2* (rs671) polymorphisms and CRC risk, but they provided inconsistent results.

The World Health Organization Global Information System on Alcohol and Health reported that total alcohol consumption in 2013 was 9·33 litres per capita per year for the Korean population, 7·55 litres per capita per year for the Japanese population and 5·79 liters per capita per year for the Chinese population[15]. Given the high alcohol consumption and high proportion of A alleles in *ADH1B* (rs1229984) and *ALDH2* (rs671) genes in the Korean population, we aimed to investigate the interactions of alcohol or methyl diets (as defined by a combined low intake of folate and high intake of alcohol) by *ADH1B* (rs1229984) and *ALDH2* (rs671) genotypes on the susceptibility of CRC development.

Materials and methods

Study population

The current study included 1039 CRC cases and 997 cancer-free controls who were consecutively admitted to two university hospitals in Seoul, Korea, from 1998 to 2004. Newly diagnosed CRC cases and their cancer site locations were reconfirmed via pathology by medical record review. Controls were individuals who had been hospitalised during the same period of time with cholelithiasis, haemorrhoids, acute appendicitis, varicose, chronic otitis media, para nasal sinusitis, cataract or other non-neoplastic surgeries.

We excluded individuals who did not have information on *ADH1B* (rs1229984) and *ALDH2* (rs671) genotypes (ten cases and twenty controls) or alcohol intake (twenty-nine cases and eighty-four controls). After exclusion, 1001 cases and 899 controls aged 30–79 years were analysed in the current study. Among 1900 patients, we partially excluded those with missing values on dietary information (five cases and 238 controls), and 996 cases and 661 controls were eligible for the analysis of methyl diets. The current study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Institutional Review Board of the participating institution. Written informed consent was obtained from every participant.

Data collection

Sociodemographic status, alcohol intake, smoking status and family history of CRC were recorded by trained nurse interviewers using structured questionnaires. Height and weight taken 2 years before diagnosis or on interview date were reported. Body mass index (BMI, kg/m^2) was calculated by dividing the weight taken 2 years before diagnosis in kilograms by the square of the height in metres.

Assessment of alcohol intake and methyl diet

Dietary intake was assessed by using a validated food frequency questionnaire[16,17]. Participants reported the frequency and amount of alcohol intake before symptoms began, and we calculated daily ethanol intake in grams per day based on the ethanol content of the beverage including beer, soju, Korean rice wine, wine and whiskey. Folate intake was categorised into tertiles based on the distribution of controls and adjusted for total energy intake by the residual regression method[18]. Alcohol intake was categorised into <0·1 g/d of low intake, 0·1 to <30 g/d of moderate intake and ≥30 g/d of high intake. We categorised into a combination of the highest folate intake (≥333·78 μg/d) and low alcohol intake (<0·1 g/d) as high methyl diets, a combination of the lowest folate intake (<141·26 μg/d) and high alcohol intake (≥30 g/d) as low-methyl diets and moderate methyl diets otherwise.

Laboratory assays

The blood samples were collected at the time of interview, and DNA extraction from buffy coat cells was conducted using a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping for *ADH1B* (rs1229984) and *ALDH2* (rs671) was performed by TaqMan assays (Applied Biosystems, Foster City, CA). The genotype distributions in controls were

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in agreement for Hardy-Weinberg equilibrium; *P* values were 0·26 for *ADH1B* (rs1229984) and 0·43 for *ALDH2* (rs671).

Statistical analysis

t Tests for continuous variables and χ^2 tests for categorical variables were used to compare cases and controls. We estimated ORs and 95 % CIs to evaluate the associations of *ADH1B* (rs1229984) and *ALDH2* (rs671) genotypes with CRC using multivariate logistic regression models. We adjusted for potential confounding factors by examining estimate change after including known risk factors for CRC; age (years, continuous); sex (men or women); pack-years of smoking (continuous); BMI, (kg/m^2, continuous); and education levels (less than high school, high school and graduate or more) in the model. Total energy intake was included for analysis of methyl diets. We considered other potential confounders including a history of bowel disease, non-steroidal anti-inflammatory drugs (NSAID) use, fibre intake, red meat intake, physical activity or a family history of CRC, but did not include these variables because inclusion of these variables did not appreciably change the results in the final model.

