Familial Cancer
DOI 10.1007/s10689-015-9793-0

SHORT COMMUNICATION

Pitfalls in the diagnosis of biallelic *PMS2* mutations

Marina Antelo[1] · Daniela Milito[1] · Jennifer Rhees[2] · Enrique Roca[1] · Miguel Barugel[1] · Miriam Cuatrecasas[3] · Leticia Moreira[3] · Maria Liz Leoz[3] · Sabela Carballal[3] · Teresa Ocaña[3] · Maria Pellisé[3] · Antoni Castells[3] · C. Richard Boland[2] · Ajay Goel[2] · Francesc Balaguer[3]

Abstract Constitutional Mismatch Repair Deficiency (CMMR-D) syndrome is an inherited childhood cancer syndrome due to bi-allelic mutations in one of the four DNA mismatch repair genes involved in Lynch syndrome. The tumor spectrum of this syndrome includes hematological, brain and Lynch syndrome associated malignancies, with an increased risk of synchronous and metachronous cancers, and signs of Neurofibromatosis type-1 syndrome such as *café-au-lait macules* during the first three decades of life. Here, we report the first Argentinian patient with CMMR-D syndrome, focusing on her history of cancer and gastrointestinal manifestations, and the challenging molecular algorithm to finally reach her diagnosis.

Keywords PMS2 · Early-onset colorectal cancer · Lynch syndrome · Constitutional mismatch repair deficiency syndrome

✉ Marina Antelo
machuantelo@hotmail.com

✉ Francesc Balaguer
fprunes@clinic.ub.es; fprunes@clinic.cat

[1] Oncology Section, Hospital of Gastroenterology "Dr. C. B. Udaondo", Buenos Aires, Argentina

[2] Division of Gastroenterology, Department of Internal Medicine, Charles Sammons Cancer Center and Baylor Research Institute, Baylor University Medical Center, Dallas, TX, USA

[3] Department of Gastroenterology, Hospital Clínic, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), University of Barcelona, Villarroel 170, 08036 Barcelona, Catalonia, Spain

Introduction

Lynch syndrome is an autosomal dominant condition caused by heterozygous germline mutations in one of the four DNA mismatch repair genes (MMRg). It presents a 20–70 % risk of developing colorectal and endometrial tumors and a significant risk for other extracolonic neoplasias. Early onset of cancer is frequently seen, but affected patients under 20 years are very rare [1]. Recently, several case reports of children and adolescents presenting with some of the Lynch associated tumors have been published [2–5], and were found to carry a bi-allelic mutation in one of the DNA MMRg. This uncommon variant of Lynch syndrome is now defined as the Constitutional Mismatch Repair Deficiency Syndrome (CMMR-D) [6, 7]. To our knowledge, up to May 2013 there were 114 patients reported worldwide with CMMR-D [8].

Case report and results

Here we report a woman that was diagnosed with a colon adenocarcinoma at age 15. During 7 years of follow up, at least fifteen colonic adenomas were removed. The upper gastrointestinal endoscopies performed during that time revealed six duodenal adenomas. A clinical diagnosis of Familial Adenomatous Polyposis was made, but germline mutational analysis for the *APC* gene was negative. Her physical examination revealed six *café au lait macules* (CALMs) and multiple Lisch nodules at her ocular examination. However, germline mutational analysis of the *NF1* gene turned out negative. At the age of 24, the patient was referred for evaluation to our High-Risk Colorectal Cancer Clinic. A renal ultrasound revealed a high-grade urothelial bladder carcinoma (T1), for which she received local chemotherapy. In her last colonoscopy, at age 25, a

