Identification of Coding Exon 3 Duplication in the BMPR1A Gene in a Patient with Juvenile Polyposis Syndrome

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Juvenile polyposis syndrome is an autosomal dominant inherited disorder characterized by multiple juvenile polyps arising in the gastrointestinal tract and an increased risk of gastrointestinal cancers, specifically colon cancer. BMPR1A and SMAD4 germline mutations have been found in patients with juvenile polyposis syndrome. We identified a BMPR1A mutation, which involves a duplication of coding exon 3 (c.230 + 452_333 + 441dup1995), on multiple ligation dependent probe amplification in a patient with juvenile polyposis syndrome. The mutation causes a frameshift, producing a truncated protein (p.D112NfsX2). Therefore, the mutation is believed to be pathogenic. We also identified a duplication breakpoint in which Alu sequences are located. These results suggest that the duplication event resulted from recombination between Alu sequences. To our knowledge, partial duplication in the BMPR1A gene has not been reported previously. This is the first case report to document coding exon 3 duplication in the BMPR1A gene in a patient with juvenile polyposis syndrome.

Key words: juvenile polyposis syndrome – BMPR1A – duplication – colon cancer

INTRODUCTION

Juvenile polyposis syndrome (JPS; OMIM 174900) is an autosomal dominant inherited disorder that predisposes a patient to the development of multiple juvenile polyps in the gastrointestinal tract (1). Affected individuals have an increased risk of colorectal and upper gastrointestinal cancers (2,3). JPS is caused by a germline mutation of the BMPR1A and SMAD4 genes, which are members of the TGF-β superfamily (4,5). Germline mutation of BMPR1A or SMAD4 has been found in ~20% of a large series of patients with JPS, respectively (6). Recently, several point mutations and large deletions in the BMPR1A and SMAD4 genes have been identified in patients with JPS on DNA sequencing and multiplex ligation dependent probe amplification (MLPA) analysis. For example, Calvac-Cerqueira et al. (7) have described 24 BMPR1A germline mutations in 102 (23.5%) of a large series of patients with JPS. Twenty-two of the 24 mutations were missense, nonsense, small insertions or small deletions, and the other two were large deletions. In this study, we identified a novel BMPR1A mutation involving a duplication of coding exon 3 of the BMPR1A gene in a 31-year-old Japanese man with JPS. Moreover, we found Alu sequences in the duplication breakpoint sequence on long-range polymerase chain reaction (PCR) and DNA sequencing. These results suggested that recombination between Alu...
elements is involved in a 1995 bp duplication event. Most BMPR1A mutations reported to date are single base substitutions and small insertions/deletions leading to missense, nonsense or frameshift mutations. To our knowledge, a partial duplication in the BMPR1A gene has not been reported previously in a patient with JPS. This is the first report to document partial duplication in the BMPR1A gene by Alu-mediated recombination in a patient with JPS.

PATIENTS AND METHODS

PATIENT AND SAMPLE COLLECTION

The patient (IV-2, Fig. 1) was a 31-year-old Japanese man who presented with gastrointestinal bleeding and abdominal pain. Endoscopic examination revealed multiple adenomatous and juvenile polyps in the colon and rectum, and four cancers were simultaneously found in the cecum, ascending colon and transverse colon. JPS was clinically diagnosed on the basis of the endoscopic findings. He and his family members (III-2, III-9, IV-1 and IV-3) were examined for germline mutations of the BMPR1A and SMAD4 genes after written informed consent had been obtained. All subjects received genetic counseling. For genetic testing, genomic DNA was isolated from peripheral blood samples obtained from the subjects, performed using a QIAamp DNA Blood Maxi or Mini Kit (QIAGEN) according to the manufacturer’s protocol.

DETECTION OF CODING EXON 3 DUPLICATION IN BMPR1A GENE

BMPR1A and SMAD4 mutations were examined using MLPA analysis, because pathogenic germline mutations were not...
found in either gene on PCR-direct sequencing. MLPA analysis was performed using a SALSA MLPA kit with P158-B1 probe mix (MRC-Holland). MLPA was carried out according to the manufacturer’s protocol, and the results were analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter).

ANALYSIS OF DUPLICATION BREAKPOINT SEQUENCE

To amplify PCR products for BMPR1A breakpoint sequencing, we designed forward primer 5'-GGG TGT ATG AAA TAT GAA GGA TCT G-3' at the 3'-side of coding exon 3 and reverse primer 5'-TCT ATG ATG GCA AAG CAA TGT CC-3' at the 5'-side of coding exon 3 in the BMPR1A gene. The breakpoint sequence was amplified by using Expand Long-Range dNTPack (Roche) according to the manufacturer’s instructions. PCR amplification consisted of initial denaturation at 92°C for 2 min, followed by 35 cycles of denaturation at 92°C for 10 s, annealing at 60°C for 15 s and extension at 68°C for 10 min. DNA fragments were separated by 1% agarose gel electrophoresis and were purified using a FastGene Gel/PCR Extraction kit (NIPPON Genetics). Purified PCR fragments were sequenced using a Big Dye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s protocol. The results were analyzed on an ABI 310 Genetic Analyzer (Applied Biosystems). Sequencing primer (5'-ATT TCC ATA GAC AGC ACC TG-3') was prepared for breakpoint sequencing.