We examined the joint association of *ADH1B* (rs1229984) and *ALDH2* (rs671) genotypes with CRC according to alcohol intake. To investigate the joint association between methyl diets and CRC stratified by *ADH1B* (rs1229984) and *ALDH2* (rs671) polymorphisms, we tested overall interaction using two-sample Wald test for top categories.

All analyses were performed using SAS 9.4 (SAS Institute Inc.). All statistical tests were two-sided, and *P* values $< 0·05$ were considered statistically significant.

Results

Baseline characteristics of the study population according to CRC status are shown in Table 1. The mean (±SD) values of age were 59·00 ± 9·60 years for cases and 55·20 ± 9·60 years for controls. Pack-years of smoking and the total amount of alcohol intake were significantly higher in cases than in controls. In addition, cases tended to have a family history of CRC than controls.

The frequency of gene polymorphisms in controls reflected their East Asian origin; for *ADH1B* (rs1229984), 57·29 % were *AA*, 37·71 % were *AG* and 5·01 % were *GG* types. For *ALDH2* (rs671) genotypes, 67·29 % were *GG*, 30·03 % were *GA* and 2·78 % were *AA* types (online Supplemental Fig. S1).

We found that total alcohol intake was significantly associated with an increased risk of CRC (Table 2); compared

Table 1 Characteristics of study participants according to colorectal cancer status

	Cases		Controls		
	n	%	*n*	%	*P**
Age (years)					<0·001
Mean		59·00		55·20	
SD		9·60		9·60	
Sex					<0·001
Men	585	58·44	444	49·39	
Women	416	41·56	455	50·61	
BMI (kg/m^2)					0·41
<18·50	30	3·00	18	2·00	
18·50– <23·00	333	33·27	321	35·71	
23·00–<25·00	283	28·27	245	27·25	
25·00≤	355	35·46	315	35·04	
Pack-years of smoking					<0·001
Mean		14·10		9·50	
SD		20·40		16·80	
Alcohol intake (g/d)					<0·001
Mean		28·60		16·70	
SD		54·00		38·30	
Education level					<0·001
Less than high school	453	45·25	285	38·62	
High school	327	32·67	365	40·60	
More than high school	221	22·08	249	27·70	
Family history of colorectal cancer					<0·001
No	941	94·01	882	98·11	
Yes	60	5·99	17	1·89	
Energy intake (kcal/d)†					<0·001
Mean		1697·96		1593·19	
SD		557·26		527·74	
Dietary folate intake (μg/d)†					0·80
Mean		224·59		223·28	
SD		107·80		95·59	

**t* test for continuous variables and χ^2 test for categorical variables.
†A total of 996 cases and 661 controls were included.

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Table 2 ORs and 95 % CIs for colorectal cancer status according to total alcohol intake

	Total alcohol intake (g/d)										
		0·1–<15		15–<30		30–<45		45–<60		≥60	
	None	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
All*												
n (case/control)	482/522	143/151		97/79		57/36		64/32		158/79		
Age-adjusted	1·00	1·11	0·85, 1·45	1·35	0·97, 1·87	1·68	1·08, 2·61	2·13	1·36, 3·34	2·19	1·62, 2·97	<.001
Multivariate	1·00	1·10	0·83, 1·46	1·32	0·92, 1·90	1·60	0·99, 2·58	2·08	1·29, 3·36	2·02	1·41, 2·87	<.001
Men†												
n (case/control)	131/131	102/95		84/76		53/36		60/30		155/76		
Age-adjusted	1·00	1·16	0·79, 1·69	1·18	0·79–1·77	1·55	0·94, 2·54	2·10	1·26, 3·50	2·21	1·52, 3·22	<.001
Multivariate	1·00	1·11	0·76, 1·63	1·14	0·76, 1·70	1·45	0·88, 2·39	2·01	1·20, 3·35	2·05	1·39, 3·01	<.001
	None	0·1–<5		5–<10		10–<20		≥20				
Women†												
n (case/control)	351/391	23/40		13/12		13/5		16/7				
Age-adjusted	1·00	0·77	0·45, 1·33	0·53	0·68, 3·46	3·84	1·32, 11·12	2·62	1·05, 6·55			0·01
Multivariate	1·00	0·75	0·43, 1·31	1·60	0·70, 3·66	3·95	1·34, 11·66	2·25	0·87, 5·82			0·02