Published online: 15 March 2015

rectal adenocarcinoma was diagnosed. While planning surgical treatment, she was hospitalized for a superior vena cava syndrome with a diagnosis of mediastinal granulocytic sarcoma. She died at age 25 during chemotherapy. Except for her maternal grand-mother with pancreatic cancer at 72 years-old, there was no other history of tumors in her family, nor known consanguinity. Immunohistochemistry staining for MLH1, MSH2, MSH6 and PMS2 proteins in her colorectal cancer tissue revealed absence of PMS2 expression both in tumor cells and in normal colonic surrounding tissue. MSI analysis was performed using five mononucleotides MSI markers and surprisingly, microsatellite stability (MSS) was observed. With the suspicion of CMMR-D due to *PMS2* biallelic mutations, we performed germline *PMS2* mutational analysis. As a first approach, we attempted to amplify PCR of exons 6, 7, 8, 10 and 11 of the PMS2 gene using primers and conditions previously established in our laboratory. No amplification product was observed from the patient's DNA. Additionally, long PCR was attempted using the primers described by Clendenning et al. [9] and no product was observed. To verify that the failed amplification was not due to DNA quality, PCR of exon 5 of MSH2 was conducted. Amplification was successful, thus suggesting that the failed amplification was specific to *PMS2*. Multiplex ligation-dependent amplification (MLPA) was performed following the manufacturer's protocol (MRC-Holland, Amsterdam, the Netherlands; probemix 007). Multiple peaks were completely absent from the patient's profile, including peaks representing exons 2, 5–11, intron 12 and exons 13–15 of the *PMS2* gene (data not shown). Products were observed for exons 1, 3 and 4 of *PMS2*, as well as for probes specifically targeting *PMS2CL* pseudogene and probes that amplify both *PMS2* and *PMS2CL*. The MLPA profile of both parents and one of two sibling revealed that all three were heterozygous carriers of the same *PMS2* rearrangement, with deletions of multiple exons. MRC-Holland was contacted for assistance in data analysis and interpretation. The most likely explanation was that the unusual profile could be due to a homozygous deletion of exons 2–15, and the observed amplification of probes for exons 3 and 4 was due to nonspecific amplification of *PMS2* pseudogenes. In line with this hypothesis, MRC-Holland modified the assay (probemix P008 version C) that tested sufficiently specific for the *PMS2* exons 3 and 4 in healthy subjects, displaying a pattern compatible with a large deletion of exons 2–15 in our patient (Fig. 1).

Fig. 1 Multiplex Ligation-dependent Probe Amplification (MLPA) results for *PMS2*. Capillary electrophoresis pattern from a heatlhy subject (**a**), proband (**b**) and sibling (**c**) Chromosomal position of the most relevant peaks are detailed [*probes that are shared by *PMS2* and *PMS2CL* (pseudo gene); #probes that amplify only *PMS2CL*]. Multiple peaks were completely absent from the patient's profile (**b**), compatible with a large deletion of exons 2–15 (only normal intensity for exon 1 peaks was observed). The pattern observed in the sibling (**c**) was compatible with a heterozygous carrier of the same *PMS2* rearrangement

