EUROPEAN JOURNAL OF CANCER 47 (2011) 965–982

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: Case series, review and follow-up guidelines

Johanna C. Herkert [a,*], *Renée C. Niessen* [a], *Maria J.W. Olderode-Berends* [a], *Hermine E. Veenstra-Knol* [a], *Yvonne J. Vos* [a], *Heleen M. van der Klift* [b], *Rene Scheenstra* [c], *Carli M.J. Tops* [b], *Arend Karrenbeld* [d], *Frans T.M. Peters* [e], *Robert M.W. Hofstra* [a], *Jan H. Kleibeuker* [e], *Rolf H. Sijmons* [a]

[a] *Department of Genetics, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*
[b] *Department of Clinical Genetics and Human Genetics, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[c] *Department of Paediatrics, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*
[d] *Department of Pathology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*
[e] *Department of Gastroenterology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*

ARTICLE INFO

Article history:
Received 18 October 2010
Accepted 20 January 2011
Available online 4 March 2011

Keywords:
Constitutional MMR-deficiency syndrome
Bi-allelic PMS2 mutation
Paediatric gastrointestinal cancer
Follow-up recommendations

ABSTRACT

Background: Bi-allelic germline mutations of one of the DNA mismatch repair genes, so far predominantly found in *PMS2*, cause constitutional MMR-deficiency syndrome. This rare disorder is characterised by paediatric intestinal cancer and other malignancies. We report the clinical, immunohistochemical and genetic characterisation of four families with bi-allelic germline *PMS2* mutations. We present an overview of the published gastrointestinal manifestations of CMMR-D syndrome and propose recommendations for gastro-intestinal screening.

Methods and Results: The first proband developed a cerebral angiosarcoma at age 2 and two colorectal adenomas at age 7. Genetic testing identified a complete *PMS2* gene deletion and a frameshift c.736_741delinsTGTGTGTGAAG (p.Pro246CysfsX3) mutation. In the second family, both the proband and her brother had multiple intestinal adenomas, initially wrongly diagnosed as familial adenomatous polyposis. A splice site c.2174+1G>A, and a missense c.137G>T (p.Ser46Ile) mutation in *PMS2* were identified. The third patient was diagnosed with multiple colorectal adenomas at age 11; he developed a high-grade dysplastic colorectal adenocarcinoma at age 21. Two intragenic *PMS2* deletions were found. The fourth proband developed a cerebral anaplastic ganglioma at age 9 and a high-grade colerectal dysplastic adenoma at age 10 and carries a homozygous c.2174+1G>A mutation.
Tumours of all patients showed microsatellite instability and/or loss of PMS2 expression.

* *Corresponding author:* Tel.: +31 50 3617100; fax: +31 50 3617230.
E-mail address: j.c.herkert@medgen.umcg.nl (J.C. Herkert).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.01.013

Conclusions: Our findings show the association between bi-allelic germline *PMS2* mutations and severe childhood-onset gastrointestinal manifestations, and support the notion that patients with early-onset gastrointestinal adenomas and cancer should be investigated for CMMR-D syndrome. We recommend yearly follow-up with colonoscopy from age 6 and simultaneous video-capsule small bowel enteroscopy from age 8.

1. Introduction

Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC, MIM 114500) is an autosomal dominant condition with high penetrance caused by heterozygous germline mutations in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, or *PMS2*. It is one of the most prevalent inherited cancer syndromes, responsible for approximately 2–5% of all colorectal cancers.[1] Patients with Lynch syndrome have a high risk of developing colorectal carcinoma and endometrial carcinoma, typically in the fourth or fifth decade. There is also an increased risk for various other tumours, i.e. cancer of the stomach, small bowel, pancreas, hepatobiliary tract, ureter and renal pyelum, ovaries, skin and brain.[2,3]

In contrast to Lynch syndrome, germline compound heterozygous or homozygous MMR gene mutations, which means inherited mutations in *both* alleles of an MMR gene, are a rare cause of paediatric colorectal cancer and other malignancies, mainly leukaemia and brain tumours.[4] These malignancies in early childhood are often associated with multiple café-au-lait maculae (CALM) and some other features of neurofibromatosis type 1 (NF1), like freckling and Lisch nodules. Since the first publications on this syndrome in 1999,[5,6] several names have been proposed, including the acronym CoLoN (Colon tumours and/or Leukaemia/Lymphoma and/or Neurofibromatosis features),[7] Lynch III syndrome,[8] childhood cancer syndrome (CCS)[9] and constitutional MMR-deficiency (CMMR-D) syndrome.[10] Up to December 2009, 97 carriers with a bi-allelic MMR defect from 53 families had been reported.[4,5,8–55]

Here, we report on five children from four Dutch families carrying bi-allelic *PMS2* germline mutations and focus on their gastrointestinal manifestations. We also present the results of an extensive literature search on the gastrointestinal and molecular data of CMMR-D syndrome patients and propose a guideline for surveillance of the gastrointestinal tract in this syndrome.

2. Patients

2.1. *Family 1*

A 2-year-old boy diagnosed with an infiltrating cerebral angiosarcoma was referred for genetic testing. Physical examination revealed multiple CALM and anal and axillary freckling. A clinical diagnosis of NF1 was suggested but *NF1* mutation analysis was negative. Two years later, magnetic resonance imaging showed a local relapse, which was surgically removed. Just before his 7th birthday, he suffered from anal blood loss and colonoscopy revealed two tubular adenomas: one with a diameter of 1.5 cm and high-grade dysplastic features in the rectum and one of 0.3 cm diameter in the sigmoid.

His family history revealed no consanguinity. The boy's mother was diagnosed with a pituitary prolactin-secreting micro-adenoma at age 30 and a moderately dysplastic colorectal adenoma at age 36. His maternal grandfather was said to have had colorectal carcinoma at age 72. The mother of this grandfather died of colorectal cancer at age 77. The paternal grandfather had oesophageal carcinoma at age 55 as did his brother at 65 years of age. Furthermore, the mother of the paternal grandfather had tongue cancer and skin cancer at an unknown age.

2.2. *Family 2*

The female proband was diagnosed at age 19 with multiple colon adenomas, some showing high-grade dysplasia. A clinical diagnosis of familial adenomatous polyposis (FAP) was made, but *APC* gene analysis revealed no mutation. A subtotal colectomy with ileo-rectal anastomosis was performed. During follow-up, a proximal jejunal adenocarcinoma, pT3N1, was found and resected. In addition, one rectal tubulovillous adenoma was observed. At age 21 she developed macroscopic haematuria. Urologic examination revealed a papillary transitional cell carcinoma of her bladder, stage pT2GIII, followed by transurethral resection and intravesical Bromocresol Blue (BCG) treatment. At age 22 endoscopy revealed two ileal and two rectal tubulovillous adenomas with low-grade dysplastic features. Finally, a rectal carcinoma was found and she died aged 23.

Her younger brother was diagnosed with a rectal high-grade dysplastic tubulovillous adenoma at age 15. Video-capsule endoscopy revealed no suspicious lesions. Double-balloon enteroscopy at age 16 showed a thickened duodenal ridge and pathological investigation indicated a duodenal tubulovillous adenoma. At esophagogastroduodenoscopy 6 months later (age 17) a duodenal adenocarcinoma of 2.7 cm, pT1N0M0, and two duodenal adenomas with high-grade dysplasia were detected. A pylorus-preserving pancreaticoduodenectomy was performed. *APC* and *MUTYH* gene testing revealed no mutations. Follow-up consisted of annual video-capsule enteroscopy and 2-yearly ileo-colonoscopy. At age 19, a jejunal adenocarcinoma, pT3N0M0, was diagnosed and surgically removed. At age 20 two colorectal tubulovillous adenomas with high-grade dysplasia were resected. One year later, at double-balloon endoscopy, multiple colorectal tubulovillous adenomas were removed and a high-grade dysplastic jejunal tubulovillous adenoma was observed followed by jejunal resection. At age 21 he developed T-cell

acute lymphoid leukaemia (T-ALL); he died at age 22. Both patients displayed no features of NF1, except for one CALM.

Until now, both parents are healthy. Their paternal grandmother died aged 56 from colorectal cancer. Her sister also suffered from colorectal cancer. The patients' maternal grandfather probably had gastric cancer with liver metastasis at age 52, whilst their maternal grandmother might have had endometrial carcinoma. She died at age 59.