*Multivariate models were adjusted for age (years, continuous), sex (men or women), pack-years of smoking (continuous), BMI (kg/m^2, continuous) and education level (less than high school, high school and more than high school).
†Multivariate models were adjusted for age (years, continuous), pack-years of smoking (continuous), BMI (kg/m^2, continuous) and education level (less than high school, high school and more than high school).

with the non-drinkers, ORs (95 % CI) were 1·60 (0·99, 2·58) for drinkers with 30–<45 g/d of alcohol intake, 2·08 (1·29, 3·36) for drinkers with 45–<60 g/d of alcohol intake and 2·02 (1·41, 2·87) for drinkers with ≥60 g/d of alcohol intake ($P_{trend} < 0·001$). When we examined the association between alcohol intake and CRC risk among men and women separately, we still found an increased risk of CRC with increasing levels of alcohol intake. Compared with non-drinkers, ORs (95 % CIs) were 2·05 (1·39, 3·01) for drinkers with ≥60 g/d of alcohol intake ($P_{trend} <$ 0·001) among men and 3·95 (1·34, 11·66) for drinkers with 10–<20 g/d and 2·25 (0·87, 5·82) for drinkers with ≥20 g/d of alcohol intake among women ($P_{trend} = 0·02$).

We found that alcohol intake varied in individuals with *ADH1B* (rs1229984) or *ALDH2* (rs671) polymorphisms in our study population (Table 3). The mean values of alcohol intake were 17·37 g/d for the *AA* genotype, 17·02 g/d for the *AG* genotype and 6·80 g/d for the *GG* genotype of *ADH1B* (rs1229984). Koreans who had the *AA* genotype of *ALDH2* (rs671) had a mean alcohol intake of 0 g/d, whereas those who had the *GG* genotype of *ALDH2* (rs671) had a mean alcohol intake of 21·67 g/d. We found that the *ADH1B* (rs1229984) polymorphism was not associated with CRC risk, but there was a suggestive inverse association for the A allele of *ALDH2* (rs671); comparing *ALDH2* (rs671) *GA/AA* with the *GG* genotype, the OR (95 % CI) was 0·83 (0·68, 1·02).

For the association between alcohol intake and CRC risk according to the combined genotypes for *ADH1B* and *ALDH2*, we found relatively increased risk in CRC among individuals with *ADH1B AG/GG* and *ALDH2 GA/AA* (online Supplemental Fig. S2). In a sensitivity analysis where we excluded individuals who did not have information on diet, we observed the similar results (data not shown).

Table 3 ORs and 95 % CIs for colorectal cancer according to *ADH1B* (rs1229984) and *ALDH2* (rs671) polymorphisms*

