

***NFI* germline mutation in a Chinese family with colon cancer**

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Abstract

Colorectal cancer (CRC) is a leading cause of cancer death worldwide. Recent advances in genomic medicine have identified novel gene mutations that contribute to an increased risk of CRC. Here, we describe a diagnosis of colon cancer in a 63-year-old woman and also in her brother. Next-generation sequencing showed that both patients harbored a germline mutation in *NFI*. The female patient also carried co-mutations in *KRAS* and *NRAS*. Furthermore, the *NFI* germline mutation was identified in a healthy offspring of the brother. The female patient received three cycles of bevacizumab plus capecitabine/oxaliplatin therapy and achieved stable disease of the primary lesion in the colon and partial response of metastasis in the right abdominal cavity. This study highlights the association of *NFI* germline mutations with colon cancer.

Keywords

Colon cancer, *NFI* germline mutation, next-generation sequencing, family pedigree, neurofibromin, bevacizumab

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Introduction

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder occurring in about 1 per 3500 individuals.¹ The main manifestations of NF1 are café-au-lait spots, skinfold freckling, neurofibromatosis, optic gliomas, and Lisch nodules of the iris.² Patients with NF1 have an

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increased risk for developing malignancies, the most common of which are malignant peripheral nerve sheath tumors and gliomas.^{3,4}

NF1 is caused by germline mutations in the *NFI* tumor suppressor gene, located on chromosome 17q11.2.^{4,5} *NFI* is one of the largest genes identified, encompassing a genome size of 282 kb including 57 constitutive exons and at least 3 alternatively spliced exons.⁶ Neurofibromin, the *NFI* gene product, belongs to the family of GTPase-activating proteins and is involved in negative regulation of the Ras-mediated signaling pathway, which controls cell growth and proliferation.⁷ Hence, a germline mutation in the *NFI* gene results in the formation of a truncated or non-functional neurofibromin that can activate or up-regulate the Ras-mediated signaling pathway, leading to the formation of benign or malignant tumors.⁸ Germline mutations in the *NFI* gene have been identified in several tumors including gliomas, malignant peripheral nerve sheath tumors, neurofibromas, pheochromocytomas, and breast cancer.^{4,9–12} Here, we report two cases of colon cancer in a brother and sister with *NFI* germline mutations.

Case Report

A 63-year-old female patient developed a transverse colon mass and a polyp of the sigmoid colon, which was discovered by colonoscopy during a physical examination on 6 December 2018 (Figure 1). On 10 December 2018, a computed tomography (CT) scan showed multiple nodules in both lungs, a thickened wall of the splenic flexure of the colon, and metastasis in the right abdominal cavity (Figure 2a). Laboratory examination revealed that the levels of CA199 and CA242 were increased to 298.25 IU/mL (normal range: <37.00 IU/mL) and 167.50 U/mL (normal range: 0.00–20.00 U/mL), respectively. Pathological examination revealed a

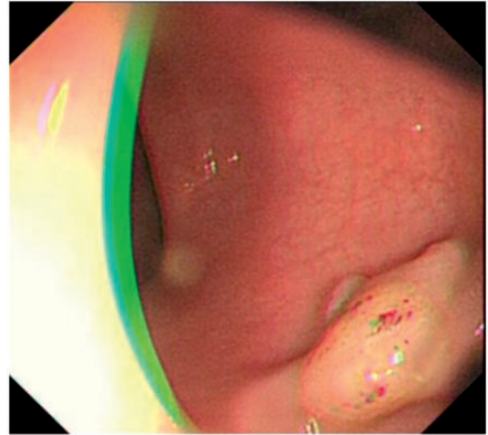


Figure 1. Colonoscopy showing a transverse colon mass and polyp of the sigmoid colon.

transverse colon adenocarcinoma with sigmoid colon tubulovillous adenomas (Figure 3). On the basis of these imaging and pathology findings, the patient was diagnosed with stage IV adenocarcinoma (cTxNxM1b). Subsequently, the patient received three cycles of treatment with bevacizumab (510 mg, ivgtt, day 1) plus capecitabine (1750 mg, bid, days 1–14)/oxaliplatin (220 mg, ivgtt, day 1) (CapOx) from 26 December 2018 to 11 February 2019. On 3 February 2019, a CT scan showed a thickened wall of the splenic flexure of the colon and metastasis in the right abdominal cavity (Figure 2b), which indicated stable disease (SD) of the primary lesion in the colon and partial response (PR) of metastasis in the right abdominal cavity. The patient's tumor tissues and blood samples were subjected to next-generation sequencing (NGS) in December 2018. A germline mutation of *NFI* L581Ffs*6 (VAF [variant allele frequency]: 24%) and somatic mutations of *KRAS* A146P (VAF: 10%), *NRAS* Q61K (VAF: 12%), *TP53* R175H (VAF: 13%), and *CD274* (PD-L1) T290M (VAF: 11%) were detected.

