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Identification of a Novel *PMS2* Alteration c.505C>G (R169G) *In Trans* with a *PMS2* Pathogenic Mutation in a Patient with Constitutional Mismatch Repair Deficiency

Maureen E. Mork^a, Ester Borrás^b, Melissa W. Taggart^c, Amanda Cuddy^d, Sarah A. Bannon^a, Y. Nancy You^{a,d}, Patrick M. Lynch^{a,e}, Pedro T. Ramirez^f, Miguel A. Rodriguez-Bigas^{a,d}, and Eduardo Vilar^{a,b,g,*}

^aClinical Cancer Genetics Program, UT MD Anderson Center, Unit 1354, 1155 Pressler St., Houston, TX 77030, USA

^bDepartment of Clinical Cancer Prevention, UT MD Anderson Cancer Center, Unit 1360, P.O. Box 301439, Houston, TX 77230-1439, USA

^cDepartment of Pathology, UT MD Anderson Cancer Center, Unit 0085, 1515 Holcombe Blvd., Houston, TX 77030, USA

^dDepartment of Surgical Oncology, UT MD Anderson Cancer Center, Unit 1484, 1400 Pressler St., Houston, TX 77030, USA

^eDepartment of Gastroenterology, Hepatology, and Nutrition, UT MD Anderson Cancer Center, Unit 1466, 1515 Holcombe Blvd., Houston, TX 77030, USA

^fDepartment of Gynecologic Oncology, UT MD Anderson Cancer Center, Unit 1362, P.O. Box 301439, Houston, TX 77230-1439, USA

^gDepartment of Gastrointestinal Medical Oncology, UT MD Anderson Center Center, Unit 426, 1515 Holcombe Blvd., Houston, TX 77030, USA

Abstract

Constitutional mismatch repair deficiency syndrome (CMMRD) is a rare autosomal recessive predisposition to colorectal polyposis and other malignancies, often childhood-onset, that is caused by biallelic inheritance of mutations in the same mismatch repair gene. Here, we describe a patient with a clinical diagnosis of CMMRD based on colorectal polyposis and young-onset endometrial cancer who was identified to have two alterations *in trans* in *PMS2*: one known pathogenic mutation (c.1831insA; p.Ile611Asnfs*2) and one novel variant of uncertain significance (c.505C>G; p.Arg169Glu), a missense alteration. We describe the clinical and molecular features in the patient harboring this novel alteration c.505C>G, who meets clinical criteria for CMMRD and exhibits molecular evidence supporting a diagnosis of CMMRD. Although experimental validation is needed to confirm its pathogenicity, *PMS2* c.505C>G likely

*Corresponding author: Eduardo Vilar, MD, PhD, Department of Clinical Cancer Prevention, Unit 1360, The University of Texas MD Anderson Cancer Center, Houston, TX 77030. Phone: (713) 745-4929; fax: (713) 794-4403; evilar@mdanderson.org.

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has functional consequences that contributes to our patient's phenotype based on the patient's clinical presentation, tumor studies, and bioinformatics analysis.

Keywords

Constitutional Mismatch Repair Deficiency; Lynch Syndrome; Variant of Uncertain Significance

Introduction

Constitutional mismatch repair deficiency syndrome (CMMRD) is a rare autosomal recessive predisposition to colorectal polyposis and a wide spectrum of malignancies, including hematological malignancies and brain tumors, often childhood-onset [1, 2]. Strikingly, CMMRD is caused by biallelic inheritance of mutations in the mismatch repair genes (MMR), which cause Lynch syndrome in a heterozygous state. Therefore, carrier parents of individuals with CMMRD will themselves have Lynch syndrome [3].

Of the four MMR genes *MLH1* (OMIM 120436), *MSH2* (OMIM 609309), *MSH6* (OMIM 600678), and *PMS2* (OMIM 600259), most reported cases of CMMRD are due to biallelic inheritance of mutations in *PMS2* followed by *MSH6* [1]. Interestingly, the clinical phenotype of Lynch syndrome patients with heterozygous *MSH6* and especially *PMS2* mutations is much less severe than those of patients with classic Lynch syndrome, with an estimated 10-19% lifetime risk for colorectal cancer (CRC) and 11-24% for endometrial cancer with *PMS2* mutations [4-6]. In addition, individuals with CMMRD caused by biallelic *PMS2* mutations often have limited or no family history of cancer [1]. Here, we describe a patient with a clinical CMMRD phenotype whose genetic testing identified a known *PMS2* pathogenic mutation (InSiGHT class 5) and a novel *PMS2* missense mutation, called a variant of uncertain significance (VUS) by the CLIA testing laboratory (InSiGHT class 3) [7]. Written informed consent was obtained from the participant in this case report for additional molecular analysis and presentation of the case.

