[Original]

Glutathione S-Transferase (GST) M1, T1, P1, N-Acetyltransferase (NAT) 1 and 2 Genetic Polymorphisms and Susceptibility to Colorectal Cancer

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Abstract: A case-control study was carried out to examine the relation between genetic polymorphisms of five genes, cigarette smoking and colorectal cancer risk. We collected blood samples from 106 colorectal cancer patients and 100 healthy persons, then analyzed them to identify genotypes for glutathione S-transferase (GST) M1, T1, P1, N-acetyltransferase (NAT) 1 and 2 by the PCR method. We also collected smoking history data from all participants by questionnaire. From statistical evaluation on various combinations of genotypes, we observed that the cancer risk of those who have both GSTM1 present genotype and GSTP1 Adenine/Adenine homozygous genotype was significantly less than those who have other combinations of genotypes for two genes. For other combinations of genes, there was no significant association between genotype and cancer risk. There was also no significant association between amount of cigarette smoking and the cancer risk. These findings suggest that it is valuable to study cancer risk when examining genotypes of more than two genes at the same time. For further study, we need to collect more samples to increase statistical reliability, and besides cigarette smoking, include the nutrition data as an environmental factor.

Key words: colorectal cancer, genetic polymorphism, GST, NAT, cigarette smoking.

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Introduction

The risk of cancer is associated with various types of exposures and host factors, including differences in carcinogen metabolism. Enzymatic activation and deactivation of carcinogens is a major principle in chemical carcinogenesis as host factor [1, 2]. Epidemiological studies contribute to understanding the xenobiotic metabolism of carcinogenesis in

humans. This type of analysis is especially suitable for those enzymes that are polymorphically expressed on a heritable basis [3–5], enabling a comparison of cancer risks among subjects with genetically deficient or an extremely active metabolism to risks among those with normal activity.

Recently, the genetic basis of polymorphic activities of glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1), glutathione S-transferase P1 (GSTP1), *N*-acetyltransferase 1 (NAT1), and *N*-acetyltransferase 2 (NAT2) have been largely elucidated [6–10]. Biological data suggest that these enzymes may be involved in carcinogenesis, and that polymorphism of the enzymes affects the susceptibility of various types of cancer.

The polymorphic GST's catalyze the conjugation of glutathione to a variety of electrophilic compounds, including carcinogens and cytotoxic drugs [11, 12]. Absence of GSTM1 activity, a μ class enzyme, which detoxifies the reactive metabolites of benzo[a]pyrene and other polycyclic aromatic hydrocarbons [13], is due to homozygous deletion of the gene [6]. A similar polymorphism of the GSTT1 gene, encoding θ class enzymes, was recently discovered [7]. GSTT1 metabolizes various potential carcinogens such as monohalomethanes that are widely used as methylating agents, pesticides and solvents [14]. Recently a polymorphic site at nucleotide 313 (an adenine to guanine substitution replacing isoleucine with valine) in the GSTP1 gene which encodes π class enzymes, was detected and found to modify the enzyme's specific activity and affinity for electrophilic substrates [8, 15]. GSTP1 is widely expressed in normal epithelial tissue, particularly abundant in the urinary, respiratory and digestive tracts [16].

Epidemiological studies have shown that a phenotype of low activity of NAT2 is associated with an increased risk of urothelial cancer among occupationally exposed dye workers [17]. The genetic alteration underlying the NAT2 polymorphisms has been determined and correlated with NAT2 enzyme activities. The four major NAT2 alleles in Japanese are represented by the wild-type allele (NAT2*4) and three low activity alleles (NAT2*5, NAT2*6 and NAT2*7). A homozygous low activity allele genotype produces a slow acetylator phenotype. Though the NAT1 gene has only been recently shown to be polymorphic and has not received as much attention as NAT2, several pilot studies have been reported for some kinds of cancer [18–20].

Cigarette smoking is one of the important environmental risk factors for cancer development [21]. Many carcinogenic compounds including benzo[a]pyrene and arylamines are present in cigarette smoke. These chemicals are substrates of GSTM1, GSTT1, GSTP1, NAT1 and NAT2 [22, 23]. Several epidemiological studies have been performed on the relation between cigarette smoking and colorectal cancer risk [24–26]. Recently, some studies tried to examine the association between a few genotypes and colorectal cancer [27–31] and a few studies investigated the interaction between a few genotypes, cigarette smoking and colorectal cancer risk [32]. The main purpose of this paper is to elucidate the relation-

ship between the genotypes of five enzymes, cigarette smoking, and the susceptibility of colorectal cancer by the case-control study.

