

Absence of *MutY* homologue mutation in patients with multiple sporadic adenomatous polyps in Korea

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Abstract

AIM: Recently, germ-line mutation in the base excision repair gene *MYH* has been identified to cause a novel autosomal recessive form of familial adenomatous polyposis (FAP). Interestingly, a striking evidence for *MYH* mutations within different ethnic groups has been demonstrated. In this study, we screened 30 patients with multiple adenomatous polyps for *MYH* mutations to assess its prevalence and ethnic specificity in Korea.

METHODS: Thirty patients (21 men and 9 women; mean age 62.3 years) with multiple adenomatous polyps were examined for *MYH* mutations. The mean number of adenomas per patient was 10.0. Sixteen exonic regions and their intronic sequences were amplified by PCR and subjected to SSCP and DNA sequencing analyses.

RESULTS: None of the patients was identified to carry any truncating or sequence alterations in *MYH*. Our screening for the mutational regions, which were recognized from Caucasian patients or affected Indian families, also failed to detect sequence substitutions.

CONCLUSION: Mutation in *MYH* may be rarely involved in the pathogenesis of multiple sporadic colorectal adenomas in Korean population, although a large-scale analysis will be required to clarify the presence of specific *MYH* variants in a subset of patients and their role in the predisposition of multiple colorectal adenomas in Korean population.

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Key words: MY; Multiple adenomatous polyps; Germ-line mutation; Familial adenomatous polyposis; Ethnic difference

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INTRODUCTION

It has been well established that genetic factors play a pivotal role in up to 35% of all colorectal cancers (CRC)^[1-3]. Familial adenomatous polyposis (FAP) is an autosomal dominant disorder with an increased predisposition to multiple colorectal adenomatous polyps, and thence to CRCs^[4]. Classic FAP is caused by inherited mutations in the adenomatous polyposis coli (*APC*) gene, which encodes a protein that plays a critical role in the regulation of colonic cell growth^[5,6]. Attenuated FAP (AFAP) is associated with smaller numbers of adenomas and is caused by mutations in the extreme of 5' or 3' ends of *APC* or in the alternatively spliced region of exon 9^[4,5].

Somatic mutations of *APC* consist of the substitution of a thymine-adenine pair for a guanine-cytosine pair (G:C→T:A), which is a typical change caused by oxidative damage to DNA^[7-11]. Oxidative DNA damage produces the stable 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxoG)^[9]. It is highly mutagenic because it tends to mispair with adenine residues, leading to an increased frequency of spontaneous G:C→T:A transversion mutations in repair-deficient bacteria and yeast cells^[10-13].

Recently, Al-Tassan *et al*^[14] reported a novel autosomal recessive form of FAP. They found that three of seven siblings from a single British Caucasian family were all compound heterozygous for two non-conservative missense variants, Y165C and G382D, in the base excision repair gene *MutY* homologue (*MYH*). This finding first implicated a defect in the base excision repair mechanism in inherited predisposition to colorectal tumors in human beings.

Base excision repair (BER) is cell's mechanism of protection against oxidative DNA damage^[15]. The BER pathway repairs mutations caused by reactive oxygen species that are generated during aerobic metabolism^[16]. Three main components of BER have been identified in human. *MutT* homologue (*MTH*) removes oxidized base from 8-oxoG:C pairs to reduce the chances of incorporation of

Table 1 Characteristics of patients

Characteristics	Patient with CRC and adenomatous polyp (n = 10)	Patient with adenomatous polyp only (n = 20)
Sex	3 males : 1 females	15 males : 5 females
Age (yr)	Mean 45.9 (range: 21 ~ 78)	Mean 51.8 (range: 44 ~ 69)
Number of adenomatous polyps	Mean 9 (range: 1 ~ 10)	Mean 7 (range: 3 ~ 100)
Family history of adenomatous polyps	0	0

CRC: colorectal cancer.

8-oxoG during DNA replication, and 8-oxoG glycosylase (OGG) initiates base-excision of 8-oxoG from 8-oxoG:C pairs^[17]. Adenine-specific DNA glycosylase *MYH* removes adenine mispaired with 8-oxoG in 8-oxoG:A pairs.^[18-20]

The prevalence of *MYH* mutations has been determined in patients with multiple colorectal polyps in Caucasian populations. However, to our knowledge, a few studies have been carried out regarding the prevalence of *MYH* mutations in Asian patients with multiple colorectal adenomatous polyps. In the present study, we therefore explored the possible implication of germ-line mutations of *MYH* in the development of multiple sporadic colorectal adenomatous polyps in Korean population.