2.3. *Family 3*

The 11-year-old male proband developed multiple adenomatous polyps in his left colon and rectum (<100). At age 21 years, multiple tubular adenomas (>30) with moderate dysplastic features were removed from the distal sigmoid till anus. A proctocolectomy was performed at age 21. A high-grade adenocarcinoma, pT1N0Mx, and 26 tubulovillous adenomas with high-grade dysplasia were found in the surgically removed tissue. At age 32, a duodenal carcinoma, pT3N0M1, a jejunal carcinoma, pT3N0M1 and 13 small gastrointestinal polyps were diagnosed and resected. At double balloon enteroscopy, 6 months later, another jejunal carcinoma, pT2N0, and an ileal adenoma with high-grade dysplasia were identified. During surgery a pan-enteroscopy was performed which identified no other lesions. At age 34 he developed a glioblastoma, for which he is currently treated. Besides his cancer history, the proband has congenital asplenia, left isomerism and a ventricle septum defect. As far as we know, these congenital anomalies are not associated with MMR defects. *MUTYH* and *APC* gene mutation analysis, routine karyotyping and array-comparative genomic hybridization analysis revealed no abnormalities.

His mother had a tubulovillous adenoma with moderate dysplastic features at age 51 and a juvenile polyp at age 55. Two of his mother's sibs had also had a few low-grade dysplastic adenomas at age 50–63. His maternal grandfather had colorectal carcinoma at age 64, and a sister of his grandfather developed colorectal cancer at 63 years of age. His father's nine siblings (now aged 50–69) had no history of cancer.

2.4. *Family 4*

At age 9 the female proband was diagnosed with a cerebral anaplastic ganglioma. She also displayed multiple CALM (>6) and freckling in her groin. *NF1* and *SPRED1* mutation analysis was negative. At age 10 she suffered from anal blood loss and a rectal adenoma with high-grade dysplasia was removed.

Her healthy parents are consanguineous and of Turkish origin. Both siblings (aged 10 and 12) are healthy. A sister of her maternal grandmother was said to have had colorectal cancer at age 56.

3. Methods

3.1. *Tumour analysis*

Microsatellite instability (MSI) analysis was performed on formalin-fixed, paraffin-embedded sections of tumours and corresponding normal tissue. Following DNA amplification using fluorescent-labelled primers, a panel of five microsatellites, including two mononucleotide repeats (BAT25, BAT26) and three dinucleotide repeats (D2S123, D5S346, D17S250), was analysed for allelic shift according to the international guidelines for evaluating MSI in families suspected of having Lynch syndrome.[56,57] If two or more markers showed instability, the tumour was classified as MSI-high.

3.2. *Immunohistochemistry*

Immunohistochemical staining for MLH1, MSH2, MSH6 and PMS2 protein expression was performed on 3 μm-thick formalin-fixed, paraffin-embedded sections of tumours of all five index patients, as described previously.[58,59] Protein expression in normal tissue adjacent to the tumour served as a positive internal control. Staining of normal tissue on the same slide was used as a positive external control.

3.3. *Mutation analysis*

DNA was isolated from peripheral blood lymphocytes using standard methods. All coding sequences of the *MLH1*, *MSH2* and *MSH6* genes were analysed by denaturing gradient gel electrophoresis (DGGE) and/or direct sequencing as previously described.[60–63] For the detection of large deletions (exonic deletions or deletions of a complete gene) and duplications by multiplex ligation-dependent probe amplification (MLPA), we used the SALSA MLPA kits P003 MLH1/MSH2 and P008 PMS2/MSH6 (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's recommended protocol, and as published elsewhere.[60] Direct sequencing of PCR products of the *PMS2* gene was performed as described by Niessen et al.[59] In addition, RT-PCR of *PMS2* was performed for family 2, 3 and 4 on RNA derived from short-term cultured lymphocytes.[64]

Any variant detected, excluding common SNPs, was analysed *in silico* using three sequence homology-based programmes: SIFT (Sorting Intolerant from Tolerant amino acid substitutions, (http://blocks.fhcrc.org/sift/SIFT.html), Align-GVGD (http://agvgd.iarc.fr/agvgd_input.php) and PolyPhen (Polymorphism Phenotyping, (http://genetics.bwh.harvard.edu/pph/). The possible effect on the function of the acceptor or donor splice site or creation of a novel splice site was analysed as well by using the RNA splice site prediction programmes from Netgene (http://www.cbs.dtu.dk/services/NetGene2/) and the Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html).

3.4. *Literature review*

To summarise the clinical and molecular data of CMMR-D syndrome patients, we performed a comprehensive literature review. PubMed and EMBASE were searched for English-language articles published between 1980 and December 2009. We primarily focused on articles in which the title/abstract included at least one of the following terms: mismatch repair defect, bi-allelic mutation or Turcot syndrome. This electronic search was completed by individually assessing the references cited in all the papers identified. Reports were only included in this analysis if CMMR-D syndrome had been genetically confirmed.

4. Results

4.1. Microsatellite analysis

The angiosarcoma tissue from the proband of family 1 showed a MSI-high phenotype. The jejunal adenocarcinoma and the bladder tumour from the index patient of family 2 and the anaplastic ganglioma of the proband of family 4 also displayed MSI.

4.2. Immunohistochemistry

Immunohistochemical staining of the MLH1, MSH2, MSH6 and PMS2 proteins showed complete loss of PMS2 staining but normal expression of MLH1, MSH2 and MSH6 in the tumours of all five patients. Normal tissue surrounding the tumours and the endothelial cells of the vessels in tumour tissue showed no PMS2 expression (Fig. 1). External controls were positive.

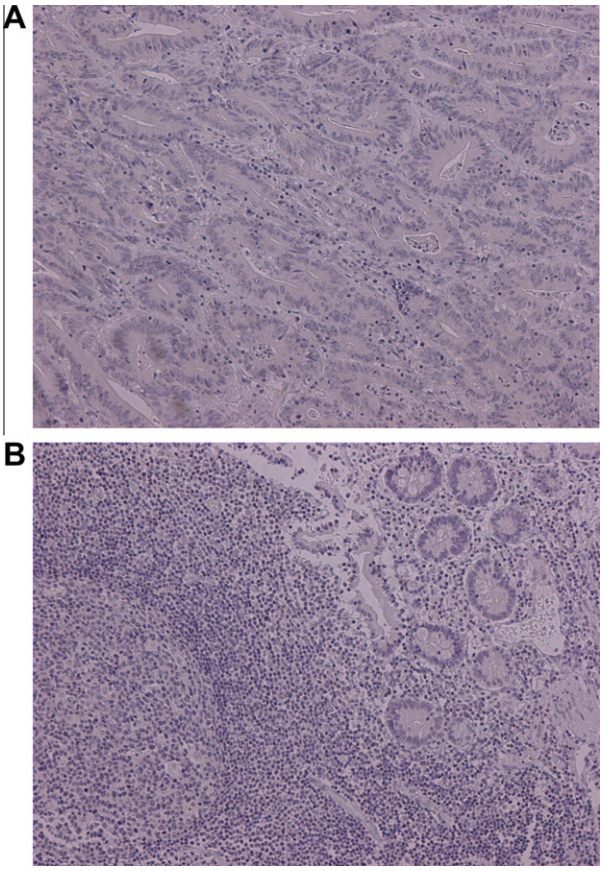

Fig. 1 – Immunohistochemistry analysis in the jejunal adenocarcinoma from the index patient of family 2. (A) Whilst MSH2, MLH1 and MSH6 staining were inconspicuous (data not shown), the nuclei are negative for PMS2 (stain: haematoxylin–eosin, magnification 20×); (B) internal control: lymph follicle with reactive germinal centre and adjacent normal colon mucosa both negative for PMS2 expression (stain: haematoxylin–eosin, magnification 20×).

4.3. Mutation analysis

Mutation analysis in the MMR genes *MLH1, MSH2, MSH6* and *PMS2* was performed. In the index patient of family 1 two pathogenic germline mutations in *PMS2* were found: a deletion of the whole *PMS2* gene and a c.736_741delinsTGTGTGTGAAG mutation in exon 7 (p.Pro246CysfsX3) (Fig. 2). This mutation leads to a frameshift in codon 246 resulting in a premature stop codon two codons downstream and it had previously been described in the Mismatch Repair Genes Variant Database (http://www.med.mun.ca/MMRvariants/). The patient inherited the deletion from his mother and the c.736_741delinsTGTGTGTGAAG mutation from his father.

