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Association between genetic polymorphisms of the base excision repair gene *MUTYH* and increased colorectal cancer risk in a Japanese population

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Running title: *MUTYH* SNPs and colorectal cancer risk in Japanese

Summary

The *MUTYH* gene encodes a DNA glycosylase that can initiate the base excision repair pathway and prevent G:C>T:A transversion by excising adenine mispaired with 8-hydroxyguanine. Biallelic germline mutations of *MUTYH* have been shown to predict familial and sporadic multiple colorectal adenomas and carcinomas, however, whether there is an association between single nucleotide polymorphisms (SNPs) of *MUTYH* and sporadic colorectal cancer (CRC) risk has remained unclear. In this study we investigated four *MUTYH* SNPs, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G, and 972G>C (Gln324His), for an association with increased CRC risk in a population-based series of 685 CRC patients and 778 control subjects from Kyushu, Japan. A statistically significant association was demonstrated between IVS1+11T and increased CRC risk (odds ratio [OR]: 1.43; 95% confidence interval [CI]: 1.012~2.030; $P = 0.042$) and one of the 5 haplotypes based on the 4 SNPs, the IVS1+11T - IVS6+35G - IVS10-2A - 972C (TGAC) haplotype containing the IVS1+11T, was demonstrated to be associated with increased CRC risk (OR: 1.43; 95% CI: 1.005~2.029; $P = 0.046$). Subsite-specific analysis showed that the TGAC haplotype was statistically significantly ($P = 0.013$) associated with an increased risk of distal colon, but not proximal colon or rectal cancer. Furthermore, IVS1+11C>T was found to be in complete linkage disequilibrium with -280G>A and 1389G>C (Thr463Thr). The results indicated that

Japanese individuals with the -280A / IVS1+11T / 1389C genotypes or the TGAC haplotype are susceptible to CRC.

Key words: MUTYH, colorectal cancer, SNP, case-control study, base excision repair (BER).

Abbreviations: CRC, colorectal cancer; BER, base excision repair; ROS, reactive oxygen stress; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; RFLP, restriction fragment length polymorphism; PCR-CTPP, PCR with confronting two-pair primers; OR, odds ratio; CI, confidence interval.

Introduction

Intracellular DNA is at risk of damage by reactive oxygen species (ROS) generated by normal metabolism and environmental exposure, and 8-hydroxyguanine (8-ohG) is one of the products induced by ROS damage and is known to be a mutagenic lesion.^(1,2) The base excision repair (BER) pathway plays an important role in repairing oxidative-damage-induced mutations, and the *MUTYH* gene encodes the glycosylase capable of initiating the BER pathway by catalyzing the removal of adenine residues mispaired with 8-ohG.⁽³⁻⁵⁾ It is indicated that defects in the BER pathway may contribute to tumorigenesis by increasing mutation frequency in oncogenes and tumor suppressor genes.⁽⁶⁾ In fact, it has been reported that some cases of autosomal recessive inherited multiple colorectal adenomatous polyposis and carcinoma with an increased frequency of somatic G:C >T:A mutations in *APC* are attributable to biallelic germline mutations in the *MUTYH* gene.⁽⁷⁻¹⁰⁾ The disease-causing mutations, Y165C, G382D, 466delE, E466X, Y90X, etc., have been reported in Caucasians, Indian, Pakistani and other ethnic groups.^(8,9,11,12) The frequency of two of the, Y165C and G382D, has been investigated in several colorectal cancer (CRC) case-control studies, and monoallelic carrier of these variants were found in 0~2.6% of the cases and 0~2.1% of the controls, biallelic carrier of these variants were found in 0~0.8% of the cases and 0% of the controls, respectively.⁽¹³⁻¹⁸⁾ However, neither of these two variants has ever been detected in East Asians, including Japanese,⁽¹⁹⁻²²⁾ suggesting that they are ethnicity-specific alleles.

Based on the above findings, we hypothesized that *MUTYH* variants other than Y165C and G382D act as low-penetrance susceptibility alleles in Japanese CRC, similar to situation previously reported for the *APC* and *CHEK2* gene variants.^(23,24)

We conducted a CRC case-control study to evaluate the significance of *MUTYH* variants in a Japanese population. In the single-nucleotide polymorphisms (SNPs) reported in the Japanese population,^(19,20) four SNPs, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G and 972G>C (Gln324His), were selected, and all 685 cases and 778 matched controls were genotyped to detect these four SNPs, and statistically significant association was found between the IVS1+11C>T SNP and increased CRC risk in the Japanese population. A haplotype-based association study was also performed, and a statistically significant association was found between the IVS1+11T - IVS6+35G - IVS10-2A - 972C (TGAC) haplotype containing the IVS1+11T allele and CRC risk. In the subsite-specific analysis, the IVS1+11C>T SNP was detected to be nearly statistically significantly associated and the TGAC haplotype was found to be statistically significantly associated with an increased risk of distal colon, but not proximal colon or rectal cancer. We also found that a novel -280G>A SNP in the 5' flanking region of *MUTYH* and a previously reported 1389G>C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C>T. Our results suggest that the -280A / IVS1+11T / 1389C or the TGAC haplotype of *MUTYH* may be novel CRC susceptibility alleles.