	Alcohol intake (g/d) among controls		Cases		Controls			
Genotypes	Mean	SD	*n*	%	*n*	%	OR	95 % CI
ADH1B (rs1229984)								
AA (ref.)	17·37	39·36	558	55·74	515	57·29	1·00	
AG	17·02	38·81	384	38·36	339	37·71	1·04	0·86, 1·26
GG	6·80	15·21	59	5·89	45	5·01	1·26	0·83, 1·91
AA (ref.)	17·37	39·36	558	55·74	515	57·29	1·00	
AG/GG	15·82	36·97	443	44·26	384	42·71	1·06	0·88,1·28
ALDH2 (rs671)								
GG (ref.)	21·67	42·52	731	73·03	604	67·19	1·00	
GA	7·15	26·11	251	25·07	270	30·03	0·84	0·68, 1·04
AA	0		19	1·90	25	2·78	0·70	0·38, 1·31
GG (ref.)	21·67	42·52	731	73·03	604	67·19	1·00	
GA/AA	6·54	25·05	270	26·97	295	32·81	0·83	0·68, 1·02

*Models were adjusted for age (years, continuous), sex (men or women), and alcohol intake (g/d, continuous).

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Table 4 Association between alcohol intake and colorectal cancer risk according to *ADH1B* (rs1229984) and *ALHD2* (rs671) polymorphisms* and folate intake†

	Total alcohol intake (g/d)								
	None		0·1–<30			≥30			
Category	N (case/control)	OR	N (case/control)	OR	95 % CI	N (case/control)	OR	95 % CI	$P_{interaction}$
Alcohol-metabolising gene									
ADH1B									
AA	270/294	1·00	134/131	1·21	0·86, 1·71	154/90	1·96	1·33, 2·88	0·97
AG/GG	212/228	1·00	106/99	1·10	0·74, 1·63	125/57	1·88	1·18, 2·99	
ALDH2									
GG	278/283	1·00	207/190	1·13	0·82, 1·57	246/131	1·71	1·15, 2·52	0·31
GA/AA	204/239	1·00	33/40	0·92	0·53, 1·59	33/16	2·25	1·14, 4·43	
Folate intake (μg/d)									
Low folate (T1)	188/113	1·00	93/47	1·31	0·81, 2·12	126/59	1·40	0·86, 2·30	0·12
High folate (T2, T3)	291/277	1·00	146/109	1·24	0·87, 1·75	152/56	2·34	1·53, 3·59	

*Models were adjusted for age (years, continuous), sex (men or women), pack-years of smoking (continuous), BMI (kg/m^2, continuous) and education level (less than high school, high school and more than high school).
†A total of 996 cases and 661 controls were included; models were adjusted for age (years, continuous), sex (men or women), pack-years of smoking (continuous), BMI (kg/m^2, continuous), education level (less than high school, high school and more than high school) and total energy intake (kcal/d, continuous); folate intake was adjusted for energy by residual methods and categorised into tertiles (<141·26, 141·26 to <333·78 and ≥333·78 μg/d).

We examined whether the associations of alcohol intake with CRC varied by *ADH1B* (rs1229984) and *ALDH2* (rs671) polymorphisms or folate intake (Table 4). Increasing alcohol intake was associated with an increased risk of CRC regardless of types of *ADH1B* (rs 1229984) and *ALDH2* (rs 671). Participants who drank ≥30 g/d of alcohol had a higher risk of CRC compared with non-drinkers in both strata of low and high folate ($P_{interaction} = 0·12$).

We found an apparent interaction of the *ALDH2* (rs671) polymorphism but not the *ADH1B* (rs1229984) polymorphism regarding the association between methyl diets and CRC risk (Fig. 1). For *ADH1B* (rs1229984) polymorphisms, ORs (95 % CIs) comparing low-methyl with high-methyl diets were 1·93 (1·10, 3·39) among individuals with the *ADH1B* (rs1229984) *AA* genotypes and 1·51 (0·79, 2·88) among those with *ADH1B* (rs1229984) *AG/GG* genotypes ($P_{interaction} = 0·58$) (Fig. 1A). For *ALDH2* (rs671) polymorphisms, ORs (95 % CIs) comparing low-methyl with high-methyl diets were 1·36 (0·82, 2·23) among those with the *ALDH2* (rs671) *GG* genotypes and 9·08 (1·93, 42·60) among those with the *ALDH2* (rs671) *GA/AA* genotypes ($P_{interaction} = 0·02$) (Fig. 1B). In the additional analysis, we categorised methyl diets by tertiles of folate intake and alcohol intake into <0·1 and ≥10 g/d and found that individuals with low-methyl diet (the lowest tertile and ≥10 g/d of alcohol) had a 4·86 times higher risk of CRC among *ALDH2 A* allele group compared with high-methyl diets, but no association in *GG* group (online Supplemental Table S2).