METHODS

Specimens

Thirty patients (21 men and 9 women; mean age, 62.3 years) with multiple sporadic colorectal adenomatous polyps were recruited from Kyung Hee University, Medical Center, Seoul, Korea. All patients were Korean and none had a family history of vertical transmission of colorectal cancer or adenomatous polyps. Five individuals who did not have any recognizable diseases were selected as healthy controls. Informed consent was taken from all patients. Ten milliliters of blood were obtained from all the patients and healthy controls for extraction of genomic DNA. The characteristics of patients are summarized in Table 1.

PCR amplification of *MYH* gene

Genomic DNA was prepared from venous blood samples using QIAGEN Genomic-tip system (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of extracted DNA was determined by a spectrophotometric measurement (Schimadzu Scientific Instruments, Inc., Concord, CA). Exons 1-16 of *MYH* and their flanking intronic sequences were amplified by PCR with 14 primer sets which cover the entire coding region of *MYH*. Primer sequences used for PCR amplification are shown in Table 2. PCR was performed with 200 ng genomic DNA as template for 38 cycles at 95°C (1 min), 58-62°C (30 s), and 72°C (30 s) in 1.5 mmol/L MgCl₂-containing reaction buffer (PCR buffer II, Perkin Elmer-Cetus, Concord, CA). Ten microliters of PCR products were electrophoresed on 20 g/L agarose gel containing ethidium bromide, visualized under ultraviolet light, and photographed.

Single strand conformation polymorphism analysis

To identify sequence alterations in the *MYH* gene, we

Table 2 Primer sequences of oligonucleotide primers used for PCR-SSCP analysis of *MYH*

Exon	Primers	Sequences (5' to 3')	Length (bp)
1	M1S	CAGAGCGCAGAGGCTTTGAACA	240
	M1AS	CTGAACGGAAGTTTCGACCCATC	
2	M2S	AATTTGGCCTCATTGTGACTGA	221
	M2AS	AATCTGCCTTTTCATGGCCAATG	
3	M3S	CACAGGCTGTGTGTCCCAAGA	259
	M3AS	CCCACCCACTGTCCCTGCTCCT	
4+5	M45S	AACTCCTCATCTGGGGTTGCAT	296
	M45AS	GGTCTGACCCATGACCCTTCCC	
6+7	M67S	ACCACCTTCACCTTGACCTTG	268
	M67AS	ACCCAAGACTCCTGGGTTCCCTA	
8	M8S	GGAACCCAGGAGTCTTGGGTGT	222
	M8AS	AAGGAGGCTGGGCACGCACAAA	
9	M9S	TTTGTGCGTGCCAGCCTGGTT	223
	M9AS	TGCTGTGAAGCAGAGCTCCAAA	
10	M10S	AAAGGAGCTCTGCTTCACAGCA	227
	M10AS	CACTCCTTAGGACTTCTCACTG	
11	M11S	GTAAGCCTACTGGGGAAGGGG	222
	M11AS	GCAGAATCTTACTCAGGTTAG	
12	M12S	GCCCTCTTGGCTTGAGTAGGGT	278
	M12AS	TCTCTTGTACTCATGCCACTG	
13	M13S	AGGGAATCGGCAGCTGAGGCCCT	226
	M13AS	AAAAGCCAACATCCTTGGCTAT	
14	M14S	TATATCCACAGGCCTATTGAA	256
	M14AS	ATATTCATGTAGAACATGTAGG	
15	M15S	GACATGAAGTTAAGGGCAGAAC	212
	M15AS	TGTTACCCAGACATTCGTTAG	
16	M16S	AACTACAAGGCCTCCTCCTTCCA	270
	M16AS	AACAACAGGATTCTCAGGGAATG	

performed nonisotopic PCR-SSCP analysis as described previously^[14]. Briefly, 20 µL of the PCR products was mixed with 5 µL of 0.5 mol/L NaOH, 10 mmol/L EDTA and 10 µL of denaturing loading buffer (950 mL/L formamide, 20 mmol/L EDTA, 0.5 g/L bromophenol blue, and 0.5 g/L xylene cyanol). After heating at 95°C for 5 min, the samples were rapidly loaded in wells pre-cooled to 4°C and run simultaneously on two 80 g/L non-denaturing polyacrylamide gels with or without 100 g/L glycerol. These two gels were run at 18-20°C and then repeated at 6-10°C in a buffer-jacketed gel apparatus (DGGE-II; Aladin Enterprises, Inc., San Francisco, CA). Following an 8 h run at 400 volts, the gels were stained with ethidium bromide and photographed under ultraviolet light.