Materials and Methods

Specimens

Blood specimens from 685 CRC cases and 778 controls were collected in a previous study. DNA was extracted from this and written informed consent was obtained from each individual patients.⁽²⁵⁾ The characteristics of the cases and controls have been described previously.⁽²⁵⁻²⁸⁾ In brief, the cases were composed of a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas, and controls were composed of individuals not diagnosed to have colorectal cancer. Other eligibility criteria were as follows: age 20 to 74 years at the time of diagnosis for the cases or at the time of selection for the controls, residents of the study area (Fukuoka City and three adjacent areas), no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and mental competence to give informed consent and participate in the interview. The number of control candidates by gender and 10-year age class was determined in accordance to the expected sex- and age-specific number of incident cases of colorectal cancer. For the reverse transcriptase-polymerase chain reaction (RT-PCR) experiment, total RNA was extracted from the non-cancerous colorectal mucosa of 6 CRC patients and converted to cDNA, as described previously.⁽¹⁹⁾ This study was approved by the Institutional Review Board (IRB) of Hamamatsu University School of Medicine (12-14,

18-4).

Target SNPs and genotyping

The six SNPs genotyped in this study were as follows: IVS1+11C>T (rs2275602), IVS6+35G>A (rs3219487), IVS10-2A>G (5'-flanking sequence: 5'-CAC TCA ACC CTG TGC CTC TC-3'; 3'-flanking sequence: 5'-GGT GGA GCA GGA ACA GCT CT-3'), 972G>C (Gln324His) (rs3219489), G382D (rs36053993), and -280G>A (5'-flanking sequence: 5'-ATT ACT ACT AAC CGT TAT GA-3'; 3'-flanking sequence: 5'-CTC CAG ACT ACA TCT CCC GC-3'). The IVS10-2A>G and -280G>A had not been presented in the SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI) Entrez system. Genotyping of the four target SNPs, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G and 972G>C (Gln324His), was performed by PCR with confronting two-pair primers (PCR-CTPP), as described previously (Fig. 1A, B),⁽¹⁹⁾ and genotyping of the G382D SNP was performed by PCR-restriction fragment length polymorphism (PCR-RFLP). Genotyping of the -280G>A SNP was performed by two independent allelic-specific PCRs (Fig. 1A, B). The PCR primers used were: IVS1+11C>T SNP: F1 (5'-AAC TAT GAG CCC GAG GCC TTC C-3'), R1 (5'-CAG CAG AAC ACG GAG GCC C-3'), F2 (5'-AGT CGT CTG TGG GTA CGC TGG AT-3'), and R2 (5'-CCA GGA GAC GGA CCG CAA G-3'); IVS6+35G>A: F1 (5'-CCA GTG TGG GTC TCA GAG G-3'), R1 (5'-CCC TAG CTC CTC

TAC CAC CTG-3'), F2 (5'-CTA GGG TAG GGG AAA TAG GAA CA-3'), and R2 (5'-CAC CCG TCA GTC CCT CTA TC-3'); IVS10-2A>G SNP, those described previously;⁽¹⁹⁾ 972G>C (Gln324His) SNP: F1 (5'-CCT GTC GGG CAG TCC TGA CG-3'), R1 (5'-CGC TGA AGC TGC TCT GAG GGC-3'), F2 (5'-CCC AGC TCC CAA CAC TGG ACA C-3'), and R2 (5'-GAG GCA GGC ACA GGT GGC AC-3'); G382D SNP: F (5'-GCC CAA ATT CTG CTG GTG C-3') and R (5'-GCC CAA CGC TGT AGT TCC TG-3'); -280G>A SNP, F (5'-TAC TGT TCT CAT GGT GCC CC-3'), R (5'-GCC TCG GGC TCA TAG TTC TAG-3'), Ra (5'-GCG GGA GAT GTA GTC TGG AGT-3'), and Rg (5'-CGG GAG ATG TAG TCT GGA GC-3'). PCR products were fractionated by electrophoresis on a 2.0% agarose gel and stained with ethidium bromide. All the cases and controls were genotyped for all of the above SNPs.

