Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: Report from the constitutional mismatch repair deficiency consortium

Doua Bakry, Melyssa Aronson, Carol Durno, Hala Rimawi, Roula Farah, Qasim Kholaif Alharbi, Musa Alharbi, Ashraf Shamvil, Shay Ben-Shachar, Matthew Mistry, Shlomi Constantini, Rina Dvir, Ibrahim Qaddoumi, Steven Gallinger, Jordan Lerner-Ellis, Aaron Pollett, Derek Stephens, Steve Kelies, Elizabeth Chao, David Malkin, Eric Bouffet, Cynthia Hawkins, Uri Tabori

*Corresponding author at: Division of Hematology/Oncology, The Hospital for Sick Children, Institute of Medical Sciences, The University of Toronto, Toronto, Ontario, Canada. Fax: +1 416 813 8024. E-mail address: uri.tabori@sickkids.ca (U. Tabori).
Abstract

Background: Constitutional mismatch repair deficiency (CMMRD) is a devastating cancer predisposition syndrome for which data regarding clinical manifestations, molecular screening tools and management are limited.

Methods: We established an international CMMRD consortium and collected comprehensive clinical and genetic data. Molecular diagnosis of tumour and germline biospecimens was performed. A surveillance protocol was developed and implemented.

Results: Overall, 22/23 (96%) of children with CMMRD developed 40 different tumours. While childhood CMMRD related tumours were observed in all families, Lynch related tumours in adults were observed in only 2/14 families ($p = 0.0007$). All children with CMMRD had café-au-lait spots and 11/14 came from consanguineous families. Brain tumours were the most common cancers reported (48%) followed by gastrointestinal (32%) and haematological malignancies (15%). Importantly, 12 (30%) of these were low grade and resectable cancers. Tumour immunohistochemistry was 100% sensitive and specific in diagnosing mismatch repair (MMR) deficiency of the corresponding gene while microsatellite instability was neither sensitive nor specific as a diagnostic tool ($p < 0.0001$). Furthermore, screening of normal tissue by immunohistochemistry correlated with genetic confirmation of CMMRD. The surveillance protocol detected 39 lesions which included asymptomatic malignant gliomas and gastrointestinal carcinomas. All tumours were amenable to complete resection and all patients undergoing surveillance are alive.

Discussion: CMMRD is a highly penetrant syndrome where family history of cancer may not be contributory. Screening tumours and normal tissues using immunohistochemistry for abnormal expression of MMR gene products may help in diagnosis and early implementation of surveillance for these children.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Cancer predisposition syndromes (CPS) are defined by the occurrence of a specific pattern of cancers in an individual with or without a family cancer history. The tumour spectrum and other clinical manifestations are usually similar between individuals with the same CPS. While specific genetic alterations have been described for many CPS, genotype/phenotype correlations are not always clearly defined. Many CPS exhibit an autosomal dominant pattern of inheritance, in which the presence of a heterozygous germline mutation of a predisposition gene often leads to early age of tumour onset. The rare occurrence of biallelic mutation of these genes usually leads to similar phenotypes, sometimes at a younger age.

Lynch syndrome is the most common form of hereditary adult-onset colorectal cancer. It is also associated with other gynaecological and urinary tract cancers [1–3]. Lynch syndrome is inherited in an autosomal dominant fashion and is caused by heterozygous mutations in one of the DNA mismatch repair (MMR) genes: MLH1, MSH2, MSH6 or PMS2 [3,4]. The MMR pathway is responsible for correcting base substitution mismatches and insertion-deletion mismatches generated during DNA replication in organisms from bacteria to mammals [5,6].

Tumours with MMR deficiency demonstrate high frequency of somatic microsatellite instability (MSI) [7,8]. Indeed, MSI is the hallmark of MMR-deficient colon cancers and this tumour genotype serves as a screening tool for diagnosing Lynch syndrome [9]. Additionally, there is a strong association between MSI and lack of expression of the mutated MMR gene [10,11].