Subjects and Methods

Subjects : Blood samples were taken from 106 patients with colorectal cancer (C18 and C19 in ICD10 classification) and 100 healthy controls who have never suffered from any type of cancer. The colorectal cancer group comprised 69 males and 37 females and the mean and standard deviation of age was 62.3 years and 12.0 respectively. They were consecutive cases at the University of Occupational and Environmental Health Hospital, Mitsubishi Chemical Hospital and Kitakyushu Medical Center, and had been histologically diagnosed from September 1991 to June 1995. Patients sampling was purely sequential in this period and no intentional selection was done for sampling. The control group consisted of 58 males and 42 females who had visited local medical clinics in Kitakyushu City between September 1993 and April 1995 for regular medical health check-ups including the collection of blood and urine specimens. The mean age was 61.7 years and the standard deviation was 16.0. No intentional sampling selection was done for the control group except for excluding a person who had a history of cancer. Though no specific age matching was carried out, there was no significant difference in the mean value between cases and controls (t and P value of Student's t -test for mean difference was 0.30 and 0.81 respectively).

All participants were interviewed regarding medical, residential and smoking history. Their smoking history was summarized as the total amount of cigarettes smoked during their lifetime up until the time of interview, taking into account changes in smoking habit with age. The amount of cigarette smoke exposure was assessed by pack-year (pack/day $\times$ years of smoking). All participants were given an explanation of the nature of the study and informed consent was obtained.

Genotyping : In order to identify the genotype of the GSTM1, GSTT1, GSTP1, NAT1 and NAT2, the polymerase chain reaction (PCR) method was used [9, 15, 33]. Genotypes and grouping of genotypes that we used in this paper are shown in Table 1. For GSTM1 and GSTT1, we classified two genotypes; present and null (deleted) genotype. For GSTP1, we classified three genotypes and called them A/A, A/G and G/G genotype in this paper. A/A genotype means Adenine/Adenine homozygous at nucleotide 313. A/G means Adenine/Guanine heterozygous, while G/G means Guanine/Guanine homozygous at nucleotide 313. When we called GSTP1 with G genotype, it referred to both A/G and G/G. For NAT1, we classified three genotypes and called them *4/*4, *4/*10 and *10/*10. *4/*4 genotype means NAT1*4 homozygous genotype composed of NAT1*4/NAT1*4 allele pair. *4/*10 genotype means heterozygous mutant genotype composed of NAT1*4/NAT1*10 allele pair, while *10/*10 means homozygous mutant genotype composed of NAT1*10/NAT1*10 allele pair. Other genotypes, for example, NAT1*3 and NAT1*11, are neglected

Table 1. Genotypes and their allele pair composition for five genes. Groupings of genotype for three genes are depicted by parentheses

Gene	Genotype	allele pair composition
GSTM1	Present	present/present or present/deleted
	Null	deleted/deleted
GSTT1	Present	present/present or present/deleted
	Null	deleted/deleted
GSTP1	A/A	Adenine/Adenine at nucleotide 313
	A/G	Adenine/Guanine at nucleotide 313
	G/G	Guanine/Guanine at nucleotide 313
NAT1	*4/*4	NAT1* 4 /NAT1* 4
	*4/*10	NAT1* 4 /NAT1*10
	*10/*10	NAT1*10/NAT1*10
NAT2	Rapid	NAT2* 4 /NAT2* 4
	Intermediate	NAT2* 4 /other type allele ¹⁾
	Slow	other type allele /other type allele

1) NAT2* 5, NAT2* 6, NAT2* 7

in this study, because there is very little possibility of them in the Japanese. For NAT2, we classified three categories and we called them rapid, intermediate and slow genotype. Rapid genotype means NAT2 *4 homozygous genotype composed of NAT2 *4/NAT2 *4 allele pair. Intermediate genotype means heterozygous genotype composed of NAT2 *4 and other types of NAT2 mutant allele; NAT2 *5, NAT2 *6, NAT2 *7, while slow genotype means homozygous mutant genotype.