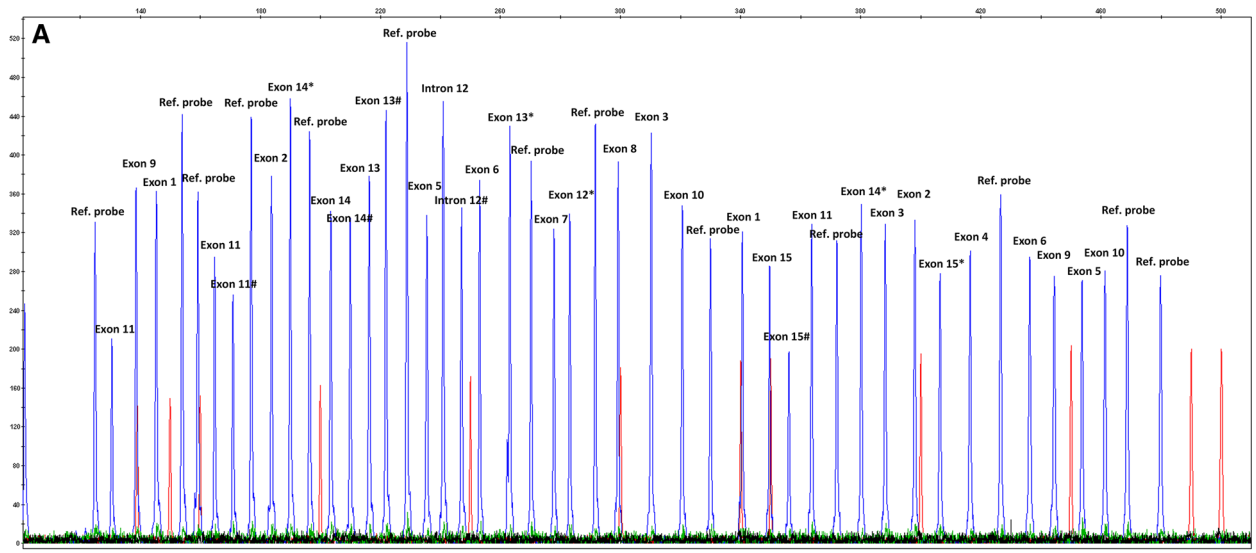
Discussion

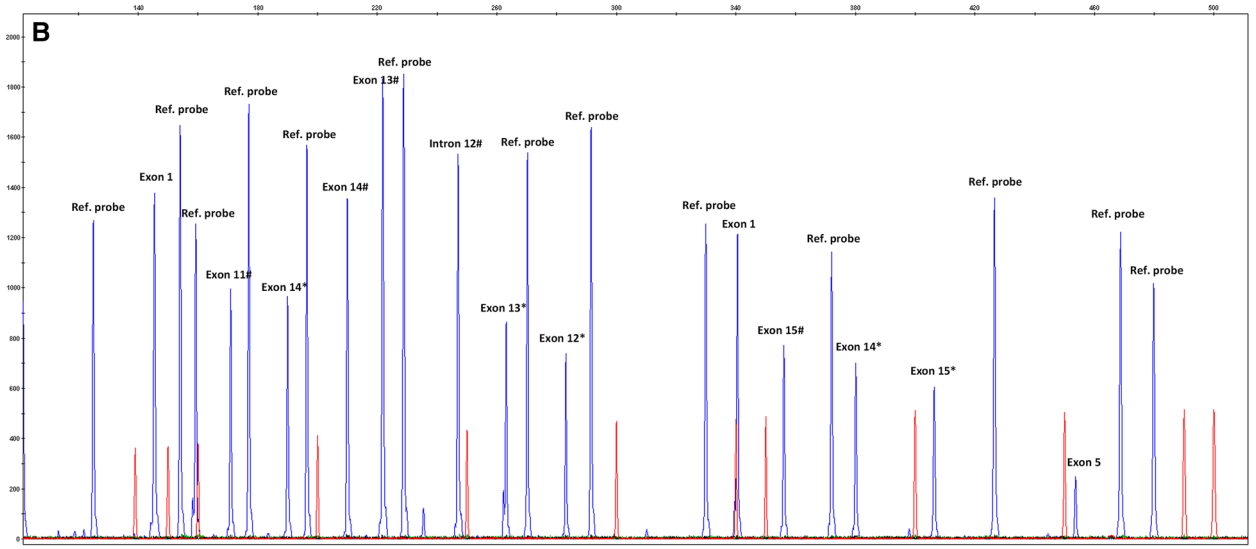
This is the first case report of an Argentinian patient with a CMMR-D syndrome and a proven *PMS2* bi-allelic mutation. She presented a wide history of tumors, which included colorectal cancers, multiples colonic adenomas, an urothelial carcinoma and a granulocytic sarcoma. This type of presentation is comparable to that reported by Herkert el al [9], in which 84 % of the families with bi-allelic *PMS2* mutations had gastrointestinal manifestations, and in which one-third of carriers had gastrointestinal cancer as the first manifestation of the CMMR-D syndrome. On the other hand, urothelial cancer is infrequent in bi-allelic MMR genes mutation carriers; we found only two case reports of urothelial cancer in the context of this syndrome, being our proband the third report worldwide [3, 10]. As far as we know, this patient represents the first reported case of a bi-allelic MMR gene mutation carrier with a granulocytic sarcoma.

