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MUTATION REPORT

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ABSTRACT

Background:

Biallelic germline mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2* cause a recessive childhood cancer syndrome characterized by early-onset malignancies and signs reminiscent of neurofibromatosis type 1 (NF1). Alluding to the underlying genetic defect we refer to this syndrome as constitutional mismatch repair-deficiency (CMMR-D) syndrome. The tumor spectrum of CMMR-D syndrome includes hematological neoplasias, brain tumors and Lynch syndrome associated tumors. Other tumor entities, such as neuroblastoma, Wilm's tumor, ovarian neuroectodermal tumor or infantile myofibromatosis have so far been found only in individual cases.

Results:

We analyzed two consanguineous families with suspected CMMR-D syndrome patients who developed rhabdomyosarcoma among other neoplasias. In the first family we identified a pathogenic *PMS2* mutation for which the affected patient was homozygous. In family 2 immunohistochemistry analysis showed isolated PMS2-expression loss in all tumors of the affected patients including the rhabdomyosarcoma and in the surrounding normal tissue. Together with the family history and microsatellite instability observed in one tumor this strongly suggests an underlying *PMS2* alteration also in family 2.

Conclusion:

Together, these two new cases show that rhabdomyosarcoma and possibly other embryonic tumors, such as neuroblastoma and Wilm's tumor, belong to the tumor spectrum of CMMR-D syndrome. Given the clinical overlap of CMMR-D syndrome with NF1, we suggest careful examination of the family history in patients with embryonic tumors and signs of NF1 as well as analysis of the tumors for loss of one of the mismatch repair genes and microsatellite instability. Subsequent mutation analysis will confirm proper diagnosis of the underlying disorder.

INTRODUCTION

The highly conserved mismatch repair (MMR) system corrects replication errors in newly synthesized DNA. In humans, mismatches and small insertion-deletion loops (IDLs) are detected by one of two heterodimers, MSH2•MSH6 (MutSα) or MSH2•MSH3 (MutSβ). MutSα is involved in the repair of base/base mismatches and misalignments of one or two nucleotides while MutSβ recognizes larger IDLs. MutSα (or MutSβ) recruits a second heterodimer, MLH1•PMS2 (also named MutLα), that possesses an endonuclease active site and allows MutLα to introduce random nicks at sites spanning the mismatch. Subsequent loading of EXO1 at the 5′ side of the mismatch leads to activation of its 5′-to-3′ exonuclease activity leading to the removal of the error containing DNA-fragment. The repair process is finalized by polymerase δ and its cofactors proliferation cell nuclear antigen (PCNA) and replication factor C (RFC) that fill in the single-stranded gap. In a final step, ligase I seals the remaining nick (reviewed in [1]). In addition to DNA repair activity, the MMR system is also involved in apoptotic response to a variety of DNA damaging agents (reviewed in [2]) and human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination [3].

Heterozygous germline loss-of-function mutations of the genes encoding the crucial components of this MMR system, *MLH1*, *MSH2*, *MSH6* or *PMS2*, cause Lynch syndrome, a well characterized dominant cancer syndrome associated with hereditary non-polyposis colorectal cancer (HNPCC) and other malignancies (reviewed in [4]). Tumors arising in these individuals result from somatic loss of the remaining wild type *MLH1*, *MSH2*, *MSH6* or *PMS2* allele, which leads to impaired MMR and accumulation of somatic mutations.

To date, some 46 families with patients harboring biallelic germline mutations of *MLH1*, *MSH2*, *MSH6* or *PMS2* have been reported (reviewed in [5]). Constitutive biallelic inactivation of one of these mismatch repair genes causes a recessive childhood cancer syndrome characterized by early-onset malignancies and café-au-lait spots (CALS) and/or other signs of neurofibromatosis type 1 (NF1).

Alluding to the underlying genetic defect we refer to this syndrome as constitutional mismatch repair-deficiency (CMMR-D) syndrome.

The tumor spectrum of the reported CMMR-D syndrome patients includes primarily hematological neoplasias, brain tumors and Lynch syndrome associated tumors [5]. So far other tumor entities, such as neuroblastoma, Wilm's tumor, ovarian neuroectodermal tumor or infantile myofibromatosis have been found only in individual cases. Herein, we report on two families with CMMR-D syndrome. In both families one affected individual developed rhabdomyosarcoma (RMS), suggesting that RMS is part of the CMMR-D syndrome tumor spectrum.