Discussion

In our case–control study, we found that alcohol intake increased the risk of CRC. When we examined the interaction of *ADH1B* (rs1229984) or *ALDH2* (rs671) polymorphisms between methyl diets and CRC, we found that the association was remarkably strong in individuals with *ALDH2* (rs671) *GA/AA* polymorphisms with low-methyl diets compared with individuals with high-methyl diets.

We previously reported that low-methyl diets were associated with an increased risk of CRC among *CC/CT* carriers of *methylenetetrahydrofolate reductase* (*MTHFR*)[19]. In the current study, we observed that this risk was nine times higher for low-methyl diets *v.* high-methyl diets among participants with the *A* allele of *ALDH2* (rs671). Likewise, a US prospective cohort study found that a combination of high alcohol and low folate intake had seventeen times higher risk of colorectal adenoma among those with *ADH1C* slow metabolized than those with fast metabolised[13], and a Japanese case–control study reported an 11·9 times higher risk of oral and pharyngeal cancer among high drinkers (≥4 units/d) with low to median folate intake compared with never drinkers with high folate intake in *ALDH2* (rs671) *GA/AA*[20]. A Taiwanese, multicenter, case–control study reported that individuals with the combination of an *AA* genotype of *ALDH2* (rs671) and ingesting 1200 or greater g of alcohol per year had a 39·76-fold higher risk of oesophageal cancer than did individuals with the combination of the *GG* genotype of *ALDH2* (rs671) and those who abstained from drinking[21]. In the same Taiwanese study, for the *ADH1B* (rs1229984) polymorphism, a 147·43 times higher risk of oesophageal cancer risk was observed for participants with the *GG* genotype of *ADH1B* (rs1229984) who drank 1200 or greater g of alcohol per year compared to non-drinkers with the *AA* genotype of *ADH1B* (rs1229984). A US Health Professional's Follow-up Study found that an increased risk of colorectal adenoma among individuals with *ADH1C* slow metaboliser and a combination of high alcohol and low folate intake[13].