Statistical analysis

Chi-squares tests were used for deviation from the Hardy-Weinberg equilibrium (HWE) among the controls, and the significance level was set at 0.05. Associations between *MUTYH* genotypes or haplotypes and risk of CRC were assessed by calculating the odds ratio (OR) and 95% confidence interval (CI). SAS version 8.2 software (SAS institute, Inc., Cary, NC) was used to perform the statistical analysis. A *P* value less than 0.05 was accepted as statistically significant in all cases. Adjustment for multiple testing was performed using

false discovery rate (FDR) principle.⁽²⁹⁾ Haplotypes were inferred by the expectation maximization algorithm with the SNPAnalyze Version 5.0 software (DYNACOM, Yokohama, Japan). Five haplotypes with a frequency of greater than 1% were selected for further statistical analysis. The linkage disequilibrium analysis of the haplotypes was performed using the SNPAnalyze Version 5.0 software (DYNACOM).

RT-PCR analysis

RT-PCR was performed for the IVS1+11C/C and C/T genotype, respectively, with 1 µl of each cDNA prepared from the non-cancerous colorectal mucosas of 6 CRC patients. The two primer pairs shown in Figure 2 were used: one pair composed of a forward primer in exon 1 and a reverse primer in exon 2, and the other pair composed of the same forward primer in exon 1 and a reverse primer in exon 3. The sequences of the primer sets are available on request.

Results

Target SNP selection

Among the variants registered in the dbSNPs of the NCBI Entrez system, there were 6

MUTYH SNPs, i.e., IVS1+11C>T (rs2275602), IVS1+1841G>A (rs3219472), IVS1+3221T>G (rs3219476), IVS6+35G>A (rs3219487), IVS14-40G>C (rs3219493) and 972G>C (Gln324His) (rs3219489), that have been detected in the Japanese population.

Among the SNPs reported in previous publications,^(19,20) 5 *MUTYH* SNPs, i.e., IVS1+11C>T (rs2275602), IVS6+35G>A (rs3219487), IVS10-2A>G (5'-flanking sequence: 5'-CAC TCA ACC CTG TGC CTC TC-3'; 3'-flanking sequence: 5'-GGT GGA GCA GGA ACA GCT CT-3'), 972G>C (Gln324His) (rs3219489), and 1389G>C (Thr463Thr) (5'-flanking sequence: 5'-CCA GGT GCT CGC TGG CTG AC-3'; 3'-flanking sequence: 5'-CAG GAG GAA TTT CAC ACC GC-3') have been reported in the Japanese population. However, since the IVS6+35G>A and IVS1+11C>T had been found to be in complete linkage disequilibrium with IVS14-40G>C and 1389G>C (Thr463Thr), respectively, the remaining 6 SNPs were initially selected as candidates. For the haplotype association analysis, a pilot study was performed by genotyping the 6 SNPs in 30 healthy Japanese individuals.

Analysis with the SNPalyze Version 5.0 software revealed the 5 haplotypes with a frequency of more than 1%, and they comprised all of the total predicted haplotype variation. As we were able to distinguish these 5 haplotypes with four SNPs, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G, and 972G>C (Gln324His), the 4 SNPs were ultimately chosen as the haplotype-tagging SNPs.

Association between the IVS1+11C>T SNP and increased risk of CRC

The 685 cases and 778 controls were genotyped for the *MUTYH* SNPs by PCR-CTPP, and the accuracy of the genotyping was verified by sequencing 5 specimens for each genotype of each SNP. The concordance rate was 100% (data not shown). The frequencies of each SNP are summarized in Table 1. The genotypic distributions of all the SNPs detected were in HWE. The IVS1+11C>T SNP, whose functional role has never been investigated, was shown to be statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI: 1.012~2.030; $P = 0.042$). After adjustments for gender, age and place of residence, the OR was estimated to be 1.46 (95% CI: 1.024~2.069; $P = 0.036$) (Table 1). The P value remained less than 0.05 after FDR adjustment (Table 1). No statistically significant differences in the frequency of any of the other three SNPs, IVS6+35G>A, IVS10-2A>G, and 972G>C, were observed between the cases and controls (Table 1). Furthermore, the association between the SNPs of *MUTYH* and the risk of CRC was examined by the anatomic subsite of the CRC. It showed the IVS1+11A/T + T/T genotypes were nearly statistically significantly associated with an increased risk of distal colon cancer risk (OR: 1.58; 95% CI: 0.984~2.544; $P = 0.058$) (Table 2). Since monoallelic mutation of G382D has recently been shown to be associated with CRC risk in Caucasians,⁽¹⁶⁾ the 685 cases and 778 control subjects were also examined for G382D, but no homozygotes or heterozygotes for this mutation were detected (data not shown). Because the complete linkage disequilibrium between IVS1+11C>T and 1389G>C had already been reported,⁽¹⁹⁾ the results suggested that

the IVS1+11C>T and 1389G>C variants of *MUTYH* may confer susceptibility to CRC in Japanese population.