CASE REPORTS

Family 1: The index patient is a 9-year old male from consanguineous Arab parents. He was diagnosed with embryonal RMS in his left nasolabial fold at the age of three years and was treated according to the German soft tissue sarcoma study trial CWS96. He relapsed one year later and was treated according to the high-risk arm of the CWS-2002-P protocol. At the age of 8 years, he required emergency surgery due to intussusception of the colon. The transverse colon was resected and a primary end-to-end anastomosis was performed. Histological workup revealed the diagnosis of a single adenocarcinoma. Lymph nodes were negative and he received FOLFOX treatment. After the fourth cycle he developed rectal bleeding and the patient was seen at our institution for a second opinion. Notably, physical examination showed several café-au-lait spots and rectal endoscopy showed no polyps or tumors. A similar case with multiple CLS and several childhood cancers due to a biallelic mutation of *PMS2* was recently diagnosed at our institution [6] and we, thus, suspected the same syndrome. Immunohistochemistry or microsatellite instability analysis of the tumors was not possible because the patient underwent surgery before presenting at our institution and a tumor specimen was unavailable. Therefore, informed consent for germline mutation analysis was obtained from the parents and *PMS2* analysis

was performed with published RNA-based methods [7]. Sequencing of RT-PCR products revealed a novel homozygous mutation, c.[219T>A]+[219T>A], leading to a premature stop codon (p.Cys73X) in *PMS2* exon 3. This finding was confirmed by sequencing of genomic DNA from this individual and is consistent with the diagnosis of CMMR-D syndrome due to a homozygous *PMS2* mutation. As expected, both parents were heterozygous carriers of the mutant *PMS2* allele (Fig. 1). Mutation analysis of DNA from two healthy siblings and members of the extended family was not possible. The absence of malignancies in the heterozygous parents is not surprising as heterozygous *PMS2* mutations are known to have a low penetrance [8, 9]. The mother's father died from a bone tumor at the age of 60 years and her brother had lung cancer. Two of the father's cousins were diagnosed with brain tumors at the ages of 21 and 50 years, respectively, and another cousin was diagnosed with bladder carcinoma at the age of 55 years.

Family 2: A 22-year-old East Indian female presented with a three-year history of anemia and a 20 pound weight loss over the previous few months. A large abdominal mass was identified on ultrasound and CT. Colonoscopy identified four synchronous adenocarcinomas of the rectum, sigmoid, transverse colon and cecum. The patient underwent total proctocolectomy with en bloc Whipple resection. Pathology confirmed the four primary colorectal cancers and identified six additional tubulovillous and villous adenomas in the rectum. The patient was also noted to have multiple CLS. Based on her history and her cutaneous features she was referred to the Familial Gastrointestinal Cancer Registry (FGICR) for genetic evaluation. Immunohistochemistry of the MLH1, MSH2, MSH6 and PMS2 proteins revealed complete lack of PMS2 expression in both normal and tumor tissue and normal expression of the other proteins. Microsatellite instability analysis of BAT25 and BAT26 of two representative tumors showed instability at BAT25 in one tumor and stability in the other. BAT26 was stable in both tumors.

Collectively these data suggest underlying biallelic *PMS2* germline mutations in the patient. Informed consent for germline mutation analysis was obtained by the patient. Unfortunately, genomic DNA extracted from blood lymphocytes of the patient failed to amplify long-range PCR products [10] and RNA extracted from puromycin treated lymphocytes of the patient was not available for cDNA sequencing [7]. Hence, no reliable germline *PMS2* analysis was possible. The proband's sister was initially diagnosed with a left occipital anaplastic astrocytoma at age 16. Four months after the astrocytoma diagnosis, she was found to have an undifferentiated sarcoma of the right pterygoid fossa. Molecular analysis of the tumor did not reveal features consistent with alveolar rhabdomyosarcoma, Ewing's sarcoma, or desmoplastic small round cell tumor, therefore the pathology suggested some embryonal RMS-like features. Initially, she was referred to a genetics clinic to be evaluated for Li-Fraumeni syndrome. The family declined genetic testing for *TP53* as well as DNA banking. The patient died at age 18 of her brain tumor. Retrospectively performed immunohistochemistry revealed complete lack of PMS2 expression in the astrocytoma and the sarcoma (Fig. 2) as well as normal tissue of the patient, confirming further the suspected biallelic *PMS2* germline mutations in both sisters. Parents of the children are second cousins and extended family history revealed complex consanguinity (Fig 3). The mother of the children reportedly died of colorectal cancer at age 28, a maternal uncle died of leukemia at age 9 and another maternal uncle died of a brain tumor at age 19. Due to the complex consanguinity, it is possible that in the mother's generation may have been biallelic *PMS2* mutation carriers as well. Confirmation of the cancers was not possible as they were treated outside of the country.

DISCUSSION

Together these patients show that RMS is part of the tumor spectrum of CMMR-D syndrome. Notably, RMS has been described previously in a patient who was closely related to three individuals in whom a homozygous *MLH1* mutation was

detected [11, 12], suggesting that this individual also has CMMR-D. Hence, the spectrum of malignancies occurring in CMMR-D syndrome patients may be extended from hematological neoplasias, brain tumors and LS-associated tumors to embryonic tumors, such as embryonic RMS and possibly also Wilm's tumor [13, 14] and neuroblastoma [15].

