Promotion Effects of Smoking in Polyp Development in Monozygotic Twins with Atypical Colorectal Polyposis

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Abstract
Smoking is a known risk factor for the development of colorectal polyps. Even in familial adenomatous polyposis and serrated polyposis syndrome, smoking is a risk factor of the development of polyps. Here, we report a case of monozygotic twins with atypical colorectal polyposis showing lots of hyperplastic polyps and adenomas and describe how the polyposis developed differently in the brothers based on the presence or absence of smoking. The case was of a set of monozygotic male twins, and the twins were in their 50s. The younger brother smoked 40 cigarettes a day since he was 16 years old. The older brother had smoked about 25 cigarettes a day since he was 16 years old but stopped smoking after he was diagnosed with polyposis. As we previously reported, we managed to remove polyps as much as possible from both twins without surgery. The median number of removed polyps (IQR: 25–75%) per colonoscopy for 20 years was 9.0 (3.5–14.8) in the older brother and 20.5 (7.5–35.5) in the younger brother. There was a significant difference between the twins ($p < 0.01$). Additionally, genetic tests found that the twins carried a rare missense variant of \textit{BRCA2}, and this variation has not been previously reported. In conclusion, these monozygotic twins with atypical colorectal polyposis showing a new variant of \textit{BRCA2} suggest that smoking is related to the development of polyposis...
of colorectal polyps. Further analysis will be required for the identified BRCA2 variant in possible involvement in the development of atypical polyposis.

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Introduction

In colorectal cancer (CRC), 35% of cases are associated with a family history, and 2–5% are hereditary CRCs with mutations in APC, MUTYH, DNA mismatch repair genes, or other related genes [1]. In contrast, colorectal polyposis is divided into adenomatous polyposis represented by familial colorectal adenoma (FAP), hamartomatous polyposis of Peutz-Jeghers syndrome, and serrated polyposis syndrome (SPS). Many of these diseases are autosomal dominant hereditary diseases, and there are many causative genes that have been reported in the literature [1]. FAP is caused by a mutation on the APC gene (5q22.2) on chromosome five, follows autosomal dominant inheritance, and causes a high rate of CRC development [2]. However, 20–40% of FAP cases are not associated with pathological variants of the APC gene; in those cases, MUTYH-associated polyposis or polymerase proofreading-associated polyposis, or unknown genetic abnormalities such as polyposis, may be considered [3]. In FAP, various studies have explored the role of environmental factors in the development of colorectal adenoma, and drinking, obesity, and smoking have been identified to promote adenoma development, while exercise has been suggested to reduce development [4]. Additionally, a meta-analysis provides strong evidence of the detrimental effect of cigarette smoking on the development of adenomatous polyps [5].

In this report, we examined a case of monozygotic twins with atypical colorectal polyposis showing a large number of hyperplastic polyps and adenomas and describe how this polyposis developed differently according to the presence or absence of smoking behavior. Additionally, we conducted a genetic analysis of these cases, which identified a rare variant of BRCA2, which has a possibility to be related with the development of atypical colorectal polyposis.