In contrast to the relatively low prevalence of tumours in Lynch syndrome patients in the first two decades of life [12,13], individuals harbouring homozygous or biallelic MMR gene mutations exhibit a distinct childhood CPS termed constitutional mismatch repair deficiency (CMMRD). These children develop haematological malignancies, brain tumours and gastrointestinal cancers. These children may have café-au-lait spots and other findings that mimic neurofibromatosis-1 (NF-1). As a result, there is often a significant delay in the diagnosis of CMMRD that prevents early implementation of potentially effective surveillance tools. Since its first description in 1999 [14,15], only a few case reports and small series of CMMRD have been reported [16–18]. Available information suggests that these individuals commonly develop cancer in the first decade of life and that survival is generally poor. However, data regarding the penetrance of the syndrome and management of these children are lacking. Furthermore, conflicting data exist regarding the utility of established molecular tools such as MSI and immunohistochemistry (IHC) in screening for CMMRD.

As a result, we established an international childhood CMMRD consortium. Genetic testing and counselling were provided and a surveillance protocol for carriers...
has been implemented. Here we report the data collected by this consortium. This information reveals novel clinical and molecular determinates of CMMRD which enable us to suggest a comprehensive approach for the management of children and families affected by this devastating CPS.

2. Methods

2.1. Patients

The international consortium of childhood CMMRD includes multiple paediatric centres worldwide. Consortium members interacted and patients/families were detected using email communication and telemedicine rounds [19]. Following institutional Research Ethics Board approval, all data were centralised in the Division of Haematology/Oncology at The Hospital for Sick Children (SickKids) and the Familial Gastrointestinal Cancer Registry (FGICR) at the Zane Cohen Centre for Digestive Diseases at Mount Sinai Hospital, in Toronto, Canada. Consent forms were obtained from the parents/guardians or the patient where applicable. Family history, demographic and clinical data were obtained from the responsible physician and/or genetic counsellor at the corresponding centres. Tumour samples were centrally reviewed (by CH) and molecular analysis was performed on available tissues. Further follow-up, genetic counselling and guidance with the surveillance protocol were provided by the genetic counsellor, the gastroenterologist and the oncologists at FGICR and SickKids.

We recruited all children (ages 0–18 years) which fulfilled the following criteria: The diagnosis of CMMRD was considered definite when a germline biallelic mutation in any of the four MMR genes (MLH1, MSH2, MSH6 and PMS2) was confirmed by sequencing. The diagnosis was considered probable if the patient had a sibling with definite CMMRD and had a colon, brain and/or haematological malignancy which stained negative for the corresponding protein found to be mutated in the sibling’s germline. The diagnosis of CMMRD was deemed possible if the patient met two criteria: a. clinical manifestations of CMMRD (café-au-lait macules, consanguinity) and affected by colon, brain or haematological malignancy; and b. at least one sibling with similar criteria of CMMRD in whom tumours stained negative for the same MMR protein. There was no limitation on age of tumour onset.

Each tumour diagnosed either symptomatically or by surveillance was considered as one neoplasm except for recurrent polyps uncovered by surveillance endoscopies which were included under gastrointestinal polyposis.

2.2. Procedures

DNA extraction, genetic testing and molecular analyses of each tissue sample were performed in clinically-approved laboratories using previously reported protocols [20–22]. The determination of novel pathogenic mutation was done using standard algorithms to predict RNA/protein disruption. Further information is available in Supplementary Methods.

A surveillance protocol for CMMRD patients was developed by the Toronto group. This includes semi-annual brain MRI, annual GI endoscopy and blood work every 4 months (Supplementary Table 1) [23].

We used SAS version 9.2 for all statistical analysis. The McNemar’s test was used to compare concordance between IHC and MSI for families with Lynch syndrome and CMMRD related tumours.