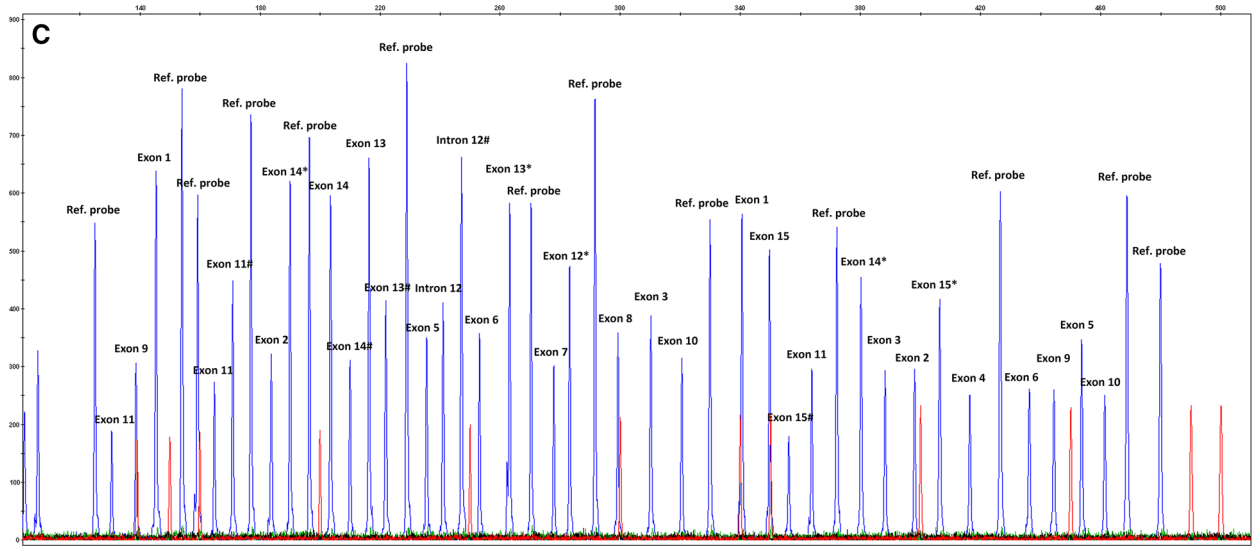
The cornerstone for Lynch syndrome diagnosis relies on tumor MSI testing and IHC for the MMR proteins. Leenen et al. [4] reported the results of molecular analyses of 21 patients with biallelic MMR mutations with gastrointestinal malignancies and MSI was demonstrated in all patients. Interestingly, brain tumors seem to behave differently, and MSS has been reported in CMMR-D patients, suggesting that *PMS2* deficiency could lead to tumorigenesis through a different mechanism. Surprisingly, in our case, colorectal cancer displayed MSS. An explanation for this finding remains elusive. In any case, our results suggest that both IHC and MSI testing should be performed when a CMMRD syndrome is suspected.

Detection of germline mutations in the *PMS2* gene is complicated by the existence of numerous pseudogenes. PCR and sequencing of *PMS2* requires a nested PCR approach for multiple exons, and detection of deletions by MLPA requires a complicated analysis that includes measurement of exon copy numbers of both the *PMS2* gene and the *PMS2CL* pseudogenes. This has been the case, and deletions in exons 3 and 4 were the most difficult to be analyzed. As a result of this testing, MRC-Holland issued an electronic warning [11] to all users to address the difficulty of the current MLPA kit to identify large rearrangements in exons 3 and 4 of the *PMS2* gene.

In the absence of a known consanguinity in the reported family, it is surprising that the patient carried an homozygous mutation. Since the penetrance of the phenotype of Lynch syndrome due to PMS2 germline mutations is


A
Ref. probe
Exon 14*
Exon 13#
Intron 12
Ref. probe
Ref. probe
Ref. probe
Exon 13*
Ref. probe
Exon 3
Exon 9
Exon 1
Ref. probe
Exon 2
Exon 8
Exon 13
Exon 6
Ref. probe
Exon 5
Intron 12#
Exon 12*
Exon 10
Exon 14*
Exon 2
Ref. probe
Ref. probe
Exon 14
Exon 14#
Exon 7
Exon 1
Exon 11
Exon 3
Ref. probe
Exon 4
Exon 11
Exon 15
Ref. probe
Exon 6
Exon 15*
Exon 9
Exon 10
Ref. probe
Exon 11#
Exon 5
Exon 11
Exon 15#


B
Ref. probe
Exon 13#
Ref. probe
Ref. probe
Ref. probe
Ref. probe
Intron 12#
Ref. probe
Ref. probe
Exon 1
Exon 14#
Ref. probe
Ref. probe
Exon 1
Ref. probe
Ref. probe
Ref. probe
Exon 11#
Exon 14*
Exon 13*
Exon 15#
Exon 12*
Exon 14*
Exon 15*
Exon 5