Case Report

The case was of a set of monozygotic male twins, and the twins were in their 50s. The younger brother underwent colonoscopy at another hospital at 30 years of age and had a large number of polyps, of which approximately 10 polyps of >5 mm in size were resected endoscopically. Of these lesions, 3 lesions were found to be high-grade dysplasia (HGD) (Intramucosal cancer in Japan), and the patient consulted us for follow-up. At that time, his older twin brother had no particular symptoms but consulted us for colonoscopy. Regarding family history, their sister was in her 40s and had pancreatic cancer, and his mother had a goiter. The younger brother smoked 40 cigarettes a day from the age of 16 years. He was 179 cm tall, weighed 75.0 kg, and had a BMI of 23.4. His older twin had smoked about 25 cigarettes a day since he was 16 years old, but he stopped after he was diagnosed with CRC and polyposis by colonoscopy. He drank a small amount of beer once or twice a week and was 177 cm tall, weighed 74.5 kg, and had a BMI of 23.8. Regarding colonoscopy, the older twin had three HGDs of 10 mm (Intramucosal cancer in Japan), one in the transverse colon and two in the sigmoid colon. All HGDs were endoscopically removed. In addition, a large number of polyps (≤10 mm) were observed in the entire colorectum (Fig. 1). The younger twin had many polyps ≤10 mm from the cecum to the transverse colon (Fig. 1). In both brothers, polyps ≥5 mm were resected.
The histopathological findings showed that approximately 30% of polyps were tubular adenomas, and about 70% of polyps were hyperplastic polyps (Fig. 1). There were no sessile serrated lesions or traditional serrated adenomas. Both brothers had more than 20 hyperplastic polyps and were diagnosed with atypical colorectal polyposis, though the number of hyperplastic polyps in both brothers met the definition of SPS (Fig. 1). After removing all polyps of 5 mm or more, a colonoscopy was performed every 6–12 months to remove as many polyps as possible. Polyps were treated for 20 years, with one to two colonoscopies performed per year; the older and younger brother underwent 32 and 30 colonoscopies, respectively. The total number of polyps removed and median number (IQR: 25–75%) (minimum-maximum) were 322, 9.0 (3.5–14.8) (0–37) in the older brother and 528, 20.5 (7.5–35.5) (0–74) in the younger brother, and the number of removed polyps per colonoscopy was significantly higher in the younger brother than the older brother ($p < 0.01$) (Fig. 2). The histopathological findings of the resected polyps in the last 5 years found that approximately 10% or less were tubular adenoma with low-grade dysplasia, while hyperplastic polyps comprised more than 90%. No subsequent cancer or HGD has been observed for either brother. The older brother did not smoke at all during the follow-up period, while the younger brother continued smoking despite cessation guidance. As for other tests, both had no esophageal abnormalities on upper gastrointestinal endoscopy, and polyps were not observed in the stomach or duodenum. The younger brother was positive for *Helicobacter pylori* infection which was eradicated. Both had a fatty liver on abdominal ultrasonography and were high serum LDL-C levels. The older brother was taking statins. Thyroid echo tests did not reveal any abnormalities. Both had a mild obesity, exceeding a BMI of 23.

Regarding genetic analysis, informed consent was obtained from the older brother, and donated peripheral blood was used for DNA extraction. This research was approved by the Ethics Committee of Saitama Medical Center, Saitama Medical University (No. 925-VII). Initially, we analyzed variants in the *APC* and *MUTYH* genes, but no pathogenic variants were found (data not shown). We then conducted a panel sequencing of 57 target genes associated with hereditary CRC and polyposis syndromes including *RNF23*, a known causative gene for SPS, as previously described, using target-captured sequencing with a slight

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**Fig. 1.** Adenomatous and hyperplastic polyps of monozygotic twins with atypical colorectal polyposis. 

- **a, b** Adenomatous and hyperplastic polyps observed in the older brother. 
  - A hyperplastic polyp (goblet cell-rich type) in the older twin (H&E, ×100). 
- **d** An adenoma with low-grade atypia in the older brother (H&E, ×100). 
- **e, f** Adenomatous and hyperplastic polyps observed in the younger brother. 
  - A hyperplastic polyp (microvesicular type) in a younger brother (H&E, ×100). 
- **h** An adenoma with low-grade atypia in a younger brother (H&E, ×100).
modification to increase the number of target genes [6, 7]. We then identified a rare missense variant, \( \text{BRCA2} \) (NM_000059.3):c.4169T>G/p.Leu1390Trp in the proband (Fig. 3). The variant was not registered in either the Genome Aggregation Database or jMORP 8.3KJPN database, indicating that this variant is very rare among healthy populations [8, 9]. In silico analyses including PolyPhen-2, PROVEAN, and SIFT predicted a deleterious effect of the variant. The variant and surrounding amino acid sequences were well conserved among various species. Notably, the variant was located within the reported nuclear export signal of \( \text{BRCA2} \) [10]. Sanger sequencing analysis of the younger brother was performed after informed consent, and it also revealed the presence of this variant. Other than the variant, we could not find any other rare variants, potentially associated with polyposis in the panel sequencing.