3. Results

Overall, 22/23 (96%) of children with CMMRD from 14 families were affected with 40 tumours (Table 1). Both genetic and clinical diagnosis of CMMRD was first confirmed on 11 probands. Seven paediatric family members were consequently diagnosed as definite CMMRD. Two individuals (MMR29, MMR30) from definite CMMRD family and who died of glioblastoma and medulloblastoma, respectively, were considered probable CMMRD since their tumours stained negative for the corresponding protein found to be mutated in other family members. Three patients were considered as possible CMMRD since they met the clinical criteria for CMMRD and had corresponding negative IHC in both their and the sibling’s tumours.

All CMMRD patients with available clinical data (n = 18) had café-au-lait spots (>6 spots) and three patients had hypopigmentation (Supplementary Fig. 1). Three individuals had axillary freckles, and one had a plexiform neurofibroma; meeting the criteria for NF-1, but lacked germline NF-1 mutations.

The penetrance of cancer in individuals with CMMRD was exceptionally high. Upon referral, four of the 23 CMMRD individuals were unaffected, however, during the study period; three of these children were diagnosed with tumours. At the time of this publication, 22 of the 23 CMMRD children (96%) have been diagnosed with cancer.

Interestingly, the family history of the CMMRD children was not typical of Lynch syndrome, with very few cancers reported in adult members. Lynch syndrome related tumours were reported in only 2/14 families whereas CMMRD related tumours were reported in all families (p = 0.0007 McNemar’s test). Of the 28 parents in the study, only one parent had a history of cancer. This case was a mother of a CMMRD child. She died of a CNS tumour at age 25. Based on the family history which included consanguinity in prior generations, it is suspected that she too had CMMRD (Supplementary Fig. 2).
Table 1
List of patients with constitutional mismatch repair deficiency (CMMRD) syndrome.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gene</th>
<th>Mutation</th>
<th>Zygosity</th>
<th>Protein effect</th>
<th>Variant classification</th>
<th>Lynch database Classification</th>
<th>Family history</th>
<th>Malignancies (age at diagnosis)</th>
<th>Neurofibromatosis type 1-like manifestations</th>
<th>Tumour tissue tested</th>
<th>Normal tissue tested</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMR1</td>
<td>PMS2</td>
<td>c.2276–7_2589+5del (Exon 14-15 deletion)</td>
<td>Hom&lt;sup&gt;4&lt;/sup&gt;</td>
<td>p.?</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>NO</td>
<td>PAX&lt;sup&gt;+&lt;/sup&gt; (5 y 10 m) Anaplastic PAX (6 y 10 m)</td>
<td>CAL&lt;sup&gt;5&lt;/sup&gt;</td>
<td>PAX, Anaplastic PAX</td>
<td>None</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR3</td>
<td>MLH1</td>
<td>c.2059C&gt;T</td>
<td>Hom</td>
<td>p.R687W</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Brother died from GI malignancy. Maternal father colon cancer at age 50 y. Proliferating T-cell lymphoma</td>
<td>CAL</td>
<td>Adenocarcinoma of colon (9 y 9 m) GI Polyposis (9 y)</td>
<td>Polyposis&lt;sup&gt;2&lt;/sup&gt;</td>
<td>high grade and low grade dysplasia</td>
<td>None</td>
</tr>
<tr>
<td>MMR4</td>
<td>MLH1</td>
<td>c.2059C&gt;T</td>
<td>Hom</td>
<td>p.R687W</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Maternal father colon cancer at age 50 y. Proliferating T-cell lymphoma</td>
<td>CAL</td>
<td>GI Polyposis (11.5 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR5</td>
<td>MLH1</td>
<td>c.2059C&gt;T</td>
<td>Hom</td>
<td>p.R687W</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Maternal father colon cancer at age 50 y. Proliferating T-cell lymphoma</td>
<td>CAL</td>
<td>GI Polyposis (11.5 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR6</td>
<td>MLH1</td>
<td>c.2059C&gt;T</td>
<td>Hom</td>
<td>p.R687W</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Maternal father colon cancer at age 50 y. Proliferating T-cell lymphoma</td>
<td>CAL</td>
<td>GBM (11 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR7</td>
<td>MSH6</td>
<td>c.3633insT</td>
<td>Hom</td>
<td>p.Val211Cyts&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Duodenal Adenocarcinoma (9 y 7 m)</td>
<td>CAL</td>
<td>T-cell lymphoma (10 y 7 m) GI Polyposis (12.5 y)</td>
<td>GBM (8 y 10 m)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR8</td>