Statistical analysis : First, the relation between the genotype and the risk of colorectal cancer was examined for each gene by chi-square tests and odds ratio evaluations. Secondly, the same statistical analysis was done for various combinations of genotypes of two different genes. Thirdly, the association of amount of cigarette smoking and the risk of cancer was examined by chi-square tests.

Results

Genotypes and cancer risk : In order to elucidate the relation between the genotype and the susceptibility of colorectal cancer, we did chi-square tests and calculated the odds ratio which indicates the relative risk. Table 2 shows frequency tables of control and cases classified by genotype for each gene with the results of chi-square tests and odds ratio evaluations. For GSTM1 and GSTT1, the frequency tables are 2×2 . For GSTP1, NAT1 and NAT2, the frequency tables are 3×2 , because these genes have 3 genotypes. However, these genotypes can be classified into two categories according to their effect on carcinogen metabolism. For GSTP1, A/G and G/G genotypes were classified under the same category, and we called

Table 2. Frequency tables of control and case classified by categories of genotype for each gene with χ^2 value and odds ratio

GSTM1					
Genotype	Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
Present [†]	58	50	2.42	0.12	1.55 (0.89-2.68)
Null	42	56			
GSTT1					
Genotype	Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
Present [†]	59	55	1.05	0.31	1.33 (0.77-2.32)
Null	41	51			
GSTP1					
Genotype	Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
A/A	76	72	not reliable		
A/G	21	34			
G/G	3	0			
A/A [†]	76	72	1.66	0.20	1.50 (0.81-2.76)
With G	24	34			
NAT1					
Genotype	Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
*4/*4	36	38	0.21	0.90	
*4/*10	42	42			
*10/*10	22	26			
*4/*4 [†]	36	38	0.001	0.98	1.01 (0.57-1.78)
With *10	64	68			
NAT2					
Genotype	Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
Rapid	49	60	1.38	0.50	
Intermediate	44	41			
Slow	7	5			
Rapid/Intermediate [†]	93	101	0.16	0.69	0.66 (0.20-2.14)
Slow	7	5			

(note : Affixed maker[†] indicates reference group for odds ratio calculation.)

it GSTP1 with G genotype. For NAT1, *4/*10 and *10/*10 genotypes were classified under the same category, and we called it NAT1 with *10. For NAT2, rapid and intermediate genotypes were classified as the same category, and we named it rapid/intermediate (See Table 1 for these grouping). There are also 2×2 frequency tables for these genes using the above groupings in Table 2.

Table 3. Frequency tables of control and case classified by categories of combination of each genotype with χ^2 value and odds ratio

GSTM1 and GSTT1						
Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTM1	GSTT1					
Present	Present	36	30	3.43	0.33	
Present	Null	22	20			
Null	Present	23	25			
Null	Null	19	31			
Present	Present [†]	36	30	3.12	0.08	1.92 (0.93-4.12)
Null	Null	19	31			

GSTM1 and GSTP1						
Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTM1	GSTP1					
Present	A/A	47	31	7.45	0.0589	
Present	with G	11	19			
Null	A/A	29	41			
Null	with G	13	15			
Present	A/A [†]	47	31	6.89	0.0086	2.15 (1.21-3.79)
Other combination		53	75			

GSTT1 and GSTP1						
Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTT1	GSTP1					
Present	A/A	45	34	3.86	0.28	
Present	with G	14	21			
Null	A/A	31	38			
Null	with G	10	13			
Present	A/A [†]	45	34	3.64	0.056	1.73 (0.98-3.05)
Other combination		55	72			

GSTM1 and NAT1						
Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTM1	NAT1					
Present	*4/*4	22	14	4.57	0.21	
Present	with *10	36	35			
Null	*4/*4	14	24			
Null	with *10	28	32			
Present	*4/*4 [†]	22	14	1.88	0.17	1.80 (0.78-4.15)
Null	with *10	28	32			

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GSTT1 and NAT1

Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTT1	NAT1					
Present	*4/*4	20	19	1.09	0.78	
Present	with *10	39	36			
Null	*4/*4	16	19			
Null	with *10	25	32			
Present	*4/*4 [†]	20	19	0.51	0.47	1.35 (0.60-3.05)
Null	with *10	25	32			

GSTP1 and NAT1

Genotype		Control	Case	χ^2	<i>P</i>	Odds ratio (95%CI)
GSTP1	NAT1					
A/A	*4/*4	27	27	1.88	0.60	
A/A	with *10	49	45			
with G	*4/*4	9	11			
with G	with *10	15	23			
A/A	*4/*4 [†]	27	27	0.996	0.32	1.53 (0.66-3.55)
with G	with *10	15	23			

(note : Affixed maker[†] indicates reference group for odds ratio calculation.)