In both the index patient of family 2 and her brother, two compound heterozygous mutations were found in *PMS2*. In exon 2, a transversion from guanine to thymine (c.137G>T) was detected resulting in an amino acid change from serine to isoleucine at codon 46 (p.Ser46Ile). This mutation has been reported in patients with Lynch syndrome and constitutional MMR-deficiency syndrome (CMMR-D)[11,13,65,66] and was not observed in 218 control chromosomes.[66] The Ser46 residue is a strongly conserved amino acid, localised in an important functional domain of the PMS2 protein (HATPase domain, http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). In addition, serine and isoleucine have different chemical–physical characteristics (Grantham Score 142). PolyPhen predicted this mutation to be probably damaging with a PSIC score of 2.490 (scores of <1.5 are predicted to be benign). SIFT analysis predicted this substitution to affect protein function with a tolerance index score of 0.00 (normalised probabilities of >0.05 are predicted to be tolerated). Align-GVGD predicted the p.Ser46Ile missense substitution as pathogenic (class C65, with a Grantham Variation of 0 and a Grantham deviation of 142). RNA splicing prediction programmes predicted that this variant would have no effect on the function of the acceptor splice site in intron 2, nor does it create a novel splice site.

The second mutation in family 2 was a guanine to adenine transversion (c.2174+1G>A) in intron 12. This nucleotide change causes one of the nucleotides of the consensus splice site at the exon–intron transition downstream of exon 12 to change, so that it is not recognised by the spliceosome. RNA analysis was performed to study the effect of c.2174+1G>A on mRNA splicing and to confirm that this mutation is located in *PMS2* and not in its highly homologous pseudogene *PMS2CL*. RT-PCR amplifying the coding region of *PMS2* exons 10–15 revealed three aberrant transcripts originating from the mutated allele. Sequencing of the RT-PCR product showed that, in one of the transcripts, exon 12 has been skipped leading to an in-frame deletion of 168 bp. In the other two transcripts, insertions of about 300 and 600 bp were observed. The exact nature of these insertions has not yet been determined. The patients' mother carried the c.2174+1G>A mutation and their father the c.137G>T (p.Ser46Ile) mutation.

In the proband of family 3 two deletions in the *PMS2* gene were found with MLPA: a deletion of exons 1–11 and a deletion of exons 5–7. RT-PCR amplifying the coding region of *PMS2* exons 1–11 showed a 640 bp deletion of exons 3–7 in the transcript, which is due to a genomic 7.5 kb deletion

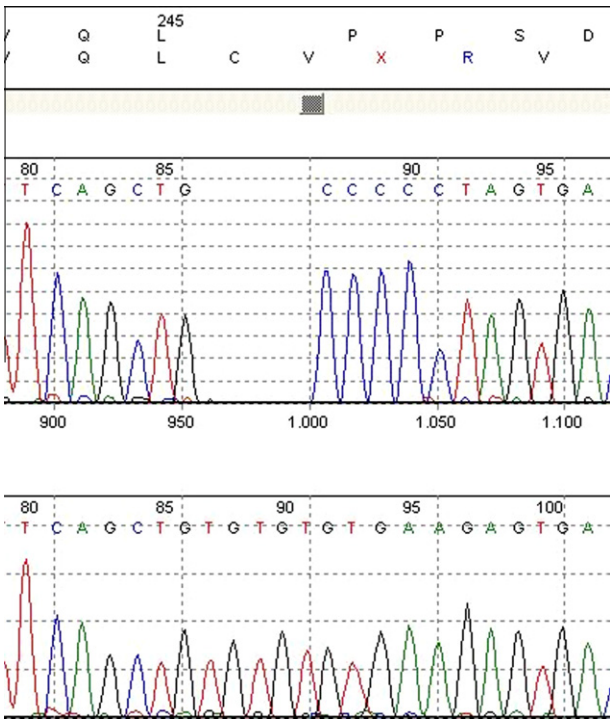

Fig. 2 – Sequencing trace for index patient of family 1 of PMS2 exon 7 showing a hemizygous c.736_741delinsTGTGTGTGAAG mutation (p.Pro246CysfsX3) leading to a frameshift in codon 246 and resulting in a premature stop codon.

harbouring the same exons. The father carries the deletion of exons 3–7, whilst the mother has the exons 1–11 deletion.

The index patient of family 4 harboured the homozygous variant c.2174+1G>A as also was detected in family 2. RT-PCR on RNA from both parents confirmed that the mutation is present in *PMS2* and not in the pseudogene. The same pattern of aberrant transcripts was found as described for family 2.

4.4. *Literature review*

The results from our literature review are summarised in Tables 1–6 and Supplemental Tables S1–S3.[4,5,8–55]

5. Discussion

Gastrointestinal cancers in young adults and children can be caused by the inheritance of mutations in both alleles of an MMR gene. Gastrointestinal cancers, mainly colorectal cancer, make up 37% of all tumours in CMMR-D syndrome patients.[10] Approximately half of the families reported with constitutional MMR deficiency have germline *PMS2* mutations (31 of 57 families, Tables 1 and 5). Of the *PMS2* families, in 26 (84%) gastrointestinal manifestations were reported. In 19 out of 60 *PMS2* homozygous or compound heterozygous mutation carriers, gastrointestinal cancer or adenomas were the first manifestation of the CMMR-D syndrome (32%). This percentage is comparable with that of all patients with CMMR-D syndrome; of the 102 CMMR-D syndrome patients, gastrointestinal manifestations were the first sign of CMMR-D syndrome in 32 of them (31%). In all CMMR-D syndrome patients, 20 individuals (20 of 102, 20%) had multiple primary CRCs, ranging from 2 to at least 10 cancers. 50 out of 75 CMMR-D syndrome patients with gastrointestinal manifestations had a homozygous or compound heterozygous *PMS2* mutation (67%). Adenomas and cancer in CMMR-D syndrome patients are more often reported in the left-sided colon compared to Lynch syndrome patients.

Here we have described four Dutch families in which five individuals carried bi-allelic, compound heterozygous or homozygous mutations of the *PMS2* gene. All five children were affected by gastrointestinal tumours and/or adenomas. PMS2 expression was absent in the patients' tumours and normal tissue. The examined tumours showed MSI.

Bi-allelic MMR gene mutations are associated with a broader spectrum of early-onset cancers than mono-allelic MMR gene mutations.[10] Cancer of the small intestine is very rare in Lynch syndrome patients, with a lifetime risk of some 4% and mean age of diagnosis of 39–53 years.[67] Small bowel cancer occurred in 11 of 102 CMMR-D syndrome patients, including three of our patients.[42] The mean age of presentation is 27 years and the youngest age at which it was diagnosed is 11 years.[19,21] Upper urothelial cancer is even rarer in bi-allelic MMR gene mutation carriers. Only one patient, who suffered from a ureter/renal pelvis carcinoma at age 15 years, has been reported so far.[9] We report a patient with papillary transitional cell carcinoma of the bladder, which, to the best of our knowledge, has not yet been reported in CMMR-D syndrome patients.

Many CMMR-D syndrome patients have features of NF1, especially CALM. It is, therefore, important to examine the skin of very young patients with gastrointestinal cancers and/or adenomas. In contrast to NF1, CALM in MMR-deficient individuals have irregular margins.[18]

Some of our patients had wrongly been diagnosed with FAP in the past. It is conceivable that, worldwide, many more patients with early-onset adenomatous polyposis have been erroneously diagnosed with FAP. In the absence of proven *APC* or *MUTYH* germline mutations, patients with childhood-onset adenomatous polyposis should be considered for MMR gene mutation testing, especially when they have features of NF1. In patients where tumour material is available, MMR gene expression and microsatellite instability should be tested first as a pre-screen method. The same approach would be appropriate for any patient with an early-onset cancer from the CMMR-D syndrome tumour spectrum.