Association between the TGAC haplotype containing the IVS1+11T and increased risk of CRC

Haplotype-based association studies are known to have greater power than individual-SNP-based association studies.⁽³⁰⁾ Haplotypes analysis were performed based on the genotyping data of the four SNPs of *MUTYH*, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G, and 972G>C. There were 5 haplotypes with a frequency greater than 1%, CGAC, CGAG, CAAG, TGAC, and CGGG (Table 3), and since the CGAC haplotype was detected in 42.7% of the controls (Table 3), the highest percentage, the CGAC haplotype was used as the reference haplotype, and the following statistical analysis was performed using the SAS system. Consistent with the results for each SNP, the TGAC haplotype containing the IVS1+11T allele was statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI: 1.005~2.029; $P = 0.046$) and after adjustment for gender, age and place of residence, the OR was 1.56 (95% CI: 1.098~2.228; $P = 0.013$) (Table 3). The P value remained less than 0.05 after FDR adjustment (Table 3). The results of subsite-specific analysis revealed a significant association between the TGAC haplotype and increased risk of distal colon cancer (OR: 1.81; 95% CI: 1.131~2.884; $P = 0.013$) (Table 4).

These results suggested that the TGAC haplotype containing the IVS1+11T SNP confers susceptibility to CRC, especially to distal colon cancer, in the Japanese population.

RT-PCR analysis and detection of a novel SNP -280G>A linked with IVS1+11C>T

The association between the IVS1+11C>T SNP of *MUTYH* and CRC risk suggested a functional difference between IVS1+11C and IVS1+11T. The IVS1+11C>T SNP is located in the boundary region between *MUTYH* exon 1 and intron 1, and many reports had suggested that gene variants in the neighborhood of the junction are often accompanied by abnormal splicing.⁽³¹⁻³³⁾ In order to investigate whether the IVS1+11C>T SNP affects the splicing of *MUTYH*, an RT-PCR analysis was performed by using cDNAs from carriers of the IVS1+11C/T and C/C genotype. However, no splicing abnormalities were detected in the cases carrying the IVS1+11C/T genotype (Fig. 2). On the other hand, during our checking of the sequences around the first exon of *MUTYH* to exclude variation at the splice site, a novel SNP of -280G>A was detected in the sample carrying the IVS1+11C>T SNP (Fig. 1B, C). Further genotyping was performed in all subjects, and -280G>A was found to be 100% linked with IVS1+11C>T. The -280G>A SNP was demonstrated to be in complete linkage disequilibrium with the IVS1+11C>T SNP ($r^2 = 1$) by the SNPalyze Version 5.0 software. Since the -280G>A SNP and 1389G>C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C>T SNP, the results suggested that the Japanese individuals

with the -280A / IVS1+11T / 1389C alleles or the -280A - IVS1+11T - IVS6+35G - IVS10-2A - 972C - 1389C haplotype were significantly associated with increased CRC risk.

Discussion

In this Japanese population-based case-control study four *MUTYH* SNPs, i.e., IVS1+11C>T, IVS6+35G>A, IVS10-2A>G, and 972G>C, were genotyped in 685 CRC cases and 778 controls. The frequency distribution of IVS1+11T and the IVS1+11T - IVS6+35G - IVS10-2A - 972C (TGAC) haplotype were significantly associated with increased CRC risk. Subsite-specific analysis showed that the IVS1+11C>T SNP was nearly statistically significantly associated ($P = 0.058$) and the TGAC haplotype were statistically significantly associated ($P = 0.013$) with an increased risk of distal colon, but not proximal colon or rectal cancer. Next, we found that the IVS1+11C>T SNP was in complete linkage disequilibrium with -280G>A. No aberrant splicing induced by IVS1+11T allele was detected by RT-PCR. Together with the previously detected 1389G>C (Thr463Thr), a SNP in complete linkage disequilibrium with IVS1+11C>T, our results suggested that individuals who have the *MUTYH* -280A / IVS1+11T / 1389C alleles or the TGAC haplotype are more susceptible to CRC in the Japanese population.