**Fig. 2.** Number of resected colorectal polyps of monozygotic twins according to the status of smoking.

**Fig. 3.** Genetic analysis of \( \text{BRCA2} \) in monozygotic twins of atypical colorectal polyposis.
Discussion

Cigarette ingredients including nicotine, tar, and benzene have been found to be carcinogenic. The association between smoking and CRC has been well reported [4, 11, 12]. As for the relationship between smoking and polyps, a meta-analysis of 42 related observational studies, including 15,354 cases and 100,011 controls, found that adenomas are developed 1.47 times more often in smokers than those who did not smoke [4]. Among 2,676 patients who underwent an all-colonoscopy at the Vanderbilt Gastroenterology Clinic and the Veterans Affairs Tennessee Valley Health System Nashville campus in the USA, the odds ratio of polyps to nonsmokers compared to current smokers was 5.0 times (95% CI: 3.3–7.3) for hyperplastic polyps only and 1.9 times (95% CI: 1.4–2.5) for adenomas only and 6.9 times (95% CI: 4.4–11.1) for both polyps [11]. A systematic review of SPS also showed that smoking is a risk factor for polyp occurrence (RR, 2.47; 95% CI, 2.12–2.87) [12]. In our case, both brothers had adenomas and hyperplastic polyps, and the younger brother who continued smoking showed accelerated polyp generation, which is consistent with these previous reports. To our knowledge, this is the first report to confirm the effects of smoking in identical twins with colorectal polyposis. Moreover, by continuing to remove polyps of 5 mm or more for 20 years as described in our previous report, we succeeded in suppressing further CRC [13]. Histopathological findings of the brothers’ colorectal polyps found that they were different from those of typical SPS and FAP and that the adenomas and hyperplastic polyps were mixed and thus considered to be a special atypical polyposis that has never been reported.

Genetic testing revealed a very rare missense variant in the BRCA2 gene of unconclusive pathogenicity or a variant of uncertain significance (VUS). In addition, we did not find any candidate variants in the genes reported to be causative for SPS or adenomatous polyposis, such as RNF43, APC, and MUTYH. Very recently, the variant BRCA2:c.4169T>G/p.Leu1390Trp was reported as a recurrent germline variant among three unrelated Chinese breast cancer patients [14]. In vitro analyses clearly demonstrated that the variant enhanced migration ability as assessed by a wound-healing test and trans-well invasion activity of breast cancer cells MCF-7 and MDA-MB-231; however, it did not show any effects on homologous recombination activity. Although genetic testing was not conducted, the twins’ younger sister had pancreatic cancer in her 40s, suggesting that she might also possess the BRCA2 variant and possibly hereditary breast and ovarian cancer syndrome. Further analysis will be required to accurately evaluate the identified BRCA2 variant and its possible involvement in the development of SPS. Although the effects of this variant on colorectal adenomas, CRC, or SPS are currently unknown, the genetic tests showed negative results for more than 50 SPS-related genes, including RNF43. Further, the younger sister of the twins developed pancreatic cancer in her 40s, and this may suggest that hereditary pancreatic cancer may also be caused by this BRCA2 variant; however, further analysis is necessary.

Here, we reported that there was a difference in the development of polyps in monozygotic twins with atypical colorectal polyposis showing a new variant of BRCA2 after middle age, with smoking considered as one of the causes. This case suggests that smoking is related to the development of colon polyps. The polyposis of the twins had both adenoma and hyperplastic polyps, and this disease may be associated with the discovered BRCA2 gene variant. The phenotype and genetic background presented should be discussed in future studies with more participants.

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Statement of Ethics

Written informed consent was obtained from the patients for publication of the details of their medical case and any accompanying images. This case report was conducted in accordance with the World Medical Association Declaration of Helsinki. This case report did not require ethical approval according to the rule of the Ethics Committee of Kyoto Prefectural University of Medicine.

Conflict of Interest Statement

All the authors had access to the data and control over the decision to publish. No financial support was provided for this study. All the authors declare no conflicts of interest associated with this study.

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Author Contributions

Naohisa Yoshida and Hideki Ishikawa conducted the colonoscopy and organized this paper. Ryohei Hirose, Ken Inoue, and Osamu Dohi analyzed the clinical data from the colonoscopy. Hidetaka Eguchi, Yasushi Okazaki, Shingo Ishiguro, and Hideyuki Ishida did the genetic analysis. Yoshito Itoh and Michihiro Mutoh gave special advice to supervise this paper.

Data Availability Statement

All data that support the findings of this study are included in this article. Further inquiries can be directed to the corresponding author.

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