<td>MSH6</td>
<td>c.3633insT</td>
<td>Hom</td>
<td>p.Val211Cyts&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Duodenal Adenocarcinoma (9 y 7 m)</td>
<td>CAL</td>
<td>T-cell lymphoblastic lymphoma (3.5 y) GI Polyposis (11.5 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR9</td>
<td>MSH6</td>
<td>c.3633insT</td>
<td>Hom</td>
<td>p.Val211Cyts&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Duodenal Adenocarcinoma (9 y 7 m)</td>
<td>CAL</td>
<td>T-cell lymphoma (10 y 7 m) GI Polyposis (12.5 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR10</td>
<td>PMS2</td>
<td>c.538-1G&gt;C/538-2_903+5del (Exon 6-8 deletion)</td>
<td>Comp Het</td>
<td>p.404&lt;sup&gt;5&lt;/sup&gt;</td>
<td>p.?</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>GI Polyposis (11.5 y)</td>
<td>T-cell lymphoblastic lymphoma (3.5 y)</td>
<td>GBM (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR11</td>
<td>PMS2</td>
<td>c.2466–2_2595+5del (Exon 15 deletion)</td>
<td>Hom</td>
<td>p.?</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Cousin died from GBM, cousin died from MB</td>
<td>CAL</td>
<td>GI Polyposis (11 y)</td>
<td>Unaffected (11 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR12</td>
<td>PMS2</td>
<td>c.2466–2_2595+5del (Exon 15 deletion)</td>
<td>Hom</td>
<td>p.?</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Cousin died from GBM, cousin died from MB, brother with GBM and T-Cell ALL</td>
<td>CAL</td>
<td>Transitional renal cell carcinoma (12 y)</td>
<td>Transitional renal cell carcinoma, lymphoma, GBM</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR13</td>
<td>PMS2</td>
<td>c.2466–2_2595+5del (Exon 15 deletion)</td>
<td>Hom</td>
<td>p.?</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Cousin died from GBM, cousin died from MB, brother with GBM and T-Cell ALL</td>
<td>CAL</td>
<td>Transitional renal cell carcinoma (12 y)</td>
<td>Transitional renal cell carcinoma, lymphoma, GBM</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR14</td>
<td>PMS2</td>
<td>c.2186–2187delCT/ c.134A&gt;C</td>
<td>Comp Het</td>
<td>p.Leu729Glns&lt;sup&gt;6&lt;/sup&gt;</td>
<td>p.Asn45Thr</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>GI Polyposis (13 y)</td>
<td>Diffuse large B cell lymphoma (15 y 2 m)</td>
<td>GBM (13 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR15</td>
<td>PMS2</td>
<td>c.2186–2187delCT/ c.134A&gt;C</td>
<td>Comp Het</td>
<td>p.Leu729Glns&lt;sup&gt;6&lt;/sup&gt;</td>
<td>p.Asn45Thr</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>GI Polyposis (13 y)</td>
<td>Diffuse large B cell lymphoma (15 y 2 m)</td>
<td>GBM (13 y)</td>
<td>Skin</td>
<td>+/- +/- +/-</td>
</tr>
<tr>
<td>MMR16</td>
<td>PMS2</td>
<td>c.3417delC</td>
<td>Hom</td>
<td>p.Thr820Alafs&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Cousin died from brain tumour, uncle died from brain tumour</td>
<td>CAL</td>
<td>Anaplastic astrocytoma</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>MMR17</td>
<td>PMS2</td>
<td>c.3417delC</td>
<td>Hom</td>
<td>p.Thr820Alafs&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Cousin died from brain tumour, uncle died from brain tumour</td>
<td>CAL</td>
<td>Anaplastic astrocytoma</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>MMR18</td>
<td>MSH6</td>
<td>c.3946G&gt;A</td>
<td>Hom</td>
<td>p.Glu1316Arg</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Mother died from brain tumour (25 y)</td>
<td>CAL</td>
<td>Anaplastic astrocytoma</td>
<td>AA (11 y)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>MMR19</td>
<td>PMS2</td>
<td>c.2458dupA</td>
<td>Hom</td>
<td>p.Thr820Alafs&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Mother died from brain tumour (25 y)</td>
<td>CAL</td>
<td>Anaplastic astrocytoma</td>
<td>AA (11 y)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>MMR20</td>
<td>PMS2</td>
<td>c.2458dupA</td>
<td>Hom</td>
<td>p.Thr820Alafs&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Pathogenic</td>
<td>Not found</td>
<td>Mother died from brain tumour (25 y)</td>
<td>CAL</td>
<td>Anaplastic astrocytoma</td>
<td>AA (11 y)</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>1</sup>Family history Malignancies (age at diagnosis)
<sup>2</sup>Neurofibromatosis type 1-like manifestations
<sup>3</sup>Tumour tissue tested
<sup>4</sup>Normal tissue tested
<sup>5</sup>Immunohistochemistry
3.1. Mutation characteristics