RESULTS AND DISCUSSION

Subject IV-2 was found to have multiple adenomatous and juvenile polyps in the colon and rectum. Juvenile polyps are observed.
hamartomatous polyps characterized by cystically dilated glands, abundant lamina propria and inflammatory cell infiltration. The juvenile polyps were diagnosed by pathologist (Fig. 2A). The presence of these juvenile polyps satisfied the clinical criteria for JPS, i.e. the presence of more than five juvenile polyps in the colorectum, juvenile polyps in the gastrointestinal tract or any number of juvenile polyps with a family history of JPS (8). The patient therefore met the clinical criteria for JPS. He was also found to develop colon cancers and subsequently, he underwent a right hemicolectomy (Fig. 2B). His father (III-2) had 12 adenomatous polyps in the colon at the age of 66 years. His mother (III-9) and younger brother (IV-3) had three adenomatous polyps in the colon at the age of 57 years and 23 years, respectively. His elder sister (IV-1) had no polyps in the colon at the age of 32 years. Colon cancer has not developed in any of these family members of Subject IV-2 until now (Fig. 1).

At first, the proband (IV-2) was screened for germline mutations of BMPRIA and SMAD4 genes after informed consent had been obtained. DNA sequencing of all exons and intron/exon boundaries in these genes was performed. Because no pathogenic mutation was detected in these genes, the genetic analysis was followed by MLPA to search for large genomic rearrangements. We detected a partial duplication (c.230+452_333+441dup1995) that included coding exon 3 in Subject IV-2 (Fig. 3). This duplication was confirmed on three independent MLPA analyses. This duplication variant was not detected in the other family members (III-2, III-9, IV-1 and IV-3) on MLPA analysis. These results support the notion that the duplication identified in Subject IV-2 is a de novo mutation.

To characterize the breakpoint sequence, we amplified genomic DNA of the breakpoint region by long-range PCR. DNA sequencing analysis was then performed. We detected
an ~2 kb band in Subject IV-2, which was not detected in the other family members (Fig. 4C). These results are consistent with the findings of MLPA analysis. Sequencing analysis of this fragment revealed that a 1995 bp duplication was located in a tandem head-to-tail orientation (Fig. 4A). We also found that the breakpoint junction contained Alu sequences FLAM-C of intron 2 and AluJb of intron 3, and these two repeat elements included 42 bp homologous sequences (Fig. 4A and B). Alu sequences are the most abundant repetitive sequence, accounting for ~10% of the human genome (9,10). Recent studies have demonstrated that Alu-mediated homologous recombination leads to genomic duplication in other human diseases, such as acute leukemia, Birt–Hogg–Dubé syndrome, and hereditary breast and ovarian cancer (11–13). It is likely that the genomic duplication in Subject IV-2 was caused by homologous recombination between FLAM-C and AluJb sequences. The duplication is expected to cause a frameshift and create a premature stop codon in the MH1 domain, lacking the protein kinase domain. BMPRIA is thought to be involved in BMP signaling by phosphorylation of intracellular SMAD1, 5 and 8 proteins, which then form complexes with SMAD4 protein (14,15). The complexes then localize to nuclei and regulate transcription of specific genes (16). The duplication was thereby predicted to cause defects of BMP-controlled intracellular signaling due to loss of the kinase domain and appears to be pathogenic.

Recently, several different types of mutations associated with inactivation of BMPRIA have been reported in JPS. van Hattem et al. (17) have described six BMPRIA mutations consisting of one missense mutation, one small deletion, one splice-site mutation and three large deletions (deletions of exon 10–11 and the entire BMPRIA gene) in 27 patients with JPS. Pyatt et al. (18) have identified eight BMPRIA mutations, including one missense, one nonsense mutation, two small deletions, two small insertions, one splice-site mutation and one small deletion/insertion in 70 patients with JPS. Aretz et al. (19) have reported 16 BMPRIA mutations in 80 unrelated families with JPS, including four missense mutations, five nonsense mutations, two small deletions, two splice-site mutations and three large deletions (deletions of coding exon 1, noncoding exon 1—coding exon 1 and the entire BMPRIA gene). In addition, germline mutations of the BMPRIA promoter region have also been described in a large JPS kindred (20). To our knowledge, no partial duplication in the BMPRIA gene has been described to date. We herein reported a novel partial duplication in the BMPRIA gene associated with Alu-mediated recombination in a Japanese patient with JPS, who showed a novel pathogenic duplication of exon 3 in the BMPRIA gene.

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**Conflict of interest statement**

None declared.

**References**