5.1. *Surveillance guidelines*

Guidelines for gastrointestinal surveillance in CMMR-D syndrome patients have only been published in a limited way so far. For heterozygous MMR mutation carriers (Lynch syndrome), colorectal surveillance (colonoscopy) is recommended every 1–2 years from age 20 to 25. Some people suggest an intermediate screening programme, such as colonoscopy, every 1–2 years starting at age 30 for heterozygous *MSH6*[68] or *PMS2* mutation carriers.[45] CMMR-D syndrome differs from Lynch syndrome, not only in tumour spectrum, age of onset, and skin characteristics, but also because it

Table 1 – Malignancies and molecular characteristics of constitutional mismatch repair-deficiency (CMMR-D) syndrome families with gastro-intestinal manifestations and compound heterozygous or homozygous *PMS2* mutations.

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
1	Patient 1 – Glioblastoma with oligodendroglial comp. (4) – B-cell NHL of terminal ileum (17) – Glioblastoma with oligodendroglial comp. (21; †21)	Patient 1 – 2 colon ad. + 1 hyperplastic polyp (13)	Patient 1 – CALM	High in glioblastoma, colon ad. and CRC	c.400C>T, p.Arg134X	c.2184_2185del, p.Leu729GlnfsX6	[17,25,49]
	Patient 2 – CRC (11) – Anaplastic glioma with oligodendroglial comp. (23)	Patient 2 – 3 ad.: sigmoid, colon and rectum (11) – Phenotypic adenomatous polyposis (14)	Patient 2 – CALM				
2	Patient 1 – Oligodendroglioma (14) – CRC (18; †18)	Patient 1 – 1 colon ad. (18)	NR	High in CRC	c.2361_2364del, p.Phe788CysfsX2	c.1221delG, p.Thr408LeufsX40	[16]
	Patient 2 – Neuroblastoma (13; †14)						
3	Patient 1 – 2× CRC (16) – PNET ovarium (21) – Endometrial ca. (23) – Brain tumour (24)	Patient 1 – 12 tubular or tubulovillous ad. (16)	Patient 1 – 6 CALM, no other NF1 features	High in CRC and ovarium	c.1169_1170ins20	c.1169_1170ins20	[46,51]
	Patient 2 – Anaplastic astrocytoma (7)	Patient 2 – 2 colon ad. (18) – 3 colon ad. (20)	Patient 2 – Multiple CALM, no other NF1 features				
	Patient 3 – ALL (4; †4)		Patient 3 – Multiple CALM				
4	Patient 1 – Duodenal ca. (16) – Giant cell glioblastoma (17)	4 tubulovillous ad. colon (16)	CALM	High in duodenal ca., low in glioblastoma	c.137G>T, p.Ser46Ile	c.1927C>T, p.Gln643X	[11]
5	Patient 1 – B-cell NHL (10; †12)		Patient 1. Multiple CALM, no other features of NF1	NA	c.2404C>T, p.Arg802X	c.2404C>T, p.Arg802X	[17,18]
	Patient 2 – SPNET (8)	Patient 2. Multiple polyps adulthood	Patient 2. Multiple CALM, no other features of NF1				
	Patient 3 – SPNET (14; †15)		Patient 3. Multiple CALM, no other features of NF1				

6	Patient 1 – T-cell leukaemia (2) – T-NHL (14) – Multiple CRC (18)	Multiple polyps adulthood	CALM	NA	c.2404C>T, p.Arg802X	c.2404C>T, p.Arg802X	[18]
7	Patient 1 – 10× CRC (23) – Duodenal ca. (25; †25)	35 adenomatous polyps (23)	CALM	High in 5 CRC and 3 ad.	Del 400 kb, including exons 9–15	Del 400 kb, including exons 9–15	[55]
8	Patient 1 – Oligodendroglioma (19) – 2× CRC (24; †25)	Patient 1. 1 colon ad. (24)		High in CRC	c.137G>T, p.Ser46Ile	c.1730dup;1732C>T, p.Arg578ValfsX3	[13]
	Patient 2 – Brain angioma (2) – CRC (20) – Endometrial ca. (24)	Patient 2. 12 colon ad. (20)	Patient 2. CALM				
9	Patient 1 – GBM (6) – CRC (15) – Jejunal ca. (15) – Urotel ca. (15; †16)	Patient 1. 20 polyps (15)	Patient 1. CALM, no other features of NF1	High in CRC	c.1768del, p.Ile590PhefsX5	c.1768del, p.Ile590PhefsX5	[9]
	Patient 2 – GBM (6; †7)		Patient 2. CALM, no other features of NF1				
	Patient 3 – GBM (9)	Patient 3. 3 colon polyps (8)					
	Patient 4 – Infantile myofibromatosis (1; †5)						
10	Patient 1 – CRC (13) – CRC (14)	Patient 1. Multiple colon polyps, 7 of which with high grade dysplasia (14)	Patient 1. Multiple CALM, axillary freckling, no other signs of NF1	High in CRC	c.812G>T, p.Gly271Val	c.812G>T, p.Gly271Val	[9]
	Patient 2 – T-NHL (10) – CRC (11)	Patient 2. 11 colon polyps (11)	Patient 2. Multiple CALM				
11	Patient 1 – T-NHL (6) – Multiple CRC (16)	Patient 1. Multiple colon ad. (16)	Patient 1. Multiple CALM	High in CRC	c.1306dup, p.Ser436LysfsX22	c.1306dup, p.Ser436LysfsX22	[29]
	Patient 2 – SPNET (9; †9)						
12	Patient 1 – CRC (14)	<10 colon ad. (14)	8 CALM, no other features of NF1	High	c.137G>T, p.Ser46Ile	c.137G>A, p.Ser46Asn	[27]

(*continued on next page*)

Table 1 – (*continued*)

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
13	Patient 1 – CRC (14) – Astrocytoma (19) – Astrocytoma (19; †21) Patient 2 – Anaplastic oligodendroglioma (11; †11)	No follow-up reported	NR	Low in CRC and astrocytoma	c.1840A>T, p.Lys614X	c.1840A>T, p.Lys614X	[23]
14	Patient 1 – 4× CRC (15) Patient 2 – Glioblastoma (8, †9)	Patient 1. >24 tubular and tubulovillous ad. (15)	Patient 1. Multiple CALM, no other features of NF1 Patient 2. Multiple CALM	NA	Del exon 7	Del exon 7	[48]
15	Patient 1 – CRC (22) – Brain tumour (23) Patient 2 – CRC (21)	No follow-up reported	NR	NA	c.2249G>A, p.Gly750Asp	Complete gene deletion	[45]
16	Patient 1 – Multiple CRC (28)	No follow-up reported	NR	NA	c.1A>G, p.Met1? (5′ truncation)	Del exons 9–10	[45]
17	Patient 1 – CRC (20) – Duodenal ca. (41) – Lymphoma (NR) Patient 2 – Brain tumour (38) Patient 3 – Brain tumour (31)	No follow-up reported	NR	NA	c.1A>G, p.Met1? (5′ truncation)	c.614A>C, p.Gln205Pro	[45]
18	Patient 1 – CRC (24) – Endometrial ca. (35) – Glioma (35) Patient 2 – CRC (26) – Glioblastoma (34) Patient 3 – Glioma (24)	No follow-up reported	NR	NA	c.1A>G, p.Met1? (5′ truncation)	c.251-2A>G, aberrant splicing	[45]

19	Patient 1 – Medulloblastoma (8) – Duodenal ca. (16) Patient 2 – T-ALL (5; †6)	Patient 1. 1 colon ad. (17)	Patient 1. Multiple CALM	High in duodenal ca.	c.949C>T, p.Gln317X	c.949C>T, p.Gln317X	[42,45]
20	Patient 1 – Giant cell glioblastoma (10) – CRC (16) – CRC (20) – Duodenal ca. (26) – Ileal ca. (30) – Endometrial ca. (31) – Ileal ca. (36) – BCCs (38–40) – Jejunal ca. (40) – Jejunal ca. (42) Patient 2 – CRC (19; †26)	Patient 1 – Multiple colon polyps (20) – Rectal polyp (31) – Gastric polyps (41) Patient 2 – Multiple colon polyps (19)	Patient 1. No CALM	High in CRC	c.989-1G>T, aberrant splicing	c.989-1G>T, aberrant splicing	[47]
21	Patient 1 – Rhabdomyosar-coma (3) – CRC (8)	No polyps	Multiple CALM	NA	c.219T>A, p.Cys73X	c.219T>A, p.Cys73X	[10,30]
22	Patient 1 – GBM (10; †?) Patient 2 – CRC (21) Patient 3 – CNS tumour (4; †4)	No follow-up reported	Patient 1. Multiple CALM	High in GBM and CRC	c.137G>T, Ser46Ile	c.804-2A>G, aberrant splicing	[22]
23	Patient 1 – Cerebral angiosarcoma (2)	2 colon ad., one with high grade dysplasia (7)	6 CALM, anal and axillary freckling	High in angiosarcoma	c.736_741delins TGTGTGTGAAG, p.Pro246CysfsX3	Complete gene deletion	This report
24	Patient 1 – Jejunal ca. (21) – Bladder ca. (21) – CRC (22; †23)	Patient 1 – Multiple colon ad. with high grade dysplasia (19) – 1 rectal tubulovillous ad. (21) – 2 ileal and 2 colorectal tubulovillous ad. (22)	Patient 1. 1 CALM, axillary freckling	High in jejunal ca. and bladder ca.	c.137G>T, p.Ser46Ile	c.2174+1G>A, aberrant splicing	This report