C
Ref. probe
Ref. probe
Ref. probe
Ref. probe
Exon 13
Intron 12#
Ref. probe
Exon 1
Exon 14*
Exon 13*
Ref. probe
Exon 14
Ref. probe
Ref. probe
Exon 1
Ref. probe
Exon 15
Ref. probe
Ref. probe
Exon 11#
Exon 12*
Exon 14*
Ref. probe
Exon 13#
Intron 12
Exon 3
Exon 15*
Exon 5
Exon 8
Exon 6
Exon 10
Exon 3
Exon 5
Exon 9
Exon 9
Exon 14#
Exon 7
Exon 11
Exon 2
Exon 6
Exon 10
Exon 2
Exon 4
Exon 11
Exon 15#
Exon 11

known to be lower than for *MLH1* of *MSH2* [12], the large rearrangement found in this family could represent a founder mutation in the Argentinian population. Further studies are needed to clarify this issue.

In conclusion, in the absence of proven *APC* germline mutations, CMMR-D syndrome should be suspected in patients with childhood CRC or colonic polyposis, especially when they have NF1 features. Further characterization of this rare and lethal syndrome, with almost 100 % mortality by age 35, is needed to establish potential benefits of surveillance, and to develop evidence-based screening guidelines.

Acknowledgments The present work was supported by a grant from the National Cancer Institute of Argentina, by Grants R01 CA72851 and CA129286 from the National Cancer Institute and the National Institutes of Health of USA, and by funds from the Baylor Research Institute to CRB and AG. FB was supported by a Grant from Fundación Alfonso Martín Escudero and from Fondo de Investigación Sanitaria (PI10/00384; PI13/00719). CIBEREHD is funded by the Instituto de Salud Carlos III. MA was supported by a "Type I and II Postgraduate Scholarship" given by the CONICET (National Council of Scientific and Technical Investigations) dependant on the Argentine Ministry of Science and Technology. DM was supported by a scholarship given by the National Cancer Institute of Argentina.

Conflict of interest None.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Jenkins MA, Baglietto L, Dowty JG et al (2006) Cancer risks for mismatch repair gene mutation carriers: a population-based early onset case-family study. Clin Gastroenterol Hepatol 4:489–498
2. Gallinger S, Aronson M, Shayan K et al (2004) Gastrointestinal cancers and neurofibromatosis type 1 features in children with a germline homozygous MLH1 mutation. Gastroenterol 126: 576–585
3. Krüger S, Kinzel M, Walldorf C et al (2008) Homozygous PMS2 germline mutations in two families with early-onset haematological malignancy, brain tumours, HNPCC-associated tumours, and signs of neurofibromatosis type 1. Eur J Hum Genet 16:62–72
4. Leenen CH, Geurts-Giele WR, Dubbink HJ et al (2011) Pitfalls in molecular analysis for mismatch repair deficiency in a family with biallelic PMS2 germline mutations. Clin Genet 80:558–565
5. Poley JW, Wagner A, Hoogmans MM et al (2007) Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. Cancer 109:2349–2356
6. Ricciardone MD, Ozçelik T, Cevher B et al (1999) Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. Cancer Res 59:290–293
7. Durno CA, Aronson M, Tabori U, Malkin D, Gallinger S, Chan HS (2012) Oncologic surveillance for subjects with biallelic mismatch repair gene mutations: 10 year follow-up of a kindred. Pediatr Blood Cancer 59:652–656
8. Wimmer K (2012) Relationship between nf1 and constitutive mismatch repair deficiency. Neurofibromatosis type 1 molecular and cellular biology, chapter 16. pp 235–251
9. Clendenning M, Hampel H, LaJeunesse J et al (2006) Long-range PCR facilitates the identification of PMS2-specific mutations. Hum Mutat 27:490–495
10. Herkert JC, Niessen RC, Olderode-Berends MJ et al (2011) Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. Eur J Cancer 47:965–982
11. http://www.mlpa.com/WebForms/WebFormMain.aspx?Tag=uczWBvH\7S\gCiIH0DhrcJq6gxcP0dAZ#uczWBvH7SgCiIH0DhrcPasz7juGGZthR6pZ0V/hlAmOKj6uGEYw
12. Senter L, Clendenning M, Sotamaa K et al (2008) The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 135:419–428