RMS is the most frequent soft tissue sarcoma in children. Cancer syndromes predisposing to RMS include Li-Fraumeni syndrome, Costello syndrome, familial retinoblastoma and Beckwith-Wiedemann syndrome [16]. An increased incidence of RMS has been reported also for NF1 [17]. As in the two reported cases, the histological subtype tends to be embryonal RMS in NF1 patients [17, 18]. Due to the clinical overlap between CMMR-D syndrome and NF1, we speculate that some individuals with RMS and the clinical (mis)diagnosis of NF1 have CMMR-D syndrome. The cancer and family history of at least one of these patients strongly suggest this possibility [5, 19]. Homozygosity of all tested microsatellite markers in the *NF1* locus may indicate parental consanguinity in another reported NF1 case with RMS rendering CMMR-D syndrome a possible alternative diagnosis also in this patient [20]. Identifying the underlying genetic alteration in patients with CMMR-D syndrome is important, since it has implications also for the wider family. There is a recurrence risk of 25% for the recessive disorder in the family and heterozygous carriers have an increased risk for Lynch syndrome-related tumors. Careful examination of the family history of the patient, analysis of the tumor(s) for loss of one of the mismatch repair proteins as well as microsatellite instability and subsequent mutation analysis will allow proper diagnosis of the underlying disorder in RMS patients with signs of NF1.

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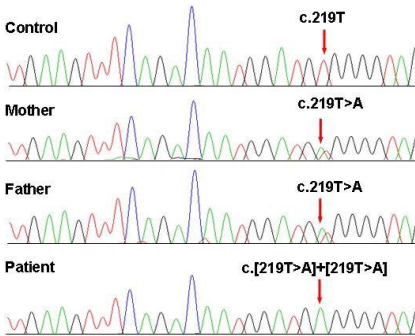
FIGURES

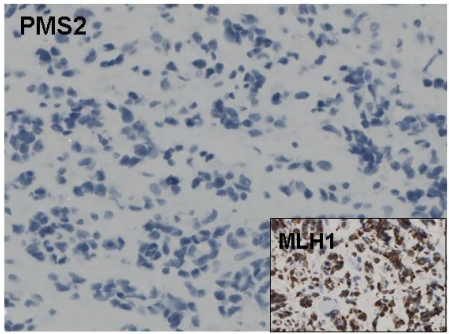
Figure 1: Sequencing results in family 1 showing heterozygosity for the mutation c.219T>A (p.C73X) in the parents and homozygosity in the patient.

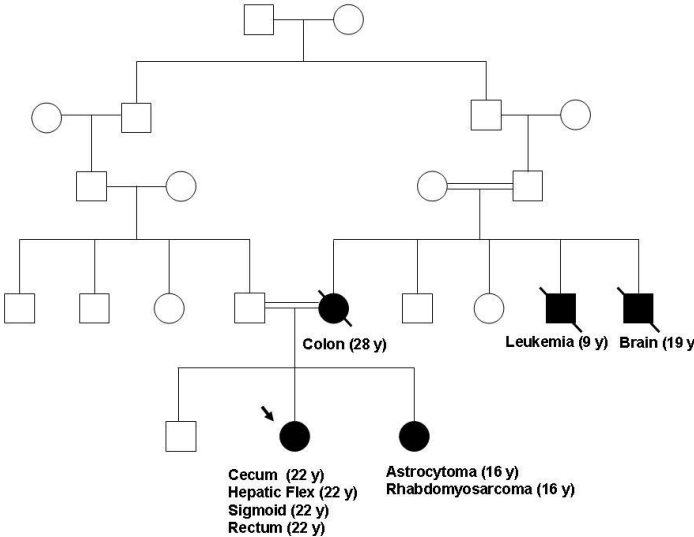
Figure 2: Immunohistochemistry analysis shows isolated lack of PMS2 expression in the rhabdomyosarcoma from patient 2 in family 2. The mismatch repair proteins MLH1 that is shown in the inset as well as MSH2 and MSH6 stain positive in the same tumor.

Figure 3: Pedigree of family 2. The index patient is marked with an arrow. The sites of tumors and the age at diagnosis in years (y) are indicated below the patient and her relatives. The sister of the index patient developed rhabdomyosarcoma at the age of 16 years.

Statement:


Control
c.219T
Mother
c.219T>A
Father
c.219T>A
Patient
c.[219T>A]+[219T>A]


PMS2
MLH1


Colon (28 y)
Leukemia (9 y)
Brain (19 y)
Cecum (22 y)
Hepatic Flex (22 y)
Sigmoid (22 y)
Rectum (22 y)
Astrocytoma (16 y)
Rhabdomyosarcoma (16 y)