(*continued on next page*)

Table 1 – (*continued*)

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
	Patient 2 – Duodenal ca. (17) – Jejunal ca. (19) – T-ALL (21; †22)	Patient 2 – 1 colorectal tubulovilleus ad. with high grade dysplasia (15) – 1 duodenal tubulovilleus ad. with low grade dysplasia (16) – 2 duodenal ad. with high grade dysplasia (17) – 2 colorectal tubulovillous ad. with high grade dysplasia (20) – Multiple colorectal tubulovillous ad. + jejunal tubulovillous ad. with high grade dysplasia (21) – 13 colorectal tubulovillous ad. with high grade dysplasia (22)	Patient 2. 1 CALM				
25	Patient 1 – CRC (21) – Duodenal ca. (32) – Jejunal ca. (32) – Jejunal ca. (32) – Glioblastoma (34)	– Multiple colorectal ad. (11) – 26 tubulovillous ad. with high grade dysplasia (21) – 13 gastrointestinal ad. (32) – 1 ileal ad. (32)	NR	NA	Del exons 1–11	Del exons 3–7	This report
26	Patient 1 – Anaplastic ganglioma (9)	– Colon ad. with high grade dysplasia (10)	>6 CALM, freckling	High in anaplastic ganglioma	c.2174+1G>A, aberrant splicing	c.2174+1G>A, aberrant splicing	This report

ad, adenoma; AML, acute myeloid leukaemia; ca, carcinoma; BCC, basal cell carcinoma; CALM, Café-au-lait maculae; CML, chronic myeloid leukaemia; comp., component; CRC, Colorectal carcinoma; Del, deletion; GBM, Glioblastoma multiforme; MDS, myelodysplastic syndrome; mo, months; MSI, Microsatellite instability; NA, not available; NF1, Neurofibromatosis type 1; NHL, Non-Hodgkin lymphoma; NR, not reported; SPNET, supratentorial primitive neuro-ectodermal tumour; T-ALL, T-cell acute lymphoid leukaemia; T-NHL, T-cell Non-Hodgkin lymphoma; WT, Wilms tumour.

Table 2 – Malignancies and molecular characteristics of constitutional mismatch repair-deficiency (CMMR-D) syndrome families with gastro-intestinal manifestations and compound heterozygous or homozygous *MLH1* mutations.

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
27	Patient 1 – CRC (35) – Sarcoma (65)	No follow up reported	NR	High in CRC and sarcoma	c.1852_1853AA>GC, p.Lys618Ala	c.546-2A>G, aberrant splicing	31
28	Patient 1 – Duodenal ca. (11; †12)	Patient 1 – No polyps	Patient 1 – Multiple CALM, no Lisch nodules	High in duodenal ca., CRC (3×) and lymphocytes	c.2059C>T, p.Arg687Trp	c.2059C>T, p.Arg687Trp	19,21
	Patient 2 – 3× CRC (9) – Glioblastoma (24)	Patient 2 – No follow-up reported	Patient 2 – 3 CALM, 1 Lisch nodule, minimal axillary freckling				
	Patient 3 – No malignancy (8)	Patient 3 – No polyps reported till 8	Patient 3 – Plexiform neurofibroma (6), 8 CALM, 1 Lisch nodule				
29	Patient 1 – CRC (22)	No follow up reported	NR	NA	c.806C>G, p.Ser269X	c.806C>G, p.Ser269X	40
30	Patient 1 – CRC (12)	No follow up reported	NR	High	c.2146G>A, p.Val716Met	c.676C>T, p.Arg226X	33

ad, adenoma; AML, acute myeloid leukaemia; ca, carcinoma; BCC, basal cell carcinoma; CALM, Café-au-lait maculae; CML, chronic myeloid leukaemia; comp., component; CRC, Colorectal carcinoma; Del, deletion; GBM, Glioblastoma multiforme; MDS, myelodysplastic syndrome; mo, months; MSI, Microsatellite instability; NA, not available; NF1, Neurofibromatosis type 1; NHL, Non-Hodgkin lymphoma; NR, not reported; SPNET, supratentorial primitive neuro-ectodermal tumour; T-ALL, T-cell acute lymphoid leukaemia; T-NHL, T-cell Non-Hodgkin lymphoma; WT, Wilms tumour.

Table 3 – Malignancies and molecular characteristics of constitutional mismatch repair-deficiency (CMMR-D) syndrome families with gastro-intestinal manifestations and compound heterozygous or homozygous *MSH2* mutations.

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
31	Patient 1 – 2× CRC (11)	Patient 1. Multiple polyps duodenum, colon, rectum (11)	Patient 1. Multiple CALM	High in CRC (both patients)	c.2006-5T>A, aberrant splicing	c.2006-5T>A, aberrant splicing	[35]
	Patient 2 – 3× CRC (12)	Patient 2. <10 polyps in colon, 2 polyps in duodenum (12)	Patient. 2 Multiple CALM				
32	Patient 1 – Astrocytoma (14) – CRC (14; †14)	Patient 1. > 100 tubulovillous ad. (14)	Patient 1. Multiple CALM, axillary freckling, no other features of NF1	High in astrocytoma and CRC, low in leukocytes	c.1906G>C, p.Ala636Pro	c.1906G>C, p.Ala636Pro	[50]
	Patient 2 – Anaplastic astrocytoma (13; †13)		Patient 2. NF1 features, details not reported				
33	Patient 1 – 3× CRC (39) – Ileal ca. (39) – CRC (48)	Patient 1. 15 colon ad., some with high grade dysplasia (39)	No features of NF1	High in all	Del exons 1–6	c.1A>G, p.Met1? (5′ truncation)	[28]
	Patient 2 – CRC (33) – Endometrial ca. (44)	Patient 2. 4 colon ad. (46)					

ad, adenoma; AML, acute myeloid leukaemia; ca, carcinoma; BCC, basal cell carcinoma; CALM, Café-au-lait maculae; CML, chronic myeloid leukaemia; comp., component; CRC, Colorectal carcinoma; Del, deletion; GBM, Glioblastoma multiforme; MDS, myelodysplastic syndrome; mo, months; MSI, Microsatellite instability; NA, not available; NF1, Neurofibromatosis type 1; NHL, Non-Hodgkin lymphoma; NR, not reported; SPNET, supratentorial primitive neuro-ectodermal tumour; T-ALL, T-cell acute lymphoid leukaemia; T-NHL, T-cell Non-Hodgkin lymphoma; WT, Wilms tumour.

Table 4 – Malignancies and molecular characteristics of constitutional mismatch repair-deficiency (CMMR-D) syndrome families with gastro-intestinal manifestations and compound heterozygous or homozygous *MSH6* mutations.