The present study is the first Japanese population-based CRC case-control study to evaluate the association between the SNPs of *MUTYH* and the risk of CRC by the anatomic

subsite of the colorectal cancer. The results demonstrate that the IVS1+11C>T SNP and TGAC haplotype confer susceptibility to distal colon cancer in the Japanese population. Since this study investigated the association between four SNPs and five haplotypes of *MUTYH* and the risk of CRC on the same set of samples, a method for multiple testing is applicable to this study. Therefore, the method of false discovery rate (FDR) was employed for all the results, and the statistical significance of the associations was found to remain essentially unchanged (Tables 1 and 3). These results coincide with the fact that tumors arising from different subsites of the colorectum differ in their population distribution, clinical features as well as genetic pathways.⁽³⁴⁻³⁷⁾ It was suggested from our results that the IVS1+11>T SNPs and TGAC haplotype of *MUTYH* may be involved in distal colon carcinogenesis and that the risk of cancer arising from each anatomic subsite of the colorectum may be modified by different genetic pathways. Further studies need to be conducted to elucidate the underlying mechanisms.

The development of CRC is a multistep, multifactor process.⁽³⁸⁾ Some studies have demonstrated that environmental factors and physical conditions may modify the genetic risk of CRC associated with SNPs.^(39,40) This may also hold true for the CRC risk associated with *MUTYH* SNPs. In the present study, adjustment was made for gender, age and place of residence to evaluate the association between the *MUTYH* SNPs and CRC risk. The adjusted OR and 95% CI remained essentially unchanged after the adjustments as compared with the values obtained without the adjustments (Tables 1 and 3). Furthermore, when the

BMI, disease history, physical activity, dietary factors, smoking and alcohol consumption status were taken into consideration, the *P* value for IVS1+11C>T was 0.058, a nearly significant value, and the *P* value for the TGAC haplotype remained less than 0.05 (data not shown). This result suggested that the association between the *MUTYH* SNPs and the risk of CRC was not significantly modified by environmental factors or the physical condition.

In the present study, the 972G/C genotype was statistically significantly associated with increased risk of proximal colon, but not distal colon or rectal cancer (Table 2). The functional analysis revealed no difference between the C/C type and G/G type,⁽⁴⁾ and the 972C allele is more frequently detected in Japanese and Chinese than in European populations as shown in the dbSNPs of the NCBI Entrez system. Taking this into consideration with our result, it could be suggested that the 972C allele may be inversely associated with the development of at least proximal colon cancer in the Japanese population. Alternatively, this inverse association of the 972C allele with the risk of proximal colon cancer in the Japanese population may arise from its interaction with other allele(s). The IVS10-2A>G SNP had been demonstrated to generate a protein without nuclear expression and the IVS10-2G allele was suggested to be associated with a low BER function in the cell nuclei and thereby, act as a risk allele for cancer. However, the results of analyses in this study revealed an OR of less than 0.7 (except for cancer of the proximal colon) for the IVS10-2G allele and the CGGG haplotype which contains the IVS10-2G allele, although the *P* value did not reach statistical significance (Tables 1-4). These results remained essentially unchanged even after

adjustments for environmental factors and physical conditions (as described above).

Investigation of some other additional clinical factors, such as pathological stage, recurrence or survival, might yield some association. On the other hand, some studies have suggested that SNPs of repair genes may be associated with reduced cancer risk or fewer recurrences, and that effective host DNA repair capacity may be associated with poorer survival.⁽⁴¹⁻⁴³⁾

These observations suggest that mutations in the repair genes may also be inversely associated with malignant alterations, in addition to their more widely recognized association with increased cancer risk. The inverse association of the IVS10-2A>G SNP detected in this study with colorectal cancer risk might be explained by the contention that individuals with the A allele may be more resistant to ROS or other stresses than individuals with the G allele, and that the A allele has a protective effect on cells with mutations, similar to the situation suggested by Wang et al. for the XRCC1 Arg194Trp variant, a SNP associated with a reduced risk for various types of cancers.⁽⁴⁴⁾ Researches on the effect of the *MUTYH* β isoforms on the cellular responses to various mutagens are expected help in clarifying this issue.

Besides the RT-PCR experiment, reporter assay was also performed to investigate whether the -280G>A and IVS1+11C>T SNPs may affect the promoter activity of *MUTYH*. The dual-luciferase reporter assay experiments detected high transcriptional activity of the region (-411 ~ +356) of *MUTYH* (data not shown). This information will be of use for future analyses. The reporter plasmids containing the wild-type and mutant sequence for the responses to oxidative stress were also investigated using the colon cancer cell line HCT116.