We detected 14 different germline MMR mutations. These included mutations in *PMS2* (8), *MSH6* (5) and *MLH1* (1). Importantly 6/14 mutations were previously unreported (Table 1). These included one missense alteration, two out-of-frame deletions, one duplication, and two truncation mutations. All CMMRD patients from non-consanguineous families were compound heterozygote (parents were heterozygous for the corresponding different mutations). *PMS2* mutations included homozygous large deletions including multiple exons.

3.2. Tumour spectrum

The 22 affected individuals with CMMRD had a total of 40 primary tumours (Table 1). Eleven patients had multiple neoplasms including three (MMR8, MMR43 and MMR51) with three metachronous tumours and two (MMR2, MMR50) with four metachronous tumours. Nineteen (48%) of these neoplasms were brain tumours, 13 (32%) gastrointestinal, 6 (15%) haematological and two other tumours (a transitional renal cell carcinoma, and one pleomorphic neurofibroma) (Table 1). Among the CNS neoplasms the most prevalent type were high grade gliomas (74%) followed by medulloblastoma/PNET (10%) and low grade glioma (16%). Most of the gliomas had a pleomorphic appearance consistent with pleomorphic xanthoastrocytoma. Five (83%) of the haematological malignancies were of T-cell origin. Importantly, we observed a high frequency of low grade and premalignant lesions. Specifically, three CNS tumours were low grade glial lesions and 9/13 gastrointestinal tumours were premalignant and dysplastic polyps.

3.3. Molecular markers of CMMRD

In order to define the best molecular tool to analyse tumours for possible CMMRD we compared two of the major methods used: IHC and MSI. IHC for the four MMR proteins (MLH1, PMS2, MSH6 and MSH2) was performed on 26 available tumours (Table 2 and Fig. 1A). Loss of the corresponding MMR protein was seen in all tumours from germline mutant CMMRD patients. These included 17 brain tumours for which sensitivity and specificity of IHC for MMR genes is not yet established. Furthermore, in contrast to Lynch syndrome tumours, all intratumoural normal tissue stained negative further indicating CMMRD. For patients where more than one tumour was available, negative staining was concordant between all tumours tested (Table 1). Taken together, IHC was 100% sensitive and specific for detecting CMMRD.