The χ^2 value and significant level *P* for each frequency table are depicted in the table. There was no statistically significant association between genotype and control-or-case. Even though the smallest *P* value of chi-square tests was 0.120 for GSTM1, it was more than 0.05, which is statistically meaningful.

Table 3 shows frequency tables of control and cases classified by the combination of genotype for two genes with the results of chi-square tests and odds ratio evaluation. Each table consists of 4×2 frequency tables. Besides this table, we made 2×2 frequency tables by proper grouping or extraction from genotype combinations.

Though there was no significant association for each 4×2 frequency table, *P* value of chi-square tests for the genotype combination of GSTM1 and GSTP1 was 0.0589. Because this was near to the meaningful value 0.05, we made 2×2 frequency tables by proper grouping for genotype combinations to increase the statistical significance. One of the new groups is one which has both GSTM1 present and GSTP1 A/A and the other is one that has other combinations; GSTM1 present and GSTP1 with G, GSTM1 null and GSTP1 A/A, GSTM1 null and GSTP1 with G. For this 2×2 frequency table, *P* value of chi-square tests was 0.0086 and odds ratio was 2.15 (confidence interval was 1.21–3.79). This shows that there is a statistically significant association of genotype combination of GSTM1 and GSTP1 with colorectal cancer incidences.

For other frequency tables of different two gene combinations, we could not observe any

Table 4. Frequency table of control and case classified by pack-years as amount of cigarettes smoked with χ^2 value and odds ratio

Pack-years	Control	Case	χ^2	<i>P</i>	Odds ratio (95% CI)
Non-smokers	45	53	4.04	0.40	
0 < pack-years ≤ 15	11	9			
15 < pack-years ≤ 30	18	17			
30 < pack-years ≤ 45	18	12			
45 < pack-years	8	15			
Non-smokers [†]	45	53	0.52	0.47	0.82 (0.47-1.41)
Smokers	55	53			

(note : Affixed maker[†] indicates reference group for odds ratio calculation.)

significant association, though some *P* values were small, that is, close to 0.05, for example, *P* = 0.056 for the 2 × 2 table of GSTT1 and GSTP1, and *P* = 0.08 for the 2 × 2 table of GSTM1 and GSTT1.

Smoking and cancer risk : In order to analyze the relation between cigarette smoking and cancer risk, we made a frequency table of control and case numbers classified by the amount of cigarette smoking. The quantity of cigarettes smoked was classified by 15 pack-year intervals; pack-year = 0 (non-smoker), 0 < pack-years ≤ 15, 15 < pack-years ≤ 30, 30 < pack-years ≤ 45, 45 < pack-years. The unit of 1 pack-year means that the person continues to smoke for one year at the rate of 1 pack (20 cigarettes) a day. The zero pack-year means that a person does not smoke at all. Table 4 shows a 5 × 2 frequency table and is followed by a 2 × 2 frequency table classified by non-smoker (pack-year = 0) and smoker (pack-years > 0).

Because the *P* values of chi-square tests for 5 × 2 and 2 × 2 tables was 0.40 and 0.47, there was no significant association between the amount of cigarettes smoked and cancer incidences.

To examine the relation between the genotype, amount of smoking and cancer risk, we made a frequency table of control and cases classified by pack-years and genotype for each gene. Table 5 shows these tables with χ^2 value and significant level *P* of chi-square tests. We must note that we omitted a frequency table for NAT2 from Table 5, because the number of NAT2 slow genotypes was too small to perform a valuable statistical analysis. For all genes, no significant association was found between genotype difference, amount of smoking and cancer incidence.

We did the same statistical analysis for the genotype combination of the two gene pair, GSTM1 and GSTP1, because there was significant association between genotype difference and cancer risk only for this pair, as shown in the previous section. Table 6 shows the frequency table for this gene pair. However, no significant association was found for this table.

