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添付資料：研究報告書

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2．研究テーマ

ADH2とALDH2遺伝子多型はアルコール消費による大腸腺腫感受性に影響を及ぼす

3．成果の概要（100字程度）

ADH2遺伝子は、性別、年齢などの交絡因子を補正して、Arg/Arg 型がHis/His型に比べて、オッズ比は有意に高くなった (OR:2.40; 95%CI: 1.00-5.78)。ALDH2 遺伝子多型に関して, 大腸腺腫の発症との関連連が見られなかったが、ALDH2 Lys+ 型とADH2 Arg+ 型の多量飲酒者はリスクが高かった(OR: 8.39; 95%CI: 1.69-41.54)。遺伝要因が飲酒量によって大腸腺腫の発症リスクに影響すると考えられた。

4．研究業績

(1) 学会における発表 ☑無 ・ □有（学会名・演題）

(2) 発表した論文 □無 ・ ☑有（雑誌名・題名）

Jiang J, Wang JW, Suzuki S, Gajalakshmi V, Kuriki K, Zhao Y, Nakamura S, Akasak S, Ishikawa H, Tokudome S. Elevated risk of colorectal cancer associated with the AA genotype of the cyclin D1 A870G polymorphism in an Indian population. Journal of Cancer Research and Clinical Oncology (2006) 132: 193-199.

—日中医学協会助成事業—

ADH2 と ALDH2 遺伝子多型はアルコール消費による大腸腺腫感受性に影響を及ぼす

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Abstract

Alcohol consumption is a probable risk factor with regard to colorectal neoplasm. Genetic polymorphisms, aldehyde dehydrogenase 2 (*ALDH2*) Glu487Lys and alcohol dehydrogenase 2 (*ADH2*) His47Arg, which have a strong impact on alcohol metabolism, are common in Japanese population but their significance for colorectal neoplasm remains to be clarified in detail. We therefore conducted a case-control study with 224 asymptomatic colorectal adenoma cases and 230 population-based controls matched for age and sex. After adjustment for sex, age, BMI, smoking status, drinking status, exercise, multivitamin consumption and family history of colorectal adenomas or cancers, the *ADH2 Arg* allele was found to be associated with increased risk, the odds ratios(ORs) being 1.41 (95%CI: 0.92-2.18) and 2.40 (1.00-5.78) for the His/Arg and Arg/Arg genotypes, respectively. Whereas no significant association was found for *ALDH2* genotypes. In heavy drinkers, having *ALDH2* Lys+ with *ADH2* Arg+ showed ORs of 8.39 (1.69-41.54), compared with non drinkers, having *ALDH2* Glu/Glu with *ADH2* His/His. In conclusion, the present study suggests the polymorphisms of *ADH2* and *ALDH2* can modify the influence of alcohol consumption on colorectal adenomas risk.

Key Words colorectal adenomas, drinking, gene-environment interaction, *ALDH2*, *ADH2*.

Introduction

The majority of colorectal cancer is thought to arise from adenomas, Alcohol consumption is a probable risk factor with respect to colorectal adenomas and colorectal cancers[1]. This increased risk might be caused by ethanol via cocarcinogenesis, induction of DNA hypomethylation, or tumor promotion, but might also result from its metabolite acetaldehyde, which was qualified as a probable carcinogen to humans. Alcohol is oxidized to acetaldehyde by the alcohol dehydrogenase enzymes (ADHs), especially by *ADH2*. Acetaldehyde is further oxidized into acetate by aldehyde dehydrogense enzymes (ALDHs), and this oxidation owes much to *ALDH2*. Encoding genes for these two representative alcohol-oxidizing capabilities and drinking behavior. Regarding *ADH2 Arg/His*, the *47His* allele represents a superactive subunit of *ADH2* that has about a 40 times higher than the less-active *Arg/Arg* from of *ADH2*. As for the *ALDH2* Glu487Lys polymorphism, the *487Lys* allele, encodes a catalytically inactive subunit. Individuals with the *ALDH2 Glu/Lys* genotype have only 6.25% of normal *ALDH2 487Glu* protein; indicating a dominant effect of *ALDH2 487Lys*. The *ADH2 47His* and *ALDH2 487Lys* alleles leading to high acetaldehyde concentrations, are clustered in East Asian populations such as Japanese and Chinese. Therefore, these two genetic polymorphisms modify the drinking habit and are expected to affect CRC and colorectal adenomas risk. However, rare evidence is available for the combined impact of *ADH2* and *ALDH2* polymorphisms on colorectal adenomas risk by alcohol drinking.

The primary aim of this case-control study is to investigate the impact of *ADH2* and *ALDH2* polymorphisms on colorectal adenomas risk. We also evaluated potential interactions between the two polymorphisms and alcohol consumption with regard

to colorectal adenomas risk.