Automated DNA sequencing

PCR amplification products were purified using the PCR purification kit (Qiagen). DNA sequencing was carried out using ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) according to the

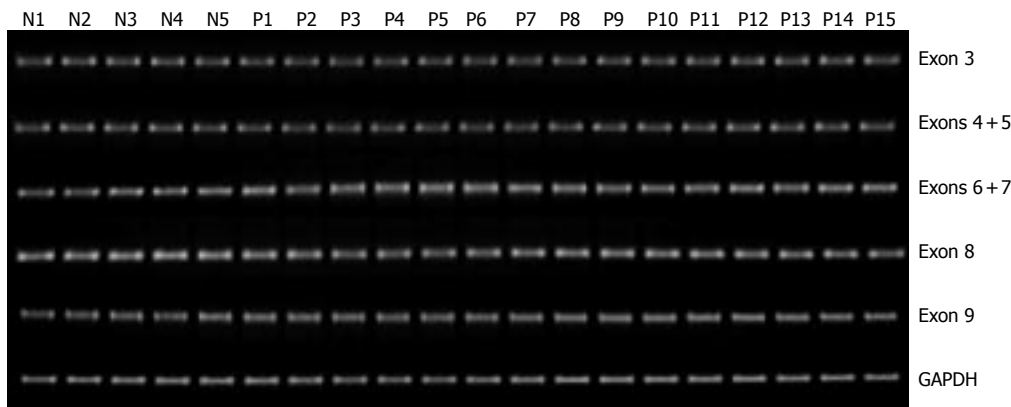


Figure 1 Genomic status of *MYH* in patients with multiple colorectal adenomas determined by quantitative DNA-PCR analysis. Representative examples for exons 3-9 amplification are shown. Two hundred nanograms of DNA obtained from 30 patients and 5 healthy controls were used as templates for PCR amplification of 16 exonic regions using 14 intronic primer sets. *GAPDH* was used as an internal control for PCR. Ten microliters of PCR product was resolved on 20 g/L agarose gel and visualized by ethidium bromide staining. No significant reduction of gene levels was identified in samples from patients as compared with normal controls. Lanes N1-N5: Normal controls; Lanes P1-P15: Patients.

manufacturer's instructions. Sequencing was carried out in both directions to confirm the findings.

RESULTS

To explore the presence of germ-line alterations of *MYH*, we, using quantitative genomic PCR analysis, initially evaluated genomic status of *MYH*. Blood DNA samples from 30 patients with multiple sporadic colorectal adenomatous polyps and 5 healthy individuals were subjected to PCR amplification of *MYH*. All of the 16 exons and flanking intronic sequences of the gene were amplified as 14 fragments using intron-specific primer sets, and their levels in the patients were compared with those in healthy controls. As shown in Figure 1, genomic levels of *MYH*, which were evaluated for 14 separate gene regions, showed no detectable difference between patients and healthy individuals, and none of the patients was found to have structural alteration within these protein-encoding regions of the gene. Although systemic analysis for the genomic status was not carried out in this study, the results suggested that our patients did not harbor germ-line deletion or structural abnormalities of the gene, which could result in loss or significant reduction of *MYH* protein function.

Using PCR-SSCP and DNA sequencing analyses, we next screened for mutations and sequence variants across the entire coding region of the *MYH* gene. To detect the *MYH* variants, such as Y165C, G382D, Y90X and E466X, which are commonly observed in Caucasian patients or affected Indian families, we performed comprehensive SSCP assay under four different running conditions^[14]. However, we could not observe any truncating *MYH* mutations in patient or control samples. Neither did we identify any likely pathogenic mutations and sequence variants in the patient samples. Representative examples of SSCP analysis are shown in Figure 2.