The patient's family medical history revealed that her older brother, aged

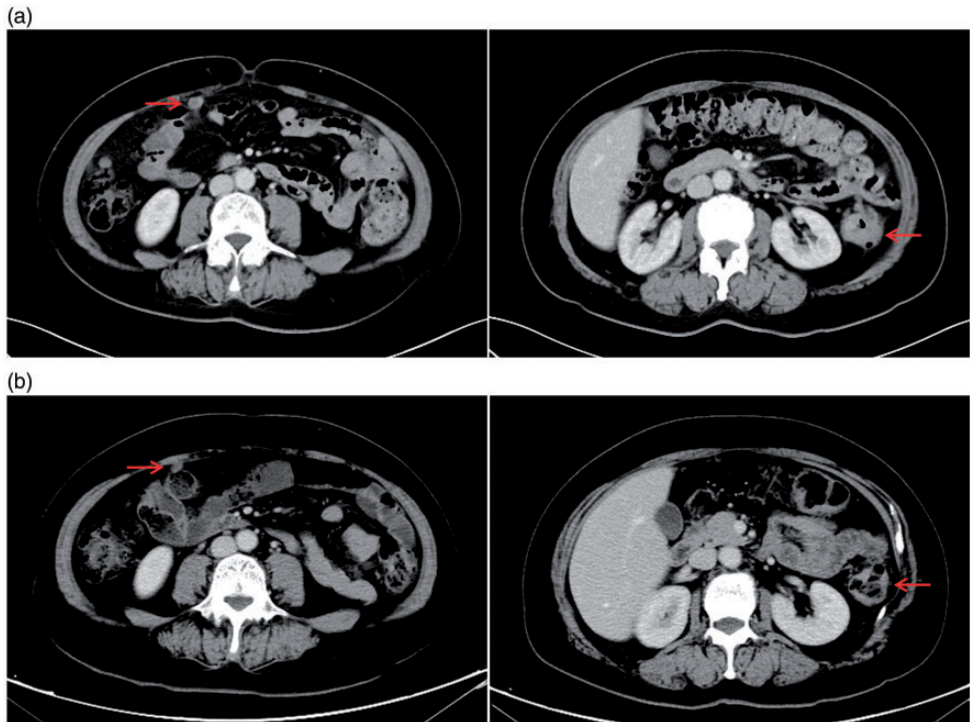


Figure 2. Computed tomography (CT) scans pre-chemotherapy (a) and post-chemotherapy (b).

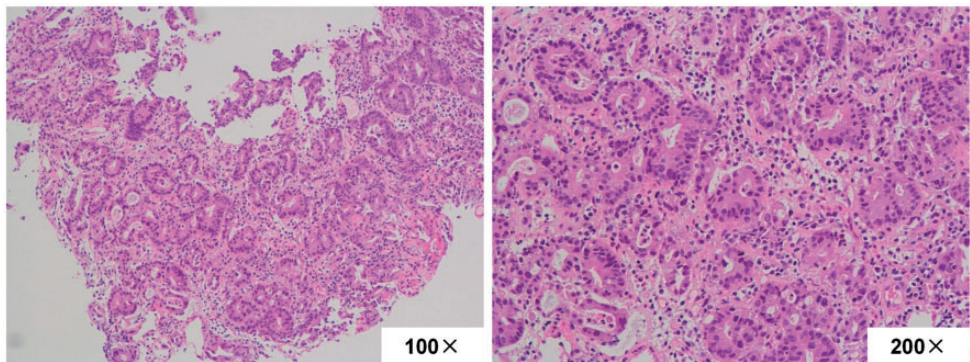


Figure 3. Hematoxylin eosin (HE) staining. Pathological examination revealed a transverse colon adenocarcinoma with sigmoid colon tubulovillous adenoma.

67 years, had also been diagnosed with sigmoid colon cancer with a tubular adenoma in a cecum polyp 7 years previously. Given the early stage of the tumor at the time of diagnosis, radical resection of the colon

cancer had been performed and no recurrence or metastases had been identified to date. The patient's first-degree relatives subsequently consented to genetic testing (Figure 4). The same *NFI* L581Ffs*6

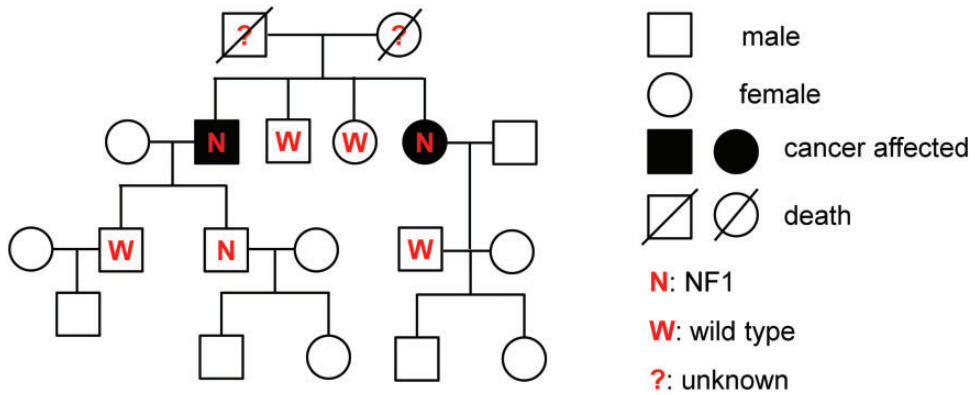


Figure 4. Family pedigree of the female patient.

germline variation (VAF: 31%) was identified in her older brother. One healthy son of the patient's older brother also carried the same germline mutation, but other healthy relatives did not. This study was approved by the medical ethics committee of Sir Run Run Shaw Hospital. Written informed consent was obtained from all individuals prior to genetic testing.