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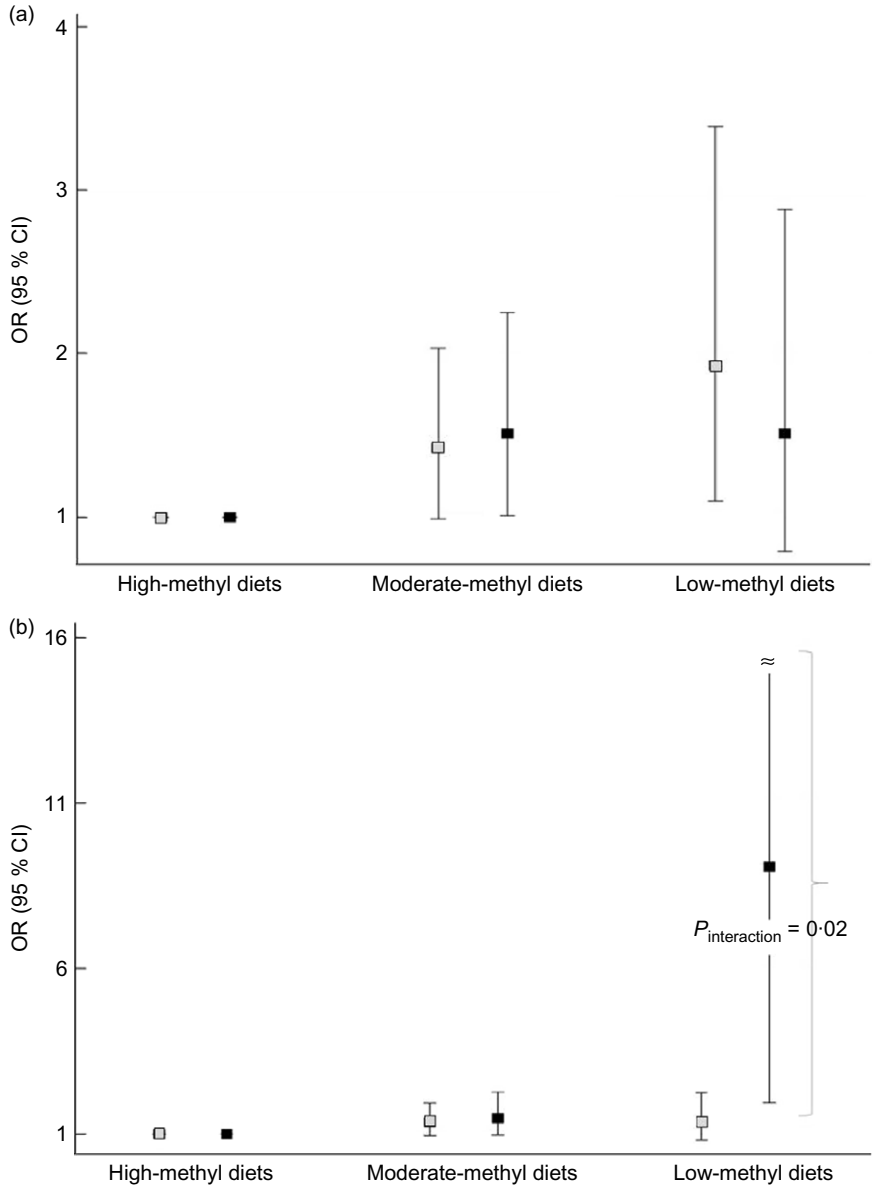

Fig. 1. Association between methyl diets and colorectal cancer risk according to *AHD1B* (rs1229984) and *ALHD2* (rs671) polymorphisms. The squares indicate the study-specific ORs, and the horizontal lines indicate the 95 % CIs. Models were adjusted for age (years, continuous), sex (men or women), pack-years of smoking (continuous), BMI (kg/m^2, continuous), education level (less than high school, high school, and more than high school) and total energy intake (kcal/d, continuous). (a) Comparing low-methyl diets with high-methyl diets, ORs (95 % CIs) were 1·93 (1·10, 3·39) among those with the *ADH1B* (rs1229984) *AA* types, 1·51 (0·79, 2·88) among those with the *ADH1B* (rs1229984) *AG/GG* genotypes ($P_{interaction}$ = 0·58). (b) Comparing low-methyl diets with high-methyl diets, ORs (95 % CIs) were 1·36 (0·82, 2·23) among those with the *ALDH2* (rs671) *GG* types, 9·08 (1·93, 42·60) among those with the *ALDH2* (rs671) *GA/AA* genotypes ($P_{interaction}$ = 0·02). All the values are presented in online Supplemental Table S1. (a) □, *ADH1B AA*; ■, *ADH1B AG/GG*; (b) □, *ALDH2 GG*; ■, *ALDH2 GA/AA*

Individuals with inactive *AA* alleles in *ALDH2* (rs671) have a low conversion of acetaldehyde to acetate and experience side effects such as facial flushing, palpitations and dizziness[22]. This reaction could lead to low alcohol intake among those with the *A* allele of *ALDH2* (rs671)[23], which is in agreement with the findings from our study. We found that Koreans who had the *GG* alleles of *ADH1B* (rs1229984) drank lower amounts of alcohol than did those with the *AA* or *AG* alleles, but previous European and East Asian studies reported higher alcohol consumption among participants who had the *GG* alleles of *ADH1B* (rs1229984)[23,24]. We cannot rule out a chance finding to explain this inconsistency.