Materials and Methods

Subject selection and data collection:

The participants and data collection method for this case-control study have been described previously in detail[2]. Briefly, 224 colorectal adenoma cases (159 men, 65 women) and 230 healthy controls (160 men, 70 women), frequency matched for age and sex, were recruited. Detailed information on demographic characteristics, personal medical history, usual physical activity, cigarette smoking and drinking habits, intake of multivitamins, and history of colorectal adenomas or cancer in the first-degree relatives were collected by trained interviewers. Alcohol use was categorized into never and ever use with consumption of <30 or ≥30 ml of ethanol per day.

Genotyping of *ALDH2* and *ADH2*:

DNA of each subject was extracted from the buffy coat fraction. Genotyping was based upon duplex PCRs with the confronting two-pair-primer (PCR-CTPP) method [3]. Briefly, four primers for the *ADH2* polymorphism and four primers for the *ALDH2* polymorphism were mixed in a 25μl volume with 0.2mMdNTPs, 0.5U of AmpliTaq Gold DNA polymerase and 2.5μl×10 PCR buffer including 15 mM Mgcl2. The results were confirmed by the PCR restriction fragment length polymorphism method using MslI for both polymorphisms.

Statistical analysis:

Differences of characteristics between cases and controls were assessed using the Chi-square test. The Hardy-Weinberg equilibrium was checked with the Chi-square test. Unconditional logistic regression analysis was employed to estimate the odds ratios (ORs) and confidence intervals (95% CIs) for the association between genotypes and risk of colorectal adenomas. Adjustments were made for matching variables (age, sex) and for possible confounders. Covariates were identified as potential confounders by examining their distribution by case-control status. The covariates were included in the model if they changed the ORs by more than 20% or significantly changed the likelihood ratio statistic ($p<0.05$) on univariate analysis. All statistical tests were two-sided and differences were considered to be statistically significant at $p < 0.05$. All analyses were performed using SAS software, version 8.20 (SAS Institute, Inc. Cary, NC, USA).

Results

Demographic and lifestyle characteristics of the 224 colorectal adenoma cases and 230 controls are shown in Table 1. Differences were observed in the distribution of cigarette smoking between cases and controls ($p = 0.001$ for males). A significantly higher proportion of cases with a family history of colorectal adenomas or cancer than controls were found ($p = 0.01$). There were no significant differences in the distribution of sex, body mass index, drinking status, vigorous exercise, or multivitamin consumption between cases and controls.

Table 2 shows genotype distributions for *ADH2* and *ALDH2* and combination, and their ORs and 95% CIs for colorectal adenomas. The genotype frequencies for all the polymorphisms were in accordance with the Hardy-Weinberg equilibrium in controls and allele frequencies were also in accordance with earlier reports in Japan. Significantly increased risk of colorectal adenomas was observed with Arg/Arg relative to His/His (OR: 2.45; 95%CI: 1.06-5.66; trend=0.005 for model 1), whereas no significant elevation of risk was observed with the *ALDH2* genotype alone. In the analysis for combination, compared with subjects having *ALDH2* Glu/Glu with *ADH2* His/His, age-sex adjusted ORs and 95% CIs for those with *ALDH2* Glu/Glu and *ADH2* Arg+, *ALDH2* Lys+ and *ADH2* His/His, and *ALDH2* Lys+ and *ADH2* Arg+ were 1.32 (0.81-2.16), 0.79 (0.49-1.29), and 1.56 (0.92-2.61), respectively. A model including confounders showed similar association.

In Table 3, the impact of combined genotypes on CRC risk stratified by alcohol drinking is presented as ORs adjusted for sex, age, BMI, smoking status, drinking status, exercise, multivitamin consumption and family history of colorectal adenomas or

cancers. In heavy drinkers, having *ALDH2* Lys+ with *ADH2* Arg+ showed ORs of 8.39 (1.69-41.54), compared with non drinkers, having *ALDH2* Glu/Glu with *ADH2* His/His.

Discussion

In present study found subjects with *ADH2* Arg allele had a higher risk of developing colorectal adenoma than those with *ADH2* His/His genotype, and also found that high alcohol consumption most markedly increased the risk of colorectal adenomas in subjects with *ADH2* Arg allele and *ALDH2* Lys allele.

Previous epidemiological studies also reported a significantly higher risk for colorectal cancer and esophageal cancer among Japanese with *ADH2* Arg allele, compared to those with *ADH2* His/His genotype[1,4,5]. There are possible explanations for the effects of *ADH2* on carcinogenesis. The high risk *ADH2* encoded by *ADH2* Arg allele might be exposed to lower dose of acetaldehyde following heavy alcohol drinking for hours. Especially, individuals with both the *ADH2* Arg allele and *ALDH2* Lys allele tend not to experience alcohol flushing, and diminished intensity of the aversive flushing response has been found to be positively associated with daily alcohol consumption [6]. Subjects who consumed alcoholic beverages and carried susceptible genotypes experienced a increase in risk of developing colorectal adenomas, much higher than those who were non-drinkers and did not carry susceptible genotypes of *ADH2* and *ALDH2*.