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
34	Patient 1 – Oligodendroglioma (10) – CRC (12; †12)	No follow-up reported	6 CALM, no other NF1 features	High in CRC, low in oligodendroglioma	c.3386_3388del, p.Cys1129_ Val1130delinsLeu	c.3386_3388del, p.Cys1129_ Val1130delinsLeu	34
35	Patient 1 – Lymphoblastic lymphoma (5) – CRC (8; †9) Patient 2 – GBM (8; †10)	No follow-up reported	Patient 1 – 8 CALM, axillary freckling, no other features of NF1 Patient 2 – 6–8 CALM, axillary freckling	High in GBM, low in lymphocytes	c.3635dupT, p.Asp1213GlyfsX2	c.3635dupT, p.Asp1213GlyfsX2	26
36	Patient 1 – CRC (19) – Endometrial ca. (24)	No polyps reported till age 29	CALM	High in CRC	c.3226C>T, p.Arg1076Cys	c.3991C>T, p.Arg1331X	37
37	Patient 1 CRC (31)	No follow-up reported	NR	NA	c.2633T>C, p.Val878Ala	c.2295C>G, p.Cys765Trp	37
38	Patient 1 – Medulloblastoma (7) – AML (10) – 2× CRC (13)	50 tubular and tubulovillous ad. (13)	CALM	NA	c.642C>G, p.Tyr214X	c. 458-1G>A, aberrant splicing	43
39	Patient 1 – Mediastinal T-NHL (2)	Patient 1. >20 colon ad. (6)	Patient 1. CALM	NA	c.226C>T, p.Gln76X	c.226C>T, p.Gln76X	32,44
	Patient 2 – Mediastinal T-NHL (1, †2)		Patient 2. CALM				
	Patient 3 – T-ALL (2)		Patient 3. CALM				
40	Patient 1 – Glioblastoma (7; †7)		Patient 1. Multiple CALM, no other features of NF1	Low in colon ad., and lymphocytes	c.1596dup, p.Glu533X	c.3261delC, p.Phe1088SerfsX2	13
		Patient 2. 11 colon ad., some with high grade dysplasia (9)	Patient 2. Multiple CALM, Lisch nodules				

(*continued on next page*)

Table 4 – (continued)

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
41	Patient 1 – 4× CRC (17)	3 tubular ad. (17)	No features of NF1	High	c.1806_1809del, p.Glu604LeufsX5	c.3226C>T, p.Arg1076Cys	39
42	Patient 1 – T-NHL (6) – CRC (13)	– Multiple tubulovillous polyps with high-grade dysplasia throughout the colon (13) – Rectal ad. (14)	Several CALM, lateral conjunctival melanosis of her left eye	High in CRC	c.691delG, p.Val231TyrfsX15	c.691delG, p.Val231TyrfsX15	41

ad, adenoma; AML, acute myeloid leukaemia; ca, carcinoma; BCC, basal cell carcinoma; CALM, Café-au-lait maculae; CML, chronic myeloid leukaemia; comp., component; CRC, Colorectal carcinoma; Del, deletion; GBM, Glioblastoma multiforme; MDS, myelodysplastic syndrome; mo, months; MSI, Microsatellite instability; NA, not available; NF1, Neurofibromatosis type 1; NHL, Non-Hodgkin lymphoma; NR, not reported; SPNET, supratentorial primitive neuro-ectodermal tumour; T-ALL, T-cell acute lymphoid leukaemia; T-NHL, T-cell Non-Hodgkin lymphoma; WT, Wilms tumour.

results in a faster transformation rate from adenoma to cancer.[69] Surviving children with bi-allelic MMR mutations require thorough periodic cancer surveillance. Gallinger et al. suggested periodic surveillance with complete blood counts, upper and lower endoscopy, chest and abdomen computerised tomography, and total body magnetic resonance imaging,[21] but did not discuss what age to start or how often upper and lower endoscopy should be performed. The youngest age at which malignant colorectal tumours were found is 8 years in two CMMR-D syndrome patients (one homozygous *MSH6* and one homozygous *PMS2* mutation carrier).[10,26,30] The youngest age at which gastrointestinal adenomas were observed is 6 years in a homozygous *MSH2* mutation carrier.[44] We, therefore, suggest yearly colonoscopy in bi-allelic MMR mutation carriers, starting at 6 years of age and yearly colonoscopy and video-capsule endoscopy, from age 8. Video-capsule endoscopy has been found to be diagnostically superior to push enteroscopy[70] or barium enterography in GI bleeding, and has recently been evaluated in children.[71] Although very young children were generally able to swallow the capsule unaided[71–74], young children (aged 6–10 years) who are undergoing bidirectional endoscopic surveillance under general anaesthesia can have the capsule placed endoscopically at the same time.

Based on the published literature, no difference in the surveillance interval for bi-allelic *PMS2/MSH6* and *MLH1/MSH2* mutation carriers can be made so far (Table 6). To determine whether the potential benefits of surveillance and treatment outweigh the emotional and physical burden of surveillance, further characterisation of the CMMR-D syndrome is needed to establish evidence-based screening guidelines.

Conflict of interest statement

None declared.

Contributions

Literature search, data extraction and writing of the first draft: Johanna C. Herkert; mutation analysis, tumour analysis and critical revision of the manuscript: Renée C. Niessen; collecting patient information, literature search, data extraction and critical revision of the manuscript: Maria J.W. Olderode-Berends; collecting patient information, data extraction and critical revision of the manuscript: Hermine E. Veenstra-Knol; mutation analysis of families 1 and 2, tumour analysis and critical revision of the manuscript: Yvonne J. Vos; mutation analysis of families 3 and 4, RNA analysis: Heleen M. van der Klift; follow-up of patients: Rene Scheenstra; mutation analysis of families 3 and 4, RNA analysis: Carli M.J. Tops; immunohistochemistry: Arend Karrenbeld; follow-up of patients: Frans T.M. Peters; critical revision of the manuscript: Robert M.W. Hofstra; follow-up of patients, critical revision of the manuscript: Jan H. Kleibeuker; collecting patient information, study supervision, drafting of the manuscript: Rolf H. Sijmons. All the authors have commented on drafts of the paper and have approved the submitted version.

Table 5 – Malignancies and molecular characteristics of constitutional mismatch repair-deficiency (CMMR-D) syndrome families with compound heterozygous or homozygous *PMS2* mutations without gastro-intestinal manifestations.

Fam.	Tumour type (age of diagnosis; death)	Polyps	NF1 features	MSI	Mutation a	Mutation b	Refs.
43	Patient 1 – Glioma (15; †16)	No follow-up reported	Patient 1. CALM	NA	c.2404C>T, p.Arg802X	c.2404C>T, p.Arg802X	[18]
	Patient 2 – ALL (15; †15)		Patient 2. CALM				
	Patient 3 – Astrocytoma (6) – Glioblastoma (7; †8)		Patient 3. CALM				
44	Patient 1 – Giant cell glioblastoma (2; †2)	No follow-up reported	Patient 1. NR	NA	c.2404C>T, p.Arg802X	c.2404C>T, p.Arg802X	[18]
	Patient 2 – T-NHL (3; †3)		Patient 2. NR				
	Patient 3 – ALL (6)		Patient 3. NR				
45	Patient 1 – ALL (6; †7)	NR	CALM	NA	c.2404C>T, p.Arg802X	c.2404C>T, p.Arg802X	[18]
46	Patient 1 – SPNET (8)	No follow-up reported	CALM	NA	c.543delT, p.Tyr181X	c.543delT, p.Tyr181X	[18]
	Patient 2 – SPNET (4; †6)						
47	Patient 1 – GBM (10; †11)	NR	>6 CALM	Low in leukocytes	c.182delA, p.Tyr61LeufsX15	c.182delA, p.Tyr61LeufsX15	[20]

ad, adenoma; AML, acute myeloid leukaemia; ca, carcinoma; BCC, basal cell carcinoma; CALM, Café-au-lait maculae; CML, chronic myeloid leukaemia; comp., component; CRC, Colorectal carcinoma; Del, deletion; GBM, Glioblastoma multiforme; MDS, myelodysplastic syndrome; mo, months; MSI, Microsatellite instability; NA, not available; NF1, Neurofibromatosis type 1; NHL, Non-Hodgkin lymphoma; NR, not reported; SPNET, supratentorial primitive neuro-ectodermal tumour; T-ALL, T-cell acute lymphoid leukaemia; T-NHL, T-cell Non-Hodgkin lymphoma; WT, Wilms tumour.

Table 6 – Overview of gastro-intestinal symptoms in constitutional mismatch repair-deficiency (CMMR-D) syndrome patients.

