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BRIEF REPORT

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A genome-wide analysis of colorectal cancer in a child with Noonan syndrome

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Abstract

Noonan syndrome (NS) is a developmental syndrome caused by germline mutations in the Ras signaling pathway. No association has been shown between NS and pediatric colorectal cancer (CRC). We report the case of CRC in a pediatric patient with NS. The patient underwent whole genome sequencing. A germline SOS1 mutation c.1310T>C (p. Ile437Thr) confirmed NS diagnosis. No known hereditary cancer syndromes were identified. Tumor analysis revealed two mutations: a TP53 missense mutation c.481G>A (p. Ala161Tyr) and NCOR1 nonsense mutation c.6052C>T (p. Arg2018*). This report highlights the complexity of Ras signaling and the interplay between developmental syndromes and cancer.

KEYWORDS

molecular genetics, Noonan syndrome, pediatric hematology/oncology, rare tumors, solid, tumors

1. | INTRODUCTION

Noonan syndrome (NS) is a developmental syndrome with an estimated prevalence of 1 in 1000–2500 and characterized by craniofacial abnormalities, cardiac defects, and cognitive delay.[1] NS is caused by germline mutations affecting the Ras signaling pathway, as are neurofibromatosis 1 (NF1) and several other syndromes that are collectively referred to as Rasopathies.[2]

The Ras signaling pathway is a ubiquitous intracellular signaling pathway that has been shown to play a central role in the pathogenesis of adult colorectal cancer (CRC). Interestingly, Rasopathies carry an inconsistent cancer predisposition ranging from an elevated risk of neurologic-type tumors in children with NF1 to a mild, almost exclusive risk for hematologic malignancies in patients of all ages with NS.[3–5] Germline mutations affecting Ras signaling pathway proteins have not been reported to carry a predisposition to CRC. Only three cases of patients with NS with CRC have ever been reported and none in the pediatric population.[3]

Abbreviations: CRC, colorectal cancer; NS, Noonan syndrome; NF1, neurofibromatosis 1; NCOR1, nuclear receptor corepressor 1

Many of the same somatically mutated genes identified to cause adult CRC have been shown to be mutated in the germline of several cancer predisposition syndromes. However, these syndromes account for lesser number of pediatric CRC cases.[6,7] Here, we report the first report of pediatric CRC in a patient with NS and the first whole-genome analysis of pediatric CRC.

2. | CLINICAL COURSE AND METHODS

A 14-year-old female with NS presented with a 4-week history of nausea, vomiting, abdominal pain, and persistent constipation. Computed tomography (CT) of the abdomen and pelvis, and barium enema demonstrated complete large bowel obstruction with transition point at the sigmoid colon (Supplementary Figure S1). The patient was admitted, sigmoidoscopy revealed the site of the obstruction to be 35 cm from the anus, and then taken to the operating room. Exploratory laparotomy revealed an obstructing colonic mass in the sigmoid colon that was resected. Lymph nodes and observed omental and pelvic peritoneal lesions were biopsied. A diverting end-colostomy was made.

TABLE 1 Clinically significant mutations identified from genome-wide analysis

Gene	Variant type	Genome locus	Exon	Nucleotide change	Amino acid change	Normal protein function	Clinical significance
SOS1	Germline	2p22.1	10	T1310C	Ile437Thr	Guanine exchange factor, Ras signaling	Diagnostic mutation for Noonan syndrome[9]
TP53	Somatic	17p13.1	4	G481A	Ala161Tyr	Tumor suppressor	>80% of *TP53* mutations in human tumors localize to the DNA binding domain[11,12]
NCOR1	Somatic	17p11.2	39	C6052T	Arg2018*	Transcriptional coregulatory protein	ID1 domain dictates retinoic acid sensitivity in APL[14] *NCOR 1* mutations may predict tamoxifen resistance in breast cancer[16,17]

Three point mutations with clinical significance were identified, one germline mutation and two somatic mutations in the tumor sample. The germline mutation in *SOS1* and somatic mutation in TP53 encoded missense mutations causing a single amino acid change in the protein product. The somatic mutation in *NCOR1* encoded a nonsense mutation causing a truncation of the protein product.

Pathology of the surgical specimens showed colon adenocarcinoma, stage T4aN2aM1 with low grade differentiation. Lymphovascular and perineural invasion was seen. Several lymph nodes (four of 29) were positive for disease, three with extracapsular extension. Metastatic adenocarcinoma was observed in the omentum and in a pelvic peritoneal lesion.

Initial genetic testing for the three major known types of inherited CRC found no mutations associated with mismatch repair, *APC* (where APC is adenomatosis polyposis coli), or *MUTYH* genes. *BRAF* V600 mutation and NRAS extended analyses were also negative. A *KRAS* gene mutation (c.38G > A) was present in the colonic mass, a contraindication to epidermal growth factor receptor (EGFR) inhibitor therapy. The patient was then enrolled in PEDS-MI-ONCOSEQ, a prospective integrative clinical sequencing that has been approved by our institutional review board.[8] The patient's parents provided informed consent and received mandatory preenrollment genetic counseling.