Table 5. Frequency table of control and case classified by pack-years as amount of cigarettes smoked and genotype with χ^2 value for each gene

GSTM1						
Pack-years	Control		Case		χ^2	P
GSTM1 genotype :	Present	Null	Present	Null		
Non-smokers	28	17	26	27	8.41 (not reliable)	0.75
0 < pack-years $\leq$ 15	7	4	4	5		
15 < pack-years $\leq$ 30	8	10	7	10		
30 < pack-years $\leq$ 45	9	9	7	5		
45 < pack-years	6	2	6	9		
Non-smokers	28	17	26	27	1.26	0.74
Smokers	30	25	24	29		
GSTT1						
Pack-years	Control		Case		χ^2	P
GSTT1 genotype :	Present	Null	Present	Null		
Non-smokers	29	16	26	27	10.81 (not reliable)	0.55
0 < pack-years $\leq$ 15	8	3	7	2		
15 < pack-years $\leq$ 30	8	10	8	9		
30 < pack-years $\leq$ 45	11	7	6	6		
45 < pack-years	3	5	8	7		
Non-smokers	29	16	26	27	1.85	0.60
Smokers	30	25	29	24		
GSTP1						
Pack-years	Control		Case		χ^2	P
GSTP1 genotype :	A/A	with G	A/A	with G		
Non-smokers	34	11	37	16	8.59 (not reliable)	0.74
0 < pack-years $\leq$ 15	9	2	5	4		
15 < pack-years $\leq$ 30	15	3	13	4		
30 < pack-years $\leq$ 45	14	4	7	5		
45 < pack-years	4	4	10	5		
Non-smokers	34	11	37	16	0.70	0.87
Smokers	42	13	35	18		
NAT1						
Pack-years	Control		Case		χ^2	P
NAT1 genotype :	*4/*4	with *10	*4/*4	with *10		
Non-smokers	17	28	18	35	14.61 (not reliable)	0.26
0 < pack-years $\leq$ 15	4	7	4	5		
15 < pack-years $\leq$ 30	4	14	6	11		
30 < pack-years $\leq$ 45	4	14	4	8		
45 < pack-years	7	1	6	9		
Non-smokers	17	28	18	35	0.86	0.84
Smokers	19	36	18	33		

Table 6. Frequency table of control and case classified by pack-years as amount of cigarettes smoked and genotype combination for GSTM1 and GSTP1 with χ^2 value

GSTM1 and GSTP1					
Pack-years	Control		Case		χ^2
GSTM1 -P1 :	Present -A/A	Others	Present -A/A	Others	
Non-smokers	24	21	16	37	6.98 (not reliable)
0 < pack-years $\leq$ 15	5	6	1	8	
15 < pack-years $\leq$ 30	8	10	5	12	
30 < pack-years $\leq$ 45	7	11	4	8	
45 < pack-years	3	5	4	11	
Non-smokers	24	21	16	37	2.01 0.57
Smokers	23	32	14	39	

Discussion

Genotypes and cancer risk : As shown in the previous section, we could not observe any significant association between genotype difference and colorectal cancer risk when examining any single gene, while there was statistically significant association for a genotype combination of two genes, that is, the colorectal cancer risk for a person who does not have GSTM1 present gene or GSTP1 A/A gene or either of them was significantly higher than for a person who has both GSTM1 present gene and GSTP1 A/A gene. It has been reported that both GSTM1 and GSTP1 genes seem to play a role in detoxification of benzo[a]pyrene [22, 23]. The result suggested the possibility that the detoxification is the most effective when both GSTM1 present gene and GSTP1 A/A gene exist, while the effectiveness decreases when one or two of them are lacking. These isozymes may contribute to detoxify putative carcinogens in the colon of humans.

Several studies have tried to examine the relation between gene polymorphisms and the risk of colorectal cancer [27–32]. Strange *et al.* [27] and Zhong *et al.* [28] studied colorectal cancer risk using the GSTM1 gene and reported the association of GSTM1 null with colorectal cancer. But Chenevix-Trench *et al.* [29] and Gertig *et al.* [31] reported opposite results, that is, there was no association between GSTM1 genotype difference and the incidence of colorectal cancer. There were a few reports other than those regarding the GSTM1 gene. Gertig *et al.* [31] and Slattery *et al.* [32] studied using GSTT1 and NAT2 gene respectively, but found no association of genotype difference with cancer risk. In our study, we expanded the number of genes to treat and performed a statistical analysis of colorectal cancer risk not only for each single gene but also for any two gene pairs. Regarding the analysis for a single gene, there was no significant association of genotype difference with

cancer risk. This supports the results by both Chenevix-Trench *et al.* [29] and Gertig *et al.* [31] and does not support results by both Strange *et al.* [27] and Zhong *et al.* [28] for GSTM1.