Patient and methods

Case report

The patient, a Caucasian female, initially presented to our institution at age 17 for surgical consultation and genetic counseling due to her personal history of multiple colon adenomas. She experienced rectal bleeding and abdominal cramping and underwent a colonoscopy that identified multiple polyps throughout the colon, many of them diminutive; 15 were biopsied and shown to be adenomas. Upper endoscopy identified fundic gland polyps with low-grade dysplasia. A detailed pedigree was taken and revealed no family history of polyposis. Her mother had not undergone colonoscopy and her father had a history of two polyps at age 42. The patient reported one brother in good health. The only cancer reported in the family was late-onset CRC in two brothers of the patient's maternal great-grandmother and CRC in her paternal great-grandfather (Figure 1a). The patient underwent comprehensive germline *APC* testing followed by *MUTYH* testing; both were normal.

The patient underwent restorative proctocolectomy with J-pouch ileorectal anastomosis. Pathology review of the surgical specimen revealed a 2 mm focus of invasive moderately differentiated adenocarcinoma involving the superficial submucosa arising from a 3 cm tubulovillous adenoma with high-grade dysplasia in the rectum and at least 20 synchronous adenomas. Thirty-eight lymph nodes were examined with no cancer present, thus rendering a diagnosis of stage I rectal cancer. Immunohistochemistry (IHC) for the MMR proteins was not performed in the rectal tumor due to insufficient tissue.

On a follow-up CT scan one year after surgery, the patient was identified to have a 4 cm retroperitoneal lymph node. Fine needle aspiration was consistent with metastatic poorly differentiated mucinous adenocarcinoma with signet ring cells. The patient underwent retroperitoneal lymphadenectomy and the IHC revealed intact expression of the four MMR proteins; however, microsatellite instability analysis (MSI) by PCR was not performed due to insufficient tissue.

The patient was followed for post-cancer surveillance at our center and at age 23 she was identified to have stage IA clear cell carcinoma of the endometrium. She underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy.

In light of the patient's diagnosis of endometrial cancer, she was re-referred to genetic counseling. Due to concern for CMMRD, genetic testing of the MMR genes was ordered. The patient was identified to have one known pathogenic mutation in *PMS2*, described as c.1831insA (p.Ile611Asnfs*2), and an additional *PMS2* alteration c.505C>G (p.Arg169Glu), that was classified as a VUS by the performing CLIA laboratory. Furthermore, the patient describes a large café-au-lait macule involving her lower limb and buttock. This clinical phenotype received a score 3, meeting the recently-proposed indication criteria for CMMRD testing [2].

The patient's parents both underwent single-site testing for these alterations; her mother was found to be the carrier of the c.1831insA *PMS2* mutation and her father was found to be the carrier of the c.505C>G *PMS2* VUS, confirming that these alterations are *in trans* in the proband.

Germline and tumor genetic testing results

We confirmed the presence of both germline *PMS2* alterations in the laboratory by LR-PCR followed by exon specific PCR and sequencing as previously described (Figure 1b) [8]. The c.1831insA (p.Ile611Asnfs*2) alteration is a frameshift mutation located in exon 11 and is consistent with a pathogenic mutation, which has been reported previously in two Lynch syndrome patients [5]. However, the *PMS2* c.505C>G (p.Arg169Glu) variant has not been reported before, although by *in silico* analysis it is predicted to generate a missense change in an amino acid residue highly conserved and located close to the ATP binding region. This alteration is classified as probably damaging by the Polyphen, SIFT and CONDEL bioinformatics algorithms [9-11]. Then, we reviewed the immunohistochemistry of available tumor sites (retroperitoneal lymph node and large tubulovillous adenoma) and normal tissues, revealing intact staining of the MMR proteins (including *PMS2*). In addition, we

performed MSI analysis in an additional adenomatous polyp with high-grade dysplasia included in the original colorectal resection, which was available for testing, revealing high-level of MSI in eight of ten markers (Figure 2a-b).

