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DOI: 10.17235/reed.2020.6974/2020
Link: PubMed (Epub ahead of print)

Please cite this article as:

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OR 6974

A novel large deletion in the APC gene associated with Gardner syndrome in a Chinese family

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Received: 19/2/2020
Accepted: 29/5/2020
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ABSTRACT

Introduction: Gardner syndrome is a hereditary disease characterized by familial adenomatous polyposis (FAP), accompanied by soft tissue tumors.

Material and methods: a Chinese FAP family was enrolled and followed-up for three years.

Results: a novel large germline fragment deletion (EX10_16DEL) of the adenomatous polyposis coli (APC) gene was identified by multiplex ligation-dependent probe amplification (MLPA). An unexpected abdominal tumor grew two years after a subtotal colectomy of the proband. The immunohistochemistry study of the abdominal tumor showed SMA(focal+), calponin(+), β-catenin(nucleus+) and CD34(focal+), CD117(-), which was consistent with a desmoid tumor.

Discussion: when a FAP related desmoid tumor appears, the possibility of Gardner syndrome should be considered. This is the first largest deletion of the APC gene in the Chinese population associated with Gardner syndrome.
**Keywords:** Gardner syndrome. APC gene. Large fragment deletion. Novel mutation. Postoperative desmoid tumor.

**INTRODUCTION**

Gardner syndrome is an autosomal dominant disease characterized by familial adenomatous polyposis (FAP) together with osteomas and soft tissue tumors, including epidermoid cysts, fibromas and desmoid tumors. Intra-abdominal desmoid, one of the most common extracolonic manifestations, is significantly correlated with the mutation site of the adenomatous polyposis coli (APC) gene and guideline-recommend surgical resection for FAP patients (1). Here we report a Chinese Gardner syndrome family with a novel large fragment deletion (EX10_16DEL) in the APC gene, identified by multiplex ligation-dependent probe amplification.

**PATIENTS AND METHODS**

**Patient information**

A Chinese FAP family spanning three generations was enrolled from the Department of Gastroenterology in the Second Xiangya Hospital, Central South University, Hunan, China. This study was approved by the review board of the Second Xiangya Hospital of Central South University. Written informed consent was obtained from the patient and family members who participated in the study. Peripheral blood was collected from the affected proband and three family members (Fig. 1A).

**Mutation analysis**

APC mutation screening was performed by Sanger sequencing for micromutations and multiplex ligation-dependent probe amplification (MLPA) for large fragment deletions/insertions. Genomic DNA was extracted from whole blood using the QIAamp Blood DNA Mini kit 51104 (Qiagen GmbH, Hilden, Germany). PCR products were analyzed on agarose gels, sequenced on an ABI 3,730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) and detected by the MLPA P043-E1 detection kit (MRC-Holland, Amsterdam, the Netherlands). The pathogenicity of the variant was interpreted according to the American College of Medical Genetics and Genomics
guideline (2). The sequencing results were compared with gene reference sequences in the UCSC hg19 to confirm potential mutations. NM_000038 was used as the reference sequence for APC gene.

Pathological histology
Immunohistochemistry was used to detect protein expressions of 15 abdominal tumor markers: CK, Vim, desmin, SMA, calponin, β-catenin, CD34, CD117, Dog-1, CD99, Bcl-2, STAT6, S100, SOX-10, Ki-67. Immunohistochemistry was performed to exclude the possibility of a gastrointestinal stromal tumor.

RESULTS
Clinical findings
The patient (III-2, Fig. 1) was a 24-year-old female, who suffered from recurrent rectal bleeding for three years with intermittent abdominal pain since 2016. Family history showed that her grandfather and father had both died of colorectal cancers and her older aunt and cousin had multiple colon polyps. Physical examination showed anemic countenance, without skin blackspots, osteomas or other significant physical signs. A routine blood examination suggested mild anemia and tumor markers were not elevated. Endoscopy showed hundreds of polyps, both in the colon and stomach, and there was no obvious abnormality in small bowel. Abdominal computed tomography (CT) scan showed that no other organs were involved in 2017. Afterward, a laparoscopic anus-retained subtotal colectomy was performed, with about 15 cm residual rectum and colon. Intraoperative exploration showed no obvious abnormality in the abdominal cavity and omentum. The histopathology of surgical specimens demonstrated adenomatous polyps with high-grade intraepithelial neoplasia in focal glands.

Genetic findings
Based on the clinical diagnosis of familial adenomatous polyposis, APC gene mutation screening was first performed in the proband. Direct DNA sequencing did not detect pathogenic germline mutations in all APC exons. APC multiplex ligation-
dependent probe amplification identified a large fragment deletion spanning seven coding exons (EX10_16DEL/CDS9_15DEL), which segregated with the FAP phenotypes in the proband and all affected family members (Fig. 1B). Unaffected family members and normal controls did not carry this deletion. The proband and affected members were followed-up under National Comprehensive Cancer Network (NCCN) guidelines for periodic physical examinations and endoscopic surveillance (1).

Pathological findings
In 2019, abdominal CT reexamination of the proband revealed multiple space-occupying lesions in the abdominal cavity; the largest was approximately 7.0 x 9.8 x 11 cm, involving the right ureter and right kidney (Fig. 2). The mass biopsy was performed under b-ultrasound localization. The immunohistochemistry of abdominal tumor biopsies showed SMA(focal+), calponin(+), β-catenin(nucleus+), CD34(focal+) and CD117(-), which suggested a desmoid tumor (Fig. 3). The patient was ultimately genetically diagnosed with Gardner syndrome with a novel large germline deletion of the APC gene.