The *ADH2* Arg allele might, under certain conditions, increase the risk for colorectal adenomas via mechanisms of the interaction between genes and drinking behavior. Our present findings can provide additional information about the role of alcohol on colorectal adenomas in Japan.

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Table 1. Characteristics of cases with colorectal adenomas and controls

	Cases (%) (n=224)	Controls (%) (n=230)	*P**
Sex			
Male	159 (71.0)	160 (69.6)	0.74
Female	65 (29.0)	70 (30.4)	
Age (years)			
50-59	77 (34.4)	58 (25.2)	0.11
60-69	116 (51.8)	135 (58.7)	
70-75	31 (13.8)	37 (16.1)	
Body mass index (kg/m2)			
<20.0	27 (12.1)	29 (12.6)	0.06
20.0-24.9	134 (59.8)	158 (68.7)	
25.0-	63 (28.1)	43 (18.7)	
Cigarette smoking (pack-years)			
0	70 (31.2)	124 (53.9)	0.001
1-20	38 (17.0)	38 (16.5)	
>20	116 (51.8)	68 (29.6)	
Drinking status			
Never	79 (35.3)	104 (45.2)	0.09
Ever	145 (64.7)	126 (54.8)	
Moderate drinkers (<30ml/d)	83 (37.0)	71 (30.9)	
heavy drinkers (30ml/d-)	62 (27.7)	55 (23.9)	
Exercise			
Yes	140 (62.5)	134 (58.3)	0.36
No	84 (37.5)	96 (41.7)	
Multivitamin consumption			
Current	53 (23.7)	45 (19.6)	0.57
Occasional	15 (6.7)	16 (6.9)	
Never	156 (69.6)	169 (73.5)	
Family history of colorectal adenomas and cancer			
No	189 (84.4)	212 (92.2)	
Yes	35 (15.6)	18 (7.8)	0.01

* Examined by *t*-test or Chi-square test.

Table2. Genotype distributions of *ADH2* and *ALDH2* polymorphisms

	Cases	Controls	Model 1		Model 2	
			OR	95%CI	OR	95%CI
ADH2						
His/His	128	154	1.00	Reference	1.00	Reference
His/Arg	78	67	1.37	0.91-2.05	1.41	0.92-2.18
Arg/Arg	18	9	2.45	1.06-5.66	2.40	1.00-5.78
ALDH2						
Glu/Glu	142	140	1.00	Reference	1.00	Reference
Glu/Lys	70	74	0.93	0.62-1.39	0.93	0.61-1.42
Lys/Lys	12	16	0.81	0.36-1.75	0.83	0.36-1.93
ALDH2 and *ADH2*						
Glu/Glu and His/His	84	94	1.00	Reference	1.00	Reference
Glu/Glu and Arg+	58	48	1.32	0.81-2.16	1.38	0.82-2.33
Lys+ and His/His	44	62	0.79	0.49-1.29	0.78	0.46-1.30
Lys+ and Arg+	38	26	1.56	0.92-2.61	1.62	0.94-2.81

Model 1 adjusted for sex and age;
Model 2 adjusted for sex, age, BMI, smoking status, drinking status, exercise, multivitamin consumptionand family history of colorectal adenomas or cancers.

Table 3. Genotype distributions of *ADH2/ALDH2* according to drinking status and their ORs and 95% CIs for colorectal adenomas

	ALDH2 Glu/Glu		*ALDH2* Lys+	
	ADH2 His/His	*ADH2* Arg+	*ADH2* His/His	*ADH2* Arg+
ORs(95%CIs) for colorectal adenomas stratified by drinking levels				
All subjects	1.00 (Reference)	1.38 (0.82-2.33)	0.78 (0.46-1.30)	1.52 (0.83-2.81)
Non-drinkers	1.01 (Reference)	1.01 (0.39-2.64)	0.77 (0.35-1.71)	1.31 (0.53-3.26)
Moderate drinkers	1.02 (Reference)	0.87 (0.35-2.17)	0.59 (0.22-1.57)	0.93 (0.29-2.98)
Heavy drinkers	1.03 (Reference)	1.59 (0.72-5.29)	0.59 (0.12-2.75)	3.07 (1.61-19.5)
ORs(95%CIs) for colorectal adenomas compared with non-drinker having *ADH2* His/His and *ALDH2* Glu/Glu				
Non-drinkers	1.00 (Reference)	0.99 (0.41-2.41)	0.97 (0.47-2.03)	1.39 (0.92-3.24)
Moderate drinkers	1.89 (0.58-3.33)	1.61 (0.72-3.65)	1.73 (0.70-4.31)	1.23 (0.45-3.37)
Heavy drinkers	1.13 (0.52-4.29)	1.94 (0.97-7.34)	0.36 (0.39-1.43)	8.39 (1.69-41.54)

ORs adjusted for sex, age, BMI, smoking status, drinking status, exercise, multivitamin consumption and family history of colorectal adenomas or cancers.