The presence of CMMRD was confirmed by germline MSI (gMSI) analysis that was performed in two germline DNA controls (NA12832 and NA10846) from NIGMS Human Genetic Cell Repository (Coriell Institute, NJ, USA) and germline DNA of the patient using 3 dinucleotide microsatellites (D17S791, D2S123, and D17S250) as previously described [12]. The gMSI ratios for D2S123, D17S250 and D17S791 in our CMMRD patient were 0.153 (0.145-0.156), 0.156 (0.154-0.161) and 0.276 (0.258-0.281), respectively, and for the DNA controls were 0.04 (0.039-0.041), 0.049 (0.048-0.051), and 0.032 (0.028-0.034), respectively. Therefore, the ratios in the patient were higher than those showed in the controls and were also within the diagnostic range for CMMRD (Figure 2c) [12].

Discussion

The c.505C>G *PMS2* alteration was identified *in trans* with a known *PMS2* pathogenic mutation in our patient with strong clinical and molecular evidence of CMMRD. Therefore, it is possible that this *PMS2* VUS (InSiGHT class 3) is contributing to our patient's clinical phenotype. Patients presenting with one pathogenic MMR mutation *in trans* with a VUS is a common clinical presentation among patients with CMMRD [13]. While the co-occurrence of a pathogenic mutation in conjunction with a VUS in a patient with CMMRD does not provide sufficient and definitive evidence on the pathogenicity of the VUS, it does raise the possibility that this alteration is responsible for the patient's phenotype.

Our patient has strong clinical evidence of CMMRD, including young-onset colorectal polyposis and endometrial cancer. In addition, her gMSI testing demonstrated the presence of genomic instability in peripheral blood, and along with the phenotype, it is consistent with a CMMRD diagnosis. Furthermore, functional validation assays using lymphoblastic cell lines (LCLs) to detect *ex vivo* MSI have been recently described as the gold standard for confirmation of pathogenicity of genetic alterations (including VUS) in the context of CMMRD [13]; however, LCLs were not available in our patient for additional testing.

The *PMS2* c.505C>G novel VUS is likely to have functional consequences in the MMR pathway. In fact, the patient's presentation is most consistent with CMMRD rather than a heterozygous *PMS2* alteration, which would be expected to cause a modest increased risk for later-onset Lynch syndrome cancers without polyposis. In addition, results derived from bioinformatics analysis are consistent with a lack of functionality in the protein secondary to this alteration. Furthermore, a heterozygous frameshift mutation such as c. 1831insA would be expected to cause absence of PMS2 protein staining upon acquisition of a second somatic hit inactivating the remaining *PMS2* allele, as has been reported previously in several patients with this specific *PMS2* alteration [5, 14]. However, in the present case, a possible explanation for the intact staining of PMS2 in the patient's malignancies could be that the second *PMS2* alteration, c.505C>G, results in a stable but nonfunctional protein, thus leading to positive staining in the tumor. This phenomenon of mutations causing intact IHC staining has been reported previously [15]. Another possibility is that this alteration may

represent a hypomorphic allele rather than a classic pathogenic mutation, similar to a *PMS2* founder mutation recently described in the Inuit population, thus causing attenuated CMMRD in the homozygous state but maintenance of mismatch repair activity in the heterozygous state [16]. Finally, an alternate explanation for our patient is the presence of an undetectable second pathogenic *PMS2* mutation; this gene is well-known for its complexity of analysis [8]. Ultimately, determining the functional impact of this VUS is critical to making recommendations for families harboring it. In the present case, we recommended a conservative approach with enhanced colon surveillance in the patient's father, who also carried the VUS, and made recommendations for germline testing of both the pathogenic *PMS2* mutation and VUS in the patient's brother. However, additional analyses including multifactorial likelihood models, segregation studies, and functional analysis based on MSI determination and tolerance to methylation in LCLs [13] are still needed to provide definitive evidence of the pathogenicity of this VUS. In summary, this case provides a novel description of the c.505C>G (p.Arg169Glu) VUS in a patient with CMMRD.

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Fig. 1.

a Pedigree demonstrating family history of cancer and segregation of the *PMS2* alterations
b Sequencing chromatograms of germline mutations detected using LR-PCR and internal PCRs of exons 5 and 11, as well as schematic illustration of the *PMS2* protein showing the known functional domains and locations of the germline mutations detected

Fig. 2.

a Immunohistochemical staining of tubulovillous adenoma demonstrating intact expression of all MMR proteins, including PMS2

b Microsatellite instability analysis by PCR demonstrating high microsatellite instability in tubulovillous adenoma

c Germline microsatellite instability analysis by PCR demonstrates the presence of a peak larger than the principal allele in the genomic DNA of our patient with CMMRD. Only one dinucleotide marker is shown for illustrative purposes