Specifics of the PEDS-MI-ONCOSEQ sequencing procedure and bioinformatics analyses have been described previously (Supplementary Material S1).[8] Nucleic acid preparation and high-throughput sequencing were performed using standard the Clinical Laboratory Improvement Amendments (CLIA) protocols. Pathogenicity of germline variants was determined through a review of the published literature and databases.

The patient received induction chemotherapy with six cycles of folinic acid (400 mg/m^2), 5-fluorouracil (400 mg/m^2, then 2400 mg/m^2 over 46 h), and oxaliplatin (85 mg/m^2) combination chemotherapy with bevacizumab (5 mg/kg). Chemotherapy was administered every 2 weeks. Oxaliplatin was discontinued after six cycles. CT scans of the neck, chest, abdomen, and pelvis showed no evidence of metastasis.

The patient continued a maintenance chemotherapy regimen of folinic acid, 5-fluorouracil, and bevacizumab every 2 weeks and did not receive radiation therapy. The patient relapsed at cycle 37, presenting with a small bowel obstruction, renal insufficiency, and bilateral hydronephrosis. CT and positron emission tomography (PET) imaging suggested progression of disease corresponding to these sites of the pelvic lesion and suspected disease in Hartmann's pouch, uterine wall, and bladder wall. The small bowel obstruction resolved and the hydronephrosis improved with the placement of bilateral ureteral stents. The family declined biopsy. The patient was treated with irinotecan 180 mg/m^2 every 2 weeks and palliative measures to maximize quality of life. The patient received three doses of irinotecan, but despite a lack of irinotecan-induced diarrhea the patient began experiencing worsening symptoms of ileus. At time of manuscript submission, the patient was managed in hospice care.

3. | RESULTS AND DISCUSSION

Integrative clinical sequencing revealed three mutations with clinical significance, one in the germline and two somatic mutations in the tumor sample (Table 1). Four somatic point mutations were also identified in the tumor sample (Supplementary Table S1). No copy number variants (CNV) focal amplification or deletions, somatic insertion/deletion mutations, driving gene fusions, outlier expressions, or pathogens were detected.

SOS1 is an important Ras pathway regulator as a guanine nucleotide exchange factor. A *SOS1* missense mutation c.1310T > C (p. Ile437Thr) was identified in the germline, inducing an amino acid substitution (I437Y) near the plekstrin homology domain (aa 444–548) (Supplementary Figure S2). This mutation has been previously reported as pathogenic for NS.[9] Of note, SOS1 loss of heterozygosity was also demonstrated in the tumor. Despite playing an important role in Ras signaling, *SOS1* has been shown to be insignificant in the development of cancer.[10]

p53, the protein product of TP53, has a well-described importance in tumor suppression, with more than half of all sporadic human cancers demonstrating p53 mutations.[11] A *TP53* missense mutation c.481G > A (p. Ala161Tyr) was identified in the tumor. This induces an amino acid substitution (A161Y) within the DNA-binding domain (aa 102–292) (Supplementary Figure S2). More than 80% of *TP53* mutations in human tumors localize to the DNA-binding domain.[12] Of note, germline *TP53* mutations cause Li-Fraumeni syndrome (LFS), which carries a very high susceptibility to cancer. However, LFS is present in only 1.3% of early onset CRC cases.[12,13]

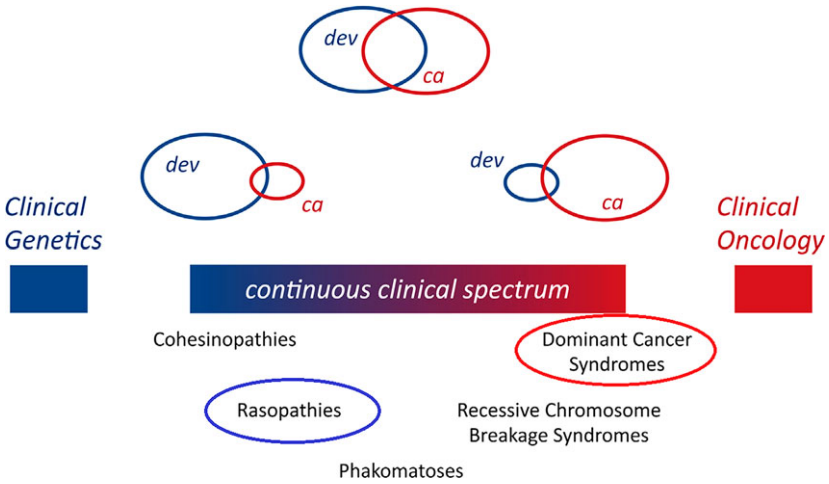

FIGURE 1 Overlap of developmental disease and cancer. A continuous clinical spectrum has been hypothesized to link genetic developmental syndromes and cancer predisposition, with developmental phenotypes possibly reflecting compensatory signaling changes. With permission from Bellacosa (2013)