ROS was induced by glucose oxidase, menadione or H₂O₂ at appropriate concentrations and treatment durations. However, the two linked SNPs, -280G>A and IVS1+11C>T, did not affect the promoter activity in our setting (data not shown). This study did not detect any functional differences in the -280G>A / IVS1+11C>T / 1389G>C SNPs, and there remains the possibility that the three SNPs might be linked with other SNPs and these SNPs might affect the susceptibility to CRC.

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Legends to figures

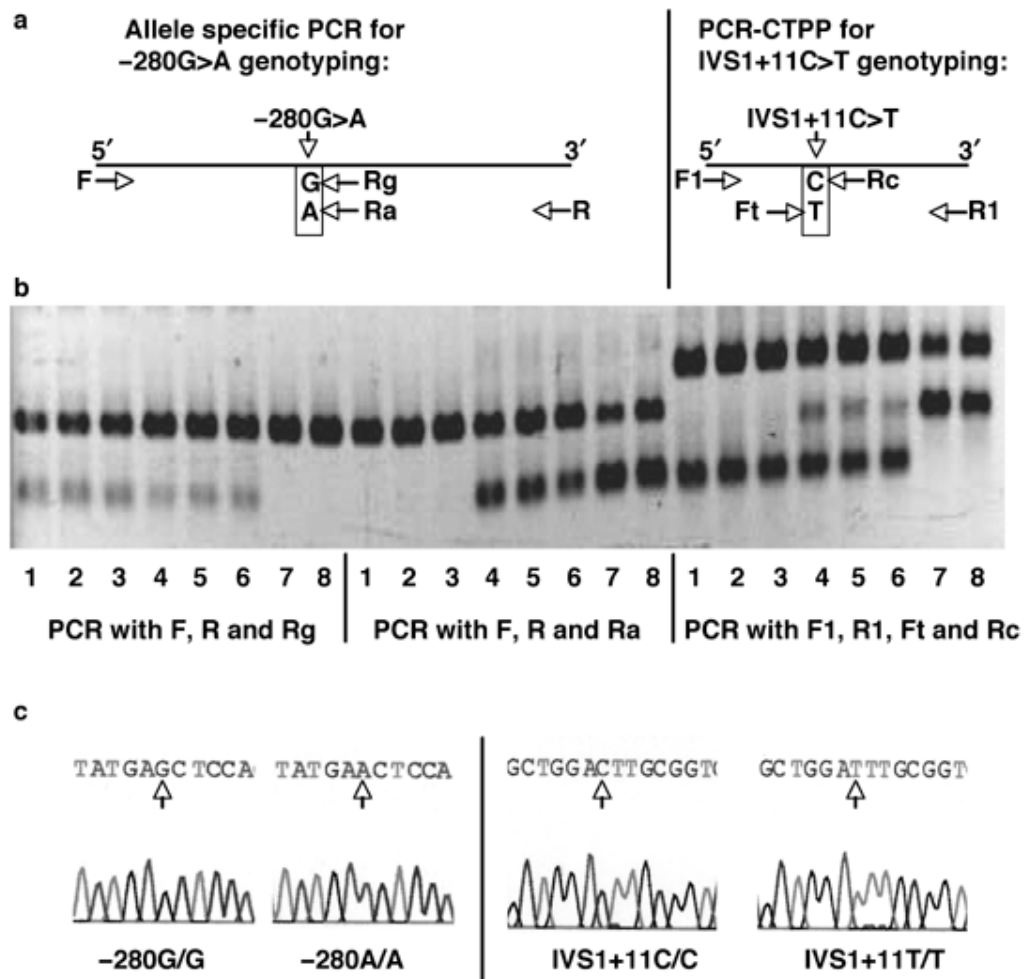


Figure 1. Genotyping of the -280G>A and IVS1+11C>T SNPs of the *MUTYH* gene. A.

The schematic diagrams of the allele-specific PCR used to genotype the -280G>A SNP (left)

and the PCR-CTPP used to genotype the IVS1+11C>T SNP (right). PCR primers are

indicated by the horizontal arrows, and F and R mean forward primer and reverse primer,

respectively. The location of each SNP is indicated by a vertical arrow. B. Agarose gel

electrophoresis of the PCR products. Eight samples, 3 from homozygous carriers of the

wild-type allele (No. 1-3), and 3 from heterozygous (No. 4-6) and 2 from homozygous (No. 7

and 8) carriers of the variation, were genotyped for -280G>A (left and middle) and

IVS1+11C>T (right). C. Sequence electropherograms of the region containing the -280G/G and A/A (left two) and IVS1+11C/C and T/T (right two). The positions of the SNPs are indicated by vertical arrows.