Regarding the analysis for any two gene pairs, we observed a significant association for GSTM1 and GSTP1 pair. Deakin *et al.* [30] studied colorectal, lung, oral and gastric cancer risk using the same analysis method for the GSTM1 and GSTT1 pair. They reported a significant association of GSTM1 present and GSTT1 null pair with colorectal cancer. Though our result for the same pair does not support Deakin's as shown in Table 3, the *P* values of chi-square tests for the 2×2 frequency table consisted of GSTM1 present and GSTT1 present pair class and GSTM1 null and GSTT1 null pair class is 0.08, which is close to 0.05. As this value is not negligible, we should continue to investigate the genotype combination of GSTM1 and GSTT1 pair in cancer risk research. Slattery *et al.* [32] studied colon cancer risk for the genotype combination of GSTM1 and NAT2 pair and reported no significant association. Our finding regarding the association of genotype combination of GSTM1 and GSTP1 pair with colorectal cancer risk has not been reported previously.

This means that a study for genotype combination of two or more than two genes is important.

Smoking and cancer risk : In our study, there was no significant association between the amount of cigarettes smoked and cancer incidences as discussed before and shown in Table 4. Some epidemiological studies have focused on the relation between cigarette smoking and the incidence of colorectal cancer or adenoma, which is considered as a precursor of cancer [24–26]. Giovannucci *et al.* [24] studied the effect of cigarette smoking amount on the incidence of colorectal cancer and adenoma, and reported a significant effect. Martinez *et al.* [25] also reported that cigarette smoking increases colorectal adenoma and Slattery observed a significant association of the amount of cigarettes smoked with increased risk of colon cancer. However, no significant association between cigarette smoking and colorectal cancer was found by Baron *et al.* [26].

A few studies tried to investigate the interaction between genotypes, smoking amount and colorectal cancer risk. Slattery *et al.* [32] reported no significant interaction between the genotype difference of GSTM1 and NAT2, cigarette smoking and colon cancer risk. Our study also did not find any significant association between these three factors. Though our result coincides with Slattery's, this does not mean that we may abandon the investigation of these interactions. A study using a large number of samples should be performed.

In our study, we collected only smoking amount data as an environmental factor. Some studies suggest the importance of food or nutrition factor in the incidence of colorectal cancer or adenoma [25, 26, 34, 35]. Giovannucci *et al.* [35] reported that alcohol intake and low methionine or folate intake increase colon cancer risk. Martinez *et al.* [25] also reported that alcohol consumption increases colorectal adenomatous polyps. Baron *et al.* [26] presented the same results, that is, the folate intake prevents colorectal adenoma and

alcohol intake increases colorectal adenoma. These reports suggest the need of further study in which the diet pattern data of participants is included.

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遺伝子多型と結腸直腸癌の発癌感受性

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要 旨: 結腸直腸癌について、発癌リスクと遺伝子変異、および喫煙の関係を調べるために症例対照研究を行った。結腸直腸癌患者 106 名、癌病歴のない対照群 100 名から採取した血液検体に対し、5種の遺伝子 (GSTM1, GSTT1, GSTP1, NAT1, NAT2) について、PCR法による遺伝子型の同定を行い、アンケート調査により各被験者の喫煙量について調べた。各遺伝子について個別に遺伝子型と癌の発症との関連性の検定を行ったが、遺伝子型の違いと発癌リスクの間に有意な関連は見出せなかった。次に、異なる2つの遺伝子間で遺伝子型の組み合わせを考慮して関連性を調べたところ、GSTM1 present型とGSTP1 Adenine/Adenine型 (313 番目ヌクレオチド) の両者を持つ者がそれ以外の組み合わせの遺伝子型を持つ者に比べ有意に結腸直腸癌の発症率が低くなることが示された。喫煙量と癌発生との関連については両者の間には有意な関連は見られなかった。遺伝子型と喫煙量と癌発生との関連についても同様に有意な関連はなかった。本研究の結果は、遺伝子型と癌発症の関連を調べる時、同時に複数の遺伝子を考慮することの有用性を示すものとなった。今後、統計学的信頼性を上げるために標本数を増やすと共に、環境要因として喫煙以外の因子、例えば食事の内容等を含めた調査が必要と考えられる。

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