	Mean age of diagnosis (y) (range)	Mean number at first colonoscopy or video-capsule enteroscopy
Gastrointestinal polyps	17 (7–46) (*n* = 25)[a] – PMS2: 15 (7–24) (*n* = 17) – MLH1: NA (*n* = 2) – MSH2: 43 (39–46) (*n* = 2) – MSH6: 13 (9–17) (*n* = 4)	8.1 (*n* = 25)[b] – PMS2: 6.6 (*n* = 17) – MLH1: 0 (*n* = 2) – MSH2: 9.5 (*n* = 2) – MSH6: 16 (*n* = 4)
Small intestinal cancer	27 (11–42) (*n* = 11) – PMS2: 28 (15–42) (*n* = 9) – MLH1: 11 (*n* = 1) – MSH2: 39 (*n* = 1) – MSH6: NA	1 (*n* of ca. = 11) – PMS2: 1 (*n* of ca. = 9) – MLH1: 1 (*n* of ca. = 1) – MSH2: 1 (*n* of ca. = 1) – MSH6: NA
Colorectal cancer	19 (8–48) (*n* = 42) – PMS2: 18 (8–26) (*n* = 25) – MLH1: 20 (9–35) (*n* = 4) – MSH2: 26 (11–48) (*n* = 6) – MSH6: 16 (8–31) (*n* = 7)	1.6 (*n* of ca. = 64)[c] – PMS2: 1.6 (*n* of ca. = 37) – MLH1: 1.5 (*n* of ca. = 6) – MSH2: 2 (*n* of ca. = 10) – MSH6: 1.6 (*n* of ca. = 11)

a The lowest age of diagnosis is 6 years in a homozygous *MSH2* mutation carrier, but it is not scored in this table, because the precise number of gastrointestinal polyps is not reported in the publication.

b The number of polyps may be an underrepresentation, because it is not often reported. In addition, when the term 'multiple gastrointestinal polyps' is used in Tables 1–5 and S1–S3, the precise number was not given in the original report.

c When multiple colorectal cancers (CRC) without a precise number were reported, they were excluded from this table. y = year. *n* = number of patients. *n* of ca = number of cancers.

Grant support

None.

Acknowledgements

We thank the patients and their family members for their cooperation. We are indebted to Jackie Senior for editing the text.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.01.013.

REFERENCES

1. Samowitz WS, Curtin K, Lin HH, et al. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001;**121**(4):830–8.
2. Koornstra JJ, Mourits MJ, Sijmons RH, et al. Management of extracolonic tumours in patients with Lynch syndrome. *Lancet Oncol* 2009;**10**(4):400–8.
3. Lynch HT, Smyrk TC, Watson P, et al. Genetics, natural history, tumour spectrum and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;**104**(5):1535–49.
4. Poley JW, Wagner A, Hoogmans MM, et al. Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. *Cancer* 2007;**109**(11):2349–56.
5. Ricciardone MD, Ozcelik T, Cevher B, et al. Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. *Cancer Res* 1999;**59**(2):290–3.
6. Wang Q, Lasset C, Desseigne F, et al. Neurofibromatosis and early onset of cancers in hMLH1-deficient children. *Cancer Res* 1999;**59**(2):294–7.
7. Bandipalliam P. Syndrome of early onset colon cancers, hematologic malignancies and features of neurofibromatosis in HNPCC families with homozygous mismatch repair gene mutations. *Fam Cancer* 2005;**4**(4):323–33.
8. Felton KE, Gilchrist DM, Andrew SE. Constitutive deficiency in DNA mismatch repair. *Clin Genet* 2007;**71**(6):483–98.
9. Kruger S, Kinzel M, Walldorf C, et al. 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* 2008;**16**(1):62–72.
10. Wimmer K, Etzler J. Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? *Hum Genet* 2008;**124**(2):105–22.
11. Agostini M, Tibiletti MG, Lucci-Cordisco E, et al. Two PMS2 mutations in a Turcot syndrome family with small bowel cancers. *Am J Gastroenterol* 2005;**100**(8):1886–91.
12. Alotaibi H, Ricciardone MD, Ozturk M. Homozygosity at variant MLH1 can lead to secondary mutation in NF1, neurofibromatosis type I and early onset leukemia. *Mutat Res* 2008;**637**(1–2):209–14.
13. Auclair J, Leroux D, Desseigne F, et al. Novel biallelic mutations in MSH6 and PMS2 genes: gene conversion as a likely cause of PMS2 gene inactivation. *Hum Mutat* 2007;**28**(11):1084–90.
14. Bertholon J, Wang Q, Galmarini CM, Puisieux A. Mutational targets in colorectal cancer cells with microsatellite instability. *Fam Cancer* 2006;**5**(1):29–34.
15. Bougeard G, Charbonnier F, Moerman A, et al. Early onset brain tumor and lymphoma in MSH2-deficient children. *Am J Hum Genet* 2003;**72**(1):213–6.
16. De Rosa M, Fasano C, Panariello L, et al. Evidence for a recessive inheritance of Turcot's syndrome caused by compound heterozygous mutations within the PMS2 gene. *Oncogene* 2000;**19**(13):1719–23.
17. De Vos M, Hayward BE, Picton S, Sheridan E, Bonthron DT. Novel PMS2 pseudogenes can conceal recessive mutations causing a distinctive childhood cancer syndrome. *Am J Hum Genet* 2004;**74**(5):954–64.
18. De Vos M, Hayward BE, Charlton R, et al. PMS2 mutations in childhood cancer. *J Natl Cancer Inst* 2006;**98**(5):358–61.
19. Durno C, Aronson M, Bapat B, Cohen Z, Gallinger S. Family history and molecular features of children, adolescents, and young adults with colorectal carcinoma. *Gut* 2005;**54**(8):1146–50.
20. Etzler J, Peyrl A, Zatkova A, et al. RNA-based mutation analysis identifies an unusual MSH6 splicing defect and circumvents PMS2 pseudogene interference. *Hum Mutat* 2008;**29**(2):299–305.
21. Gallinger S, Aronson M, Shayan K, et al. Gastrointestinal cancers and neurofibromatosis type 1 features in children with a germline homozygous MLH1 mutation. *Gastroenterology* 2004;**126**(2):576–85.
22. Giunti L, Cetica V, Ricci U, et al. Type A microsatellite instability in pediatric gliomas as an indicator of Turcot syndrome. *Eur J Hum Genet* 2009;**17**(7):919–27.
23. Gururangan S, Frankel W, Broaddus R, et al. Multifocal anaplastic astrocytoma in a patient with hereditary colorectal cancer, transcobalamin II deficiency, agenesis of the corpus callosum, mental retardation, and inherited PMS2 mutation. *Neuro Oncol* 2008;**10**(1):93–7.
24. Hackman P, Tannergard P, Osei-Mensa S, et al. A human compound heterozygote for two MLH1 missense mutations. *Nat Genet* 1997;**17**(2):135–6.
25. Hamilton SR, Liu B, Parsons RE, et al. The molecular basis of Turcot's syndrome. *N Engl J Med* 1995;**332**(13):839–47.
26. Hegde MR, Chong B, Blazo ME, et al. A homozygous mutation in MSH6 causes Turcot syndrome. *Clin Cancer Res* 2005;**11**(13):4689–93.
27. Jackson CC, Holter S, Pollett A, et al. Cafe-au-lait macules and pediatric malignancy caused by biallelic mutations in the DNA mismatch repair (MMR) gene PMS2. *Pediatr Blood Cancer* 2008;**50**(6):1268–70.
28. Kets CM, Hoogerbrugge N, van Krieken JH, et al. Compound heterozygosity for two MSH2 mutations suggests mild consequences of the initiation codon variant c.1A>G of MSH2. *Eur J Hum Genet* 2009;**17**(2):159–64.
29. Kratz CP, Niemeyer CM, Juttner E, et al. Childhood T-cell non-Hodgkin's lymphoma, colorectal carcinoma and brain tumor in association with cafe-au-lait spots caused by a novel homozygous PMS2 mutation. *Leukemia* 2008;**22**(5):1078–80.
30. Kratz CP, Holter S, Etzler J, et al. Rhabdomyosarcoma in patients with constitutional mismatch-repair-deficiency syndrome. *J Med Genet* 2009;**46**(6):418–20.
31. Liu T, Tannergard P, Hackman P, et al. Missense mutations in hMLH1 associated with colorectal cancer. *Hum Genet* 1999;**105**(5):437–41.
32. Marafie MJ, Al Awadi S, Al Mosawi F, et al. Impact of 226C>T MSH2 gene mutation on cancer phenotypes in two HNPCC-associated highly-consanguineous families from Kuwait: emphasis on premarital genetic testing. *Fam Cancer* 2009;**8**(4):289–98.