A potential mechanism explaining the high risk of CRC with high alcohol intake is attributed to the prolongation of ethanol's contact with colon mucosa and the slow conversion of the carcinogenic acetaldehyde to acetate. Slow digestion of ethanol could lead to an unfavourable

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response to ethanol such as intestinal inflammation. For example, ethanol in the intestinal tract destroys proteins that make a tight junction linking cells in the intestinal wall, making it easier for intestinal bacteria to pass through the mucosal plasma membrane. This process makes the intestinal wall vulnerable to inflammation, which may be related to cancer development[25,26].

In contrast, folate has a key nutrient as an essential coenzyme for the pathway in DNA methylation, repair and synthesis. THF, the active form of folate, is required to convert homocysteine into methionine, forming SAM for DNA methylation. THF is used to convert to 5,10-methylene-THF, which is required for DNA synthesis involved in the synthesis of *thymidylate* and *purine*[3,27]. Therefore, folate deficiency can contribute to impaired DNA methylation and generate abnormal DNA synthesis and repair[28,29].

Alcohol may disrupt the intestinal absorption of dietary folate and folate supply to tissues, blocking the normal storage and release by the liver[30]. Genetic polymorphisms in *ADH1B* (rs1229984) and *ALDH2* (rs671) determined the efficiency of these enzymes in oxidising alcohol after drinking. Among individuals with the *A* allele of *ADH1B* (rs1229984), alcohol may be absorbed into the stomach in a short time and distributed into the bloodstream, resulting in rapid production of acetaldehyde in the liver. The low activity of *ALDH2* (rs671) polymorphisms delays the processing of acetaldehyde in the body, thus leading to longer acetaldehyde exposure in the colon[9,31]. Prolonged exposure of acetaldehyde among individuals with low activity of *ALDH2* (rs671) polymorphisms may lead to elevate antagonistic activity of alcohol against folate, resulting in inhibition of folate absorption and disturbance in DNA synthesis, repair and methylation. The disturbance could be worsened when folate intake is low.

There are several strengths and limitations in our study. Our study included a large sample size and is the first, to our knowledge, to explore the joint association between methyl diets and CRC risk according to the *ADH1B* (rs1229984) or *ALDH2* (rs671) polymorphisms in East Asian populations. The relatively higher variation in alcohol intake and the high proportion of *ADH1B* (rs1229984) and *ALDH2* (rs671) variants in the Korean population allowed us to explore the interaction by these genotypes even among individuals who experience side effects such as facial flushing. However, we had limitations in our study. First, relatively low prevalence of *ALDH2 A* allele among those with low-methyl diets led to the wide range of CIs. However, given that individuals with *ALDH2 A* allele rarely drank high amount of alcohol, increased risk of CRC among those with low-methyl diets and *ALDH2 A* allele may warrant the presentation of the data in this group. Further studies need to be replicated. Second, we did not consider nutrients involved in one-carbon metabolism other than folate due to a lack of information. Third, measurement error in alcohol intake and other covariates may exist. We cannot rule out the possibility that unmeasured or residual confounding factors were present, although we adjusted for potential confounding factors. Forth, we cannot rule out the possibility of selection bias due to the exclusion of imbalanced missing values between cases and controls in the dietary analysis. However, we found the similar results for alcohol and genetic polymorphisms in the sensitivity analysis where we excluded individuals who did not have information on diet. Lastly, given that 74 % of women were non-drinkers in our study, we could not conduct sex-specific analyses for the joint effects of alcohol and genetic polymorphisms on CRC risk.

In summary, our data suggest that low-methyl diets increased the risk of CRC among individuals with a rare variant (inactive form) of *ALDH2* (rs671) genotypes. Our study results need to be replicated in further epidemiologic studies.

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Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S136898001900452X

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