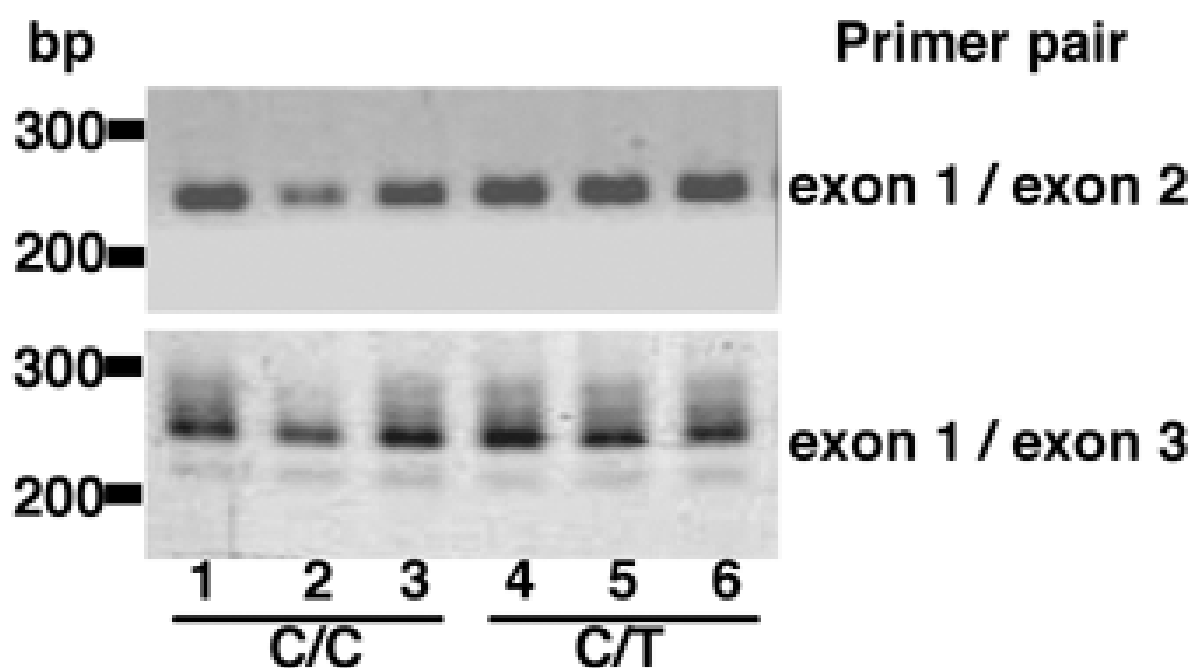


Figure 2. RT-PCR analysis. RT-PCR was performed with a set of primers located at exon 1 and exon 2 (upper panel) and a set of primers located at exon 1 and exon 3 (lower panel). cDNAs from 3 homozygous carriers of the wild-type allele (lanes 1-3) and 3 heterozygous carriers of the variation (lanes 4-6) were used as the templates.

Table 1. Genotypes of the four *MUTYH* SNPs and risk of colorectal cancer

Variation [†]	Genotype	No. of controls (%) / cases (%)	Not adjusted		Adjusted [‡]		
			OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	FDR adjusted <i>P</i> value [§]
IVS1+11C>T	C/C	714 (91.8) / 607 (88.6)	1.00 (reference)	-	1.00 (reference)	-	-
	C/T + T/T	64 (8.2) / 78 (11.4)	1.43 (1.012~2.030)	0.042	1.46 (1.024~2.069)	0.036	0.036
IVS6+35G>A	G/G	628 (80.7) / 539 (78.7)	1.00 (reference)	-	1.00 (reference)	-	-
	G/A	143 (18.4) / 140 (20.4)	1.14 (0.880~1.480)	0.321	1.14 (0.878~1.485)	0.321	0.963
	A/A	7 (0.9) / 6 (0.9)	1.00 (0.334~2.990)	0.998	0.97 (0.320~2.926)	0.953	>1.0
IVS10-2A>G	A/A	741 (95.2) / 662 (96.6)	1.00 (reference)	-	1.00 (reference)	-	-
	A/G + G/G	37 (4.8) / 23 (3.4)	0.70 (0.409~1.183)	0.178	0.67 (0.390~1.139)	0.138	0.276
972G>C	G/G	215 (27.6) / 194 (28.3)	1.00 (reference)	-	1.00 (reference)	-	-
(Gln324His)	G/C	395 (50.8) / 350 (51.1)	0.98 (0.771~1.250)	0.883	0.96 (0.751~1.223)	0.733	>1.0
	C/C	168 (21.6) / 141 (20.6)	0.93 (0.692~1.251)	0.632	0.90 (0.670~1.220)	0.511	>1.0

[†]Nucleotide +1 is the A of the ATG-translation initiation codon.

