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

Hansoo Kim, Hyo-Jong Kim, Sung-Gil Chi, Sang-Kil Lee, Gwang-Ro Joo, Seok-Ho Dong, Byung-Ho Kim, Young-Woon Chang, Jung-II Lee, Rin Chang

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

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 sporoic 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 (Shimadzu 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 performed nonisotopic PCR-SSCP analysis as described previously. 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

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**Table 1 Characteristics of patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient with CRC and adenomatous polyp (n = 10)</th>
<th>Patient with adenomatous polyp only (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>3 males : 1 females</td>
<td>15 males : 5 females</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>Mean 65.3 (range: 21-78)</td>
<td>Mean 51.8 (range: 44-69)</td>
</tr>
<tr>
<td>Number of adenomatous polyps</td>
<td>Mean 9 (range: 1-10)</td>
<td>Mean 7 (range: 3-100)</td>
</tr>
<tr>
<td>Family history of adenomatous polyps</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CRC: colorectal cancer.

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

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primers</th>
<th>Sequences (5’ to 3’)</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1S</td>
<td>CAGACGGCGAGGCTTGAGCA</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>M2S</td>
<td>TGACAGGGATCTTGAGCA</td>
<td>221</td>
</tr>
<tr>
<td>3</td>
<td>M3S</td>
<td>CCAACGGACGGCATGCGCC</td>
<td>259</td>
</tr>
<tr>
<td>4+5</td>
<td>M4S</td>
<td>AACTCCCTATGCTGGTCCTC</td>
<td>296</td>
</tr>
<tr>
<td>6+7</td>
<td>M67S</td>
<td>ACCACCTCCACCCTGATGTC</td>
<td>268</td>
</tr>
<tr>
<td>8</td>
<td>M8S</td>
<td>GAAGGCAAGAGGCTTGGG</td>
<td>222</td>
</tr>
<tr>
<td>9</td>
<td>M9S</td>
<td>TTTGCAGCTCCAGCTTGTT</td>
<td>223</td>
</tr>
<tr>
<td>10</td>
<td>M10S</td>
<td>AAGGGAGCTGTCCGACAGCA</td>
<td>227</td>
</tr>
<tr>
<td>11</td>
<td>M11S</td>
<td>GTAGCCCTACTGCGGAGG</td>
<td>222</td>
</tr>
<tr>
<td>12</td>
<td>M12S</td>
<td>GCGAGATCTTACAGGTGT</td>
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<tr>
<td>13</td>
<td>M13S</td>
<td>TCTTGTGATCTAGCAGTCT</td>
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<tr>
<td>14</td>
<td>M14S</td>
<td>AAAGCAGGACATCTGCTCC</td>
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<td>15</td>
<td>M15S</td>
<td>GACATGAGTGAAGGCGGAG</td>
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<tr>
<td>16</td>
<td>M16S</td>
<td>TGTCACAGGAGATTCAGT</td>
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</tr>
<tr>
<td>16</td>
<td>M16AS</td>
<td>AACAACAGGGATTCTAGAG</td>
<td>270</td>
</tr>
</tbody>
</table>

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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 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 bialleic 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. 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. Specific MYH mutations appear to be identified in different ethnic groups. A question still remains as to how frequently 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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