Nuclear receptor corepressor 1 (NCOR1) is the cornerstone of an epigenetic complex that affects cell differentiation in several cell types via modulation of chromatin histone deacetylation.[14] A *NCOR1* somatic nonsense mutation c.6052C > T (p. Arg2018*) was identified, causing significant protein truncation (Supplementary Figure S2). The C-terminal end of NCOR1 contains two separate nuclear receptor-interacting domains, ID1 (aa 2032–2115) and ID2 (aa 2212–2273). Motifs within these regions have been shown to be necessary for binding to nuclear hormone receptors. NCOR1 also plays an important role in acute promyelocytic leukemia therapy. Retinoic acid competes with NCOR1 for transcription factor RAR alpha binding.[15] Recently, large-scale genomic studies have identified NCOR1 driver mutations in breast cancer and hepatocarcinoma.[16,17]

Of note, no mutations affecting the Wnt signaling pathway were identified in the germline or the tumor. The Wnt pathway plays an important pathogenic role in CRC, with 93% of all CRC tumors affecting this pathway have mutations.[6,11] Germline mutations in this pathway cause hereditary CRC syndromes including familial adenomatous polyposis and juvenile polyposis.

The genetics of developmental syndromes have offered important insight into cancer, and the overlapping manifestations been described as a continuous spectrum (Figure 1).[6,7,18,19] Variations in genotype likely disrupt development by affecting the interplay between different signal transduction and epigenetic pathways. Subsequent compensation may explain survival as well as nonintuitive cancer risks.[2,18–20] With the advancement of genetic testing and tissue pipelines, future whole-genome studies could identify the pathway changes of therapeutic value.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

AUTHOR CONTRIBUTIONS

Rahul M. Prasad, MD participated in the procedure and postoperative care, collected clinical data, performed the literature search, and prepared the manuscript; Rajen J. Mody, MD managed postoperative care, collected clinical data, participated in manuscript revision, and conducted final review; Melisa Mullins, MD, PhD and Zaher Naji, MD managed postoperative care, collected clinical data, and participated in manuscript revision; George Myers, DO was the assisting surgeon and participated in manuscript revision; James D. Geiger, MD was the lead surgeon who conducted the procedure, participated in manuscript revision, and conducted final review. All authors read and approved the final manuscript.

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REFERENCES

1. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet*. 2013;381(9863):333–342.
2. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer*. 2007;7(4):295–308.
3. Jongmans MC, van der Burgt I, Hoogerbrugge PM, et al. Cancer risk in patients with Noonan syndrome carrying a PTPN11 mutation. *Eur J Hum Genet*. 2011;19(8):870–874.
4. Kratz CP, Franke L, Peters H, et al. Cancer spectrum and frequency among children with Noonan, Costello, and cardio-facio-cutaneous syndromes. *Br J Cancer*. 2015;112(8):1392–1397.
5. Kratz CP, Rapisuwon S, Reed H, Hasle H, Rosenberg PS. Cancer in Noonan, Costello, cardiofaciocutaneous and LEOPARD syndromes. *Am J Med Genet C Semin Med Genet*. 2011;157C(2):83–89.
6. Carethers JM, Jung BH. Genetics and genetic biomarkers in sporadic colorectal cancer. *Gastroenterology*. 2015;149(5):1177–1190, e1173.
7. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546–1558.
8. Mody RJ, Wu YM, Lonigro RJ, et al. Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. *JAMA*. 2015;314(9):913–925.
9. Lepri F, De Luca A, Stella L, et al. SOS1 mutations in Noonan syndrome: molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. *Hum Mutat*. 2011;32(7):760–772.
10. Swanson KD, Winter JM, Reis M, et al. SOS1 mutations are rare in human malignancies: implications for Noonan syndrome patients. *Genes Chromosomes Cancer*. 2008;47(3):253–259.
11. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487(7407):330–337.
12. Bieging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. *Nat Rev Cancer*. 2014;14(5):359–370.
13. Yurgelun MB, Masciari S, Joshi VA, et al. Germline TP53 mutations in patients with early-onset colorectal cancer in the colon cancer family registry. *JAMA Oncol*. 2015;1(2):214–221.
14. Martinez-Iglesias OA, Alonso-Merino E, Gomez-Rey S, et al. Autoregulatory loop of nuclear corepressor 1 expression controls invasion, tumor growth, and metastasis. *Proc Natl Acad Sci USA*. 2016;113(3):E328–E337.
15. Wong MM, Guo C, Zhang J. Nuclear receptor corepressor complexes in cancer: mechanism, function and regulation. *Am J Clin Exp Urol*. 2014;2(3):169–187.
16. Fujimoto A, Furuta M, Totoki Y, et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. *Nat Genet*. 2016;48(5):500–509.
17. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534(7605):47–54.
18. Bellacosa A. Developmental disease and cancer: biological and clinical overlaps. *Am J Med Genet A*. 2013;161A(11):2788–2796.
19. Kato S, Lippman SM, Flaherty KT, Kurzrock R. The conundrum of genetic "drivers" in benign conditions. *J Natl Cancer Inst*. 2016;108(8). https://doi.org/10.1093/jnci/djw036
20. Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci*. 2011;36(6):320–328.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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