33. Marcos I, Borrego S, Urioste M, Garcia-Valles C, Antinolo G. Mutations in the DNA mismatch repair gene MLH1 associated with early-onset colon cancer. *J Pediatr* 2006;**148**(6): 837–9.
34. Menko FH, Kaspers GL, Meijer GA, et al. A homozygous MSH6 mutation in a child with cafe-au-lait spots, oligodendroglioma and rectal cancer. *Fam Cancer* 2004;**3**(2):123–7.
35. Muller A, Schackert HK, Lange B, et al. A novel MSH2 germline mutation in homozygous state in two brothers with colorectal cancers diagnosed at the age of 11 and 12 years. *Am J Med Genet A* 2006;**140**(3):195–9.
36. Ostergaard JR, Sunde L, Okkels H. Neurofibromatosis von Recklinghausen type I phenotype and early onset of cancers in siblings compound heterozygous for mutations in MSH6. *Am J Med Genet A* 2005;**139A**(2):96–105.
37. Plaschke J, Linnebacher M, Kloor M, et al. Compound heterozygosity for two MSH6 mutations in a patient with early onset of HNPCC-associated cancers, but without hematological malignancy and brain tumor. *Eur J Hum Genet* 2006;**14**(5):561–6.
38. Raevaara TE, Gerdes AM, Lonnqvist KE, et al. HNPCC mutation MLH1 P648S makes the functional protein unstable, and homozygosity predisposes to mild neurofibromatosis type 1. *Genes Chromosomes Cancer* 2004;**40**(3):261–5.
39. Rahner N, Hoefler G, Hogenauer C, et al. Compound heterozygosity for two MSH6 mutations in a patient with early onset colorectal cancer, vitiligo and systemic lupus erythematosus. *Am J Med Genet A* 2008;**146A**(10):1314–9.
40. Rey JM, Noruzinia M, Brouillet JP, et al. Six novel heterozygous MLH1, MSH2, and MSH6 and one homozygous MLH1 germline mutations in hereditary nonpolyposis colorectal cancer. *Cancer Genet Cytogenet* 2004;**155**(2):149–51.
41. Ripperger T, Beger C, Rahner N, et al. Constitutional mismatch repair deficiency and childhood leukemia/ lymphoma – report on a novel biallelic MSH6 mutation. *Haematologica* 2010;**95**(5):841–4.
42. Roy S, Raskin L, Raymond VM, et al. Pediatric duodenal cancer and biallelic mismatch repair gene mutations. *Pediatr Blood Cancer* 2009;**53**(1):116–20.
43. Scott RH, Mansour S, Pritchard-Jones K, et al. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic MSH6 mutations. *Nat Clin Pract Oncol* 2007;**4**(2):130–4.
44. Scott RH, Homfray T, Huxter NL, et al. Familial T-cell non-Hodgkin lymphoma caused by biallelic MSH2 mutations. *J Med Genet* 2007;**44**(7):e83.
45. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* 2008;**135**(2):419–28.
46. Shalon L, Markowitz J, Bialer M, et al. Ovarian neoplasm and endometrioid carcinoma in a patient with Turcot syndrome. *J Pediatr Gastroenterol Nutr* 1997;**25**(2):224–7.
47. Sjursen W, Bjornevoll I, Engebretsen LF, et al. A homozygote splice site PMS2 mutation as cause of Turcot syndrome gives rise to two different abnormal transcripts. *Fam Cancer* 2009;**8**(3):179–86.
48. Tan TY, Orme LM, Lynch E, et al. Biallelic PMS2 mutations and a distinctive childhood cancer syndrome. *J Pediatr Hematol Oncol* 2008;**30**(3):254–7.
49. Taylor MD, Perry J, Zlatescu MC, et al. The hPMS2 exon 5 mutation and malignant glioma. Case report. *J Neurosurg* 1999;**90**(5):946–50.
50. Toledano H, Goldberg Y, Kedar-Barnes I, et al. Homozygosity of MSH2 c.1906G → C germline mutation is associated with childhood colon cancer, astrocytoma and signs of neurofibromatosis type I. *Fam Cancer* 2009;**8**(3):187–94.
51. Trimbath JD, Petersen GM, Erdman SH, et al. Cafe-au-lait spots and early onset colorectal neoplasia: a variant of HNPCC? *Fam Cancer* 2001;**1**(2):101–5.
52. Vilkki S, Tsao JL, Loukola A, et al. Extensive somatic microsatellite mutations in normal human tissue. *Cancer Res* 2001;**61**(11):4541–4.
53. Wagner A, Reddingius RE, Kros JM, et al. Wilms tumor and gliablastoma in a child with double MLH1 germline mutation. *Fam Cancer* 2003;**2**:57.
54. Whiteside D, McLeod R, Graham G, et al. A homozygous germ-line mutation in the human MSH2 gene predisposes to hematological malignancy and multiple cafe-au-lait spots. *Cancer Res* 2002;**62**(2):359–62.
55. Will O, Carvajal-Carmona LG, Gorman P, et al. Homozygous PMS2 deletion causes a severe colorectal cancer and multiple adenoma phenotype without extraintestinal cancer. *Gastroenterology* 2007;**132**(2):527–30.
56. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;**58**(22):5248–57.
57. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;**96**(4):261–8.
58. Berends MJ, Wu Y, Sijmons RH, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. *Am J Hum Genet* 2002;**70**(1):26–37.
59. Niessen RC, Kleibeuker JH, Westers H, et al. PMS2 involvement in patients suspected of Lynch syndrome. *Genes Chromosomes Cancer* 2009;**48**(4):322–9.
60. Gille JJ, Hogervorst FB, Pals G, et al. Genomic deletions of MSH2 and MLH1 in colorectal cancer families detected by a novel mutation detection approach. *Br J Cancer* 2002;**87**(8):892–7.
61. Wu Y, Nystrom-Lahti M, Osinga J, et al. MSH2 and MLH1 mutations in sporadic replication error-positive colorectal carcinoma as assessed by two-dimensional DNA electrophoresis. *Genes Chromosomes Cancer* 1997;**18**(4):269–78.
62. Wu Y. *Design and application of DGGE-based mutation detection systems for genes involved in HNPCC*. Groningen: University of Groningen; 1999.
63. Wu Y, Berends MJ, Mensink RG, et al. Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am J Hum Genet* 1999;**65**(5):1291–8.
64. van der Klift HM, Tops CM, Bik EC, et al. Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. *Hum Mutat* 2010;**31**(5):578–87.
65. Clendenning M, Hampel H, LaJeunesse J, et al. Long-range PCR facilitates the identification of PMS2-specific mutations. *Hum Mutat* 2006;**27**(5):490–5.
66. Nakagawa H, Lockman JC, Frankel WL, et al. Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 2004;**64**(14):4721–7.
67. Koornstra JJ, Kleibeuker JH, Vasen HF. Small-bowel cancer in Lynch syndrome: is it time for surveillance? *Lancet Oncol* 2008;**9**(9):901–5.
68. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. *JAMA* 2006;**296**(12):1507–17.

69. Rijcken FE, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. *Gut* 2002;**50**(3):382–6.
70. Leighton JA, Sharma VK, Hentz JG, et al. Capsule endoscopy versus push enteroscopy for evaluation of obscure gastrointestinal bleeding with 1-year outcomes. *Dig Dis Sci* 2006;**51**(5):891–9.
71. Postgate A, Hyer W, Phillips R, et al. Feasibility of video capsule endoscopy in the management of children with peutz-jeghers syndrome: a blinded comparison with barium enterography for the detection of small bowel polyps. *J Pediatr Gastroenterol Nutr* 2009;**49**(4):417–23.
72. Antao B, Bishop J, Shawis R, Thomson M. Clinical application and diagnostic yield of wireless capsule endoscopy in children. *J Laparoendosc Adv Surg Tech A* 2007;**17**(3):364–70.
73. Fritscher-Ravens A, Scherbakov P, Bufler P, et al. The feasibility of wireless capsule endoscopy in detecting small intestinal pathology in children under the age of 8 years: a multicentre European study. *Gut* 2009;**58**(11):1467–72.
74. Yoo JH, Tarbox J, Granpeesheh D. Using stimulus fading to teach a young child with autism to ingest wireless capsule endoscopy. *Gastrointest Endosc* 2008;**67**(7):1203–4.