DISCUSSION

Germ-line mutation of the *MYH* gene has been known to

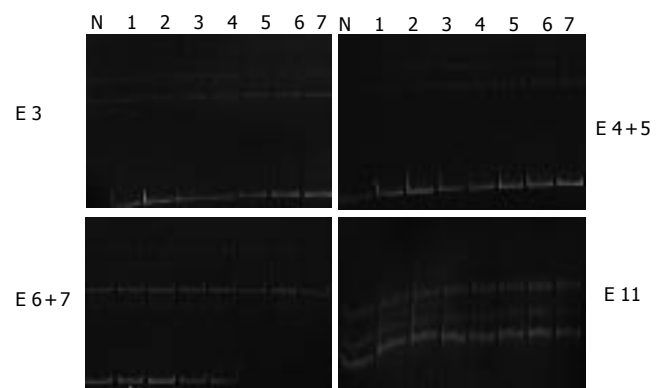


Figure 2 Non-isotopic PCR-SSCP analysis of *MYH*. For detection of sequence alterations, all of the 16 exons and flanking intronic sequences were amplified by PCR as 14 separate fragments, and 20 μ L of PCR product was subjected to non-isotopic SSCP analysis. Genomic DNA isolated from the healthy controls was used as normal controls. None of patient samples showed abnormal migration shift of single strand DNA molecules. Lane N: Normal control; Lanes 1-7: Patients; Lane E: Exons.

predispose persons in a variety of European populations to recessive inheritance of multiple colorectal adenomatous polyposis and classic FAP^[4, 22]. In addition, all patients with biallelic *MYH* mutations have an increased predisposition to CRCs.

MYH mutation presents with a distinguished genetic pathway in developing colorectal tumors. Many questions regarding its role in pathogenesis of multiple colorectal adenomatous polyps and CRCs still remain open. For example, it has not been identified why germ-line *MYH* mutations are associated with tumors of gastrointestinal tract, or why *MYH* polyposis differs in its phenotype and inheritance from classical FAP^[21].

It is difficult to distinguish between patients with *APC* mutations and those with *MYH* germ-line mutation on the basis of clinical features or pathological findings. *MYH* mutations seem to be a more common cause of the multiple adenoma phenotypes than are *APC* mutations^[23-26]. They are, however, a less common cause of classic colorectal adenomatous polyposis.

Jones *et al*^[4] could not identify any pathogenic variants in BER genes *OGG1* or *MTH1* in cases with colorectal tumors. Thus, there is a possibility that these genes are less frequently mutated than *MYH*. It is also possible that those mutations do not predispose to tumors in humans due to unknown mechanism. However, further studies are required to determine whether *OGG1* or *MTH1* are involved in CRC predisposition.

On the basis of previous studies, genetic analysis of *MYH* mutation should be considered for patients with a phenotype resembling FAP or AFAP, when no clear evidence of vertical transmission is noted. However, whether it is worthwhile to perform genetic testing in the family of patients with *MYH* mutations is a question that still remains unclear.

In the present study, we failed to detect any truncating or sequence alterations of the *MYH* gene in Korean patients with multiple colorectal adenomas. Although patient numbers enrolled in this study are too small to exclude the association of *MYH* mutations with development of multiple colonic adenoma in Korean population, our data suggests the presence of ethnic difference in contribution of *MYH* mutations to disease development between the Korean patients and the white patients. Previous studies identified four British families that were either homozygous for Y165C or compound heterozygous for Y165C/G382D, three Indian families that were homozygous for E466X, and a single Pakistani family that was homozygous for Y90X^[4]. Specific *MYH* mutations appear to be identified in different ethnic groups. A question still remains as to how frequently *MYH* mutations contribute to the phenotype of apparently sporadic AFAP/FAP. Further studies of patients from distinct geographical and ethnic groups will help to define the possible ethnic differences in actual mutations.

In conclusion, our observations suggest that *MYH* mutations are not common in patients with multiple colorectal adenomas in Korean population. Considering that number and histology of colorectal polyps are the cornerstones of detection of many CRC predisposition conditions, further larger-scale evaluation including the histologic analyses of the polyps, is required to determine whether *MYH* polymorphisms play a role in predisposition of a subset of multiple sporadic colorectal adenomas in Korean population.

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