Discussion

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death worldwide, with over 1.8 million new cases and 881,000 deaths estimated in 2018.¹³ Among patients with CRC, 75% of cases were sporadic while 25% represented a family history of cancer.¹⁴ In addition, approximately 30% of all CRC cases are an inherited form of the disease, including Lynch syndrome, familial adenomatous polyposis, Li-Fraumeni syndrome, MUTYH-associated polyposis, Peutz-Jeghers syndrome, and Juvenile polyposis syndrome,¹⁵ resulting from highly penetrant inherited gene mutations. Some germline mutations are associated with an increased risk of CRC, including *APC*, *MSH2*, *MLH1*, and *STK11*.¹⁵

In the present case study, a patient and her brother had a diagnosis of colon cancer. Of note, both patients were diagnosed with intestinal polyps and pathological assessment revealed tubulovillous adenomas. Most tubular adenomas have a low risk of becoming malignant, with only a small proportion progressing to high-grade dysplasia or cancer.¹⁶ At present, the most prominent carcinogenic gene associated with familial colorectal adenomatous polyposis is the *APC* gene.¹⁵ Although the classical Mendelian syndrome (Lynch syndrome and familial adenomatous polyposis) is related to high-penetrance gene inheritance,¹⁵ many other genes that exhibit moderate penetrance have not been associated with a risk of cancer.¹⁷ *NFI* mutations are predicted to cause nonsense mutation-mediated mRNA degradation in the cell, which may result in an abnormality or functional loss of the *NFI* gene.⁵ Following a report by Li et al.¹⁸ in which 1 out of 22 colorectal tumors had mutated *NFI*, *NFI* has been suggested to play a role in colorectal tumorigenesis. Loss of heterozygosity in the *NFI* gene has been identified in primary colorectal tumors.¹⁹ Patients carrying *NFI* mutations had significantly shorter progression-free survival compared with those having wild-type *NFI*.²⁰ The *NFI*

L581Ffs*6 mutation detected in the present case study was a heritable germline variation, and a first-degree relative of the patient had a 50% probability of carrying the same variation. Our patient and her brother carried the same germline mutation of *NFI*. As expected, one of two of the brother's sons also carried the germline mutation at the same variant site. Accordingly, it could be speculated that the *NFI* germline mutation is a heritable variation that may contribute to the occurrence of familial polyposis and increase the risk of colon cancer.

The large size of the *NFI* gene, the existence of pseudogenes, and the diversity of mutation types means that traditional mutation detection in patients with a *NFI* mutation is a complicated, time-consuming, and laborious process.²¹ However, the development of NGS technology has been able to mitigate deficiencies in the single Sanger sequencing method to some extent.²² In addition, possible genotype–phenotype correlations are rarely identified in *NFI*.²³ Although a number of *NFI* manifestations have been described,² none were found in the female patient or in other relatives with *NFI* mutations, further supporting the absence of a clear relationship between specific mutations and clinical features. The present case highlights the importance of diagnosis in patients with hereditary cancer syndromes, especially for asymptomatic family members.

NFI mutations have been found in 45% to 93% of melanomas that promote connective tissue proliferation.²⁴ *NFI* deletions in melanoma often co-occur with mutations of the Ras pathway, such as *RASA2*, *PTPN11*, and *SOS1*, indicating that mutations in *NFI* may co-associate with additional activating alterations in the RAS/MAP kinase pathway.²⁵ In the present case, a patient with an *NFI* germline mutation also carried a co-mutation of *KRAS* and *NRAS*, indicating that *NFI* loss and

RAS were not mutually exclusive in colon cancer. Bevacizumab is a monoclonal antibody against vascular endothelial growth factor (that has a significant clinical benefit in patients with metastatic CRC harboring either mutant or wild-type *K-RAS*,²⁶ and combination therapy of CapOx plus bevacizumab has shown promising activity and a favorable safety profile in this patient population.²⁷ In our case study, the female patient received bevacizumab plus CapOx therapy and showed SD of the primary lesion in the colon and PR of metastasis in the right abdominal cavity.

In summary, we identified a germline *NFI* L581Ffs*6 mutation in three members of a Chinese family, two of whom had been diagnosed with CRC. Given the heredity of *NFI* germline mutations, it is necessary to closely follow up with family members of patients who carry such a mutation. Clarification of the pathogenic role of germline mutations may facilitate the planning of appropriate preventive strategies for family members harboring high-risk mutations.


Declaration of conflicting interest

Yihong Zhang and Mei Yang are employees of OriginMed. The other authors declare that there is no conflict of interest.

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