DISCUSSION
APC-associated polyposis conditions include: FAP, attenuated FAP, Gardner syndrome and Turcot syndrome. FAP, as the classical and central phenotype of the above disease, is a hereditary colon cancer predisposition syndrome in which hundreds of thousands of adenomatous colonic polyps develop. Gardner syndrome is the association of colonic adenomatous polyposis, osteomas or soft tissue tumors (including epidermoid cysts, fibromas, desmoid tumors) (3).

Desmoid tumor (DT) is a rare histologically benign mesenchymal neoplasm with an aggressive growth and no transferability. It is difficult to diagnose early and complete treatment. In a previous study, DT was the top five most common cause of death among deceased FAP patients, and was also one of the most frequent manifestations of FAP (4,5). The prevalence of desmoid tumor in FAP is around 10 to 25 % (6,7) and the prevalence of FAP among DT varied between 7.5-16 % (8). The
incidence of DT in FAP was highest in the second and third decades of life, with 80% occurring by age 40 (7). DT was classified as three types: abdominal wall, intra-abdominal and extra-abdominal (chest, head and neck region and extremities) (9). Intra-abdominal desmoids were more common. The minimum age of onset was three years old and DT might be the primary manifestation that occurs earlier than colon polyps (10). Intra-abdominal desmoids are usually asymptomatic and are only found when they have grown significantly. The patient did not have positive abdominal signs until the mass grew quite large due to the undetectable location in the pelvic cavity and unpalpable from the abdomen. Surgical treatment of DT was recommended, despite the high postoperative recurrence rate (11). The risk of DT is increased when there is a positive family history of desmoids or previous intra-abdominal surgery. Approximately 65% of DT in FAP occur within the abdomen or abdominal wall (7). Abdominal desmoid tumors may occur spontaneously or following abdominal surgery. It was thought that iatrogenic surgical injuries in the abdomen cause abnormal monoclonal proliferation of fibroblasts and lead to the development of desmoid fibromas. DT can be divided into sporadic desmoid and FAP-related desmoid. FAP-associated desmoid are best evaluated by CT scan with a recommended CT scoring system (12). The CT scores of the neonatal abdominal tumor in the patient were concordant with the pathological diagnosis of a desmoid tumor in this case. Independent predictors for desmoid occurrence in FAP patients include previous abdominal surgery, an APC 3' pathogenic variant of codon 1061 and 1309-1580, a family history of desmoid tumors and female gender (13). Previous pregnancy was regarded as the most important risk factor (14). However, the patient was not pregnant. Furthermore, other researchers consider that DT can develop regardless of the APC mutation site and there might be a relationship between the genotype and DT severity (15). A comparison of sporadic and FAP-associated desmoid-type fibromatoses led to the opinion that the risk of death due to the desmoid tumor was low, whereas it represented a substantial cause of death among FAP patients (16). The NCCN guidelines state that abdominal palpation is insufficient for desmoid and more close follow-up of extracolonic manifestations is required. Previously reported pathogenic variants of the APC gene in the Chinese population
revealed a total of 82 different pathogenic variants from 127 Chinese FAP families. Nine were large deletion or duplication variants (7.09%), which showed no significant difference in western and Chinese patient populations (17,18). The previously reported deletions of the APC gene seldom include the promoter region or exon 16, as the promoter region is important for initiation of transcription and the last coding exon is necessary for regulation and localization of β-catenin (19). Mutations at the 3’ end of the APC gene are highly associated with aggressive fibromatosis and mutational analysis plays an important role in diagnosing β-catenin-negative mesenteric desmoids (20). To the best of our knowledge, this is the largest deletion of the APC gene reported in Chinese population with Gardner syndrome. It has been previously reported in The Human Gene Mutation Database (HGMD) [CG058197, CG075764] (21,22), although the phenotypic character of an abdominal desmoid was not described.

The possibility of Gardner syndrome should be considered for FAP patients with a postoperative abdominal tumor, despite negative physical signs. Genetic testing, abdominal CT scan and tumor histopathology should be further performed. This is the largest deletion of the APC gene reported in the Chinese population associated with Gardner syndrome, which enriches the APC gene mutation database and genotype-phenotype relationship.
ACKNOWLEDGEMENTS

We would like to thank all subjects for participating in this study.

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Fig. 1. A. Pedigree of the family. B. Multiplex ligation-dependent probe amplification of the $APC$ in the proband revealed seven exons deletions.
Fig. 2. Abdominal CT images. A. Multiple nodules were found in the lower abdominal cavity, with size of around 7.0 x 9.8 x 11 cm. The CT value was about 42 HU and the enhanced scan showed non-homogenous mild enhancement. B. The right ureter was involved in the mass.
Fig. 3. Pathological images (x100). A. HE staining. B. Immunohistochemical staining of β-catenin (nucleus+). C. Immunohistochemical staining of SMA(focal+). D. Immunohistochemical staining of calponin(+). E. Immunohistochemical staining of CD34(focal+). F. Immunohistochemical staining of CD117(-).