[‡]Adjustment was made for gender, 5-year age class, and residential area.

[§]False Discovery Rate (FDR) adjusted *P* value.

Table 2. *MUTYH* genotypes and the risk of CRC stratified by anatomic subsite

Variation [†]	Genotype	Proximal colon [‡] (n = 150)			Distal colon [‡] (n = 232)			Rectum [‡] (n = 290)		
		No.	OR (95% CI)	P value	No.	OR (95% CI)	P value	No.	OR (95% CI)	P value
IVS1+11C>T	C/C	133	1.00 (reference)	-	203	1.00 (reference)	-	259	1.00 (reference)	-
	C/T + T/T	17	1.50 (0.843~2.664)	0.169	29	1.58 (0.984~2.544)	0.058	31	1.36 (0.861~2.154)	0.187
IVS6+35G>A	G/G	122	1.00 (reference)	-	178	1.00 (reference)	-	229	1.00 (reference)	-
	G/A	28	1.03 (0.655~1.629)	0.887	51	1.27 (0.884~1.835)	0.195	58	1.07 (0.757~1.510)	0.703
	A/A	0	-	0.984	3	1.52 (0.383~6.056)	0.551	3	1.15 (0.286~4.581)	0.848
IVS10-2A>G	A/A	143	1.00 (reference)	-	226	1.00 (reference)	-	281	1.00 (reference)	-
	A/G + G/G	7	0.98 (0.422~2.268)	0.959	6	0.48 (0.197~1.156)	0.101	9	0.62 (0.295~1.319)	0.216
972G>C (Gln324His)	G/G	34	1.00 (reference)	-	72	1.00 (reference)	-	83	1.00 (reference)	-
	G/C	95	1.57 (1.020~2.426)	0.040	105	0.78 (0.553~1.108)	0.167	144	0.90 (0.655~1.247)	0.538
	C/C	21	0.78 (0.434~1.405)	0.409	55	0.99 (0.654~1.484)	0.943	63	0.94 (0.639~1.392)	0.768

[†]Nucleotide +1 is the A of the ATG-translation initiation codon.

[‡]Adjustment was made for gender, 5-year age class, and residential area.

Table 3. Haplotype frequency based on the four *MUTYH* SNPs and risk of colorectal cancer

Haplotype [†]				Frequency (%) [‡]		Not adjusted		Adjusted [§]		
IVS1+11C>T	IVS6+35G>A	IVS10-2A>G	972G>C	Control	Case	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	FDR adjusted <i>P</i> value [¶]
C	G	A	C	42.9	40.4	1.00 (reference)	-	1.00 (reference)	-	-
C	G	A	G	40.9	41.5	0.93 (0.791~1.089)	0.360	1.10 (0.936~1.293)	0.248	0.992
C	A	A	G	9.8	10.7	1.08 (0.840~1.397)	0.537	1.18 (0.910~1.519)	0.215	0.645
T	G	A	C	4.0	5.8	1.43 (1.005~2.029)	0.046	1.56 (1.098~2.228)	0.013	0.013
C	G	G	G	2.5	1.6	0.63 (0.370~1.079)	0.090	0.66 (0.387~1.136)	0.135	0.270

[†]Nucleotide +1 is the A of the ATG-translation initiation codon.

[‡]Inferred common haplotypes with frequency >1% were listed.

[§]Adjustment was made for gender, 5-year age class, and residential area.

[¶]False Discovery Rate (FDR) adjusted *P* values.

Table 4. *MUTYH* haplotypes and the risk of CRC stratified by anatomic subsite

Haplotype [†]				Proximal colon [‡]		Distal colon [‡]		Rectum [‡]	
IVS1+11C>T	IVS6+35G>A	IVS10-2A>G	972G>C	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value
C	G	A	C	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
C	G	A	G	1.13 (0.855~1.484)	0.397	1.10 (0.871~1.389)	0.424	1.06 (0.856~1.311)	0.595
C	A	A	G	1.00 (0.634~1.587)	0.990	1.27 (0.885~1.816)	0.196	1.15 (0.822~1.594)	0.424
T	G	A	C	1.61 (0.904~2.879)	0.106	1.81 (1.131~2.884)	0.013	1.36 (0.854~2.166)	0.195
C	G	G	G	0.99 (0.430~2.296)	0.988	0.43 (0.166~1.116)	0.083	0.62 (0.296~1.316)	0.216

[†]Nucleotide +1 is the A of the ATG-translation initiation codon.

[‡]Adjustment was made for gender, 5-year age class, and residential area.