MSI was tested on 28 tumours and the paired normal tissues and was neither specific nor sensitive for detection of...
CMMRD in childhood tumours. In total, 20/28 lesions were MSI-stable. Specifically, out of the 14 brain tumours only one exhibited an MSI-high genotype (Table 2, Fig. 1B). Gastrointestinal lesions revealed MSI-stable in low grade polyps and MSI-high in severely dysplastic polyps and colon cancers (n = 5). Patients with multiple cancers such as colon carcinoma, lymphoma and brain tumours (n = 6) exhibited a different MSI genotype in different tumour tissues (Fig. 2B). Taken together, agreement between IHC and CMMRD was significantly better than MSI (p < 0.0001, McNemar’s test).

Since individuals with CMMRD have lost expression of one MMR protein in their intratumoural normal cells, we tested whether IHC, which showed 100% sensitivity in tumours, might be a robust clinical tool to detect biallelic gene deficiency (CMMRD) in normal tissue. We initially used normal colon and skin biopsies from individuals known to have the mutation (n = 5). All normal tissues tested revealed negative IHC for the mutated gene while staining positive for the other MMR genes. To further support this observation, we stained skin biopsies from multiple members of a family where one individual was diagnosed as having a possible PMS2 mutation by negative tumour staining and clinical examination. Immunostaining of the skin biopsies revealed positive stains in unaffected family members.

**Table 2**

<table>
<thead>
<tr>
<th>Malignancies</th>
<th>MSI (H/S)</th>
<th>IHC (negative/positive)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain tumours</td>
<td>1/14</td>
<td>17/0</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2/1</td>
<td>2/0</td>
<td></td>
</tr>
<tr>
<td>GI low grade dysplasia</td>
<td>0/5</td>
<td>2/0</td>
<td></td>
</tr>
<tr>
<td>GI high grade dysplasia</td>
<td>3/0</td>
<td>3/0</td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td>2/0</td>
<td>1/0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0/1</td>
<td>1/0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8/20</td>
<td>26/0</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

MSI, microsatellite instability; IHC, immunohistochemistry; GI, gastrointestinal; H/S, high/stable.

Fig. 1. Molecular diagnosis of mismatch repair (MMR) deficiency in tumour tissue of constitutional mismatch repair deficiency (CMMRD) patients. (A) Immunohistochemistry (IHC) of glioblastoma (GBM) from a patient with PMS2 biallelic mutation. MSH6, MSH2 and MLH1 exhibit positive staining. PMS2-negative staining. (B) Microsatellite instability (MSI) of three tumours from one patient with CMMRD. Note similar pattern in the blood, GBM and renal cell carcinoma (RCC) confirming MSI-stable site. The lymphoma from the same patient has a different pattern suggesting MSI-high.
including the parents \((n = 6, \text{ Fig. 2})\). However, both the index patient and his unaffected sister, who had café-au-lait spots, stained negative for the protein. Consequently, genetic testing confirmed that these two children harboured a homozygous PMS2 mutation (CMMRD), while the rest of the family members \((n = 6)\) harboured heterozygote mutation or wild-type gene.

3.4. Surveillance protocol data

We previously reported the feasibility of a surveillance protocol on one family with CMMRD [23]. The surveillance protocol was developed by paediatric and adult gastroenterology, paediatric neuro-oncology and the cancer genetics teams. Here we report additional data for this surveillance protocol on a larger group of patients with CMMRD. In total, 39 asymptomatic lesions were detected. These included two malignant gliomas, two gastrointestinal carcinomas and six cases of gastrointestinal polyposis (Table 1). Additional 29 dysplastic polyps were detected in subsequent endoscopies (Fig. 4). Importantly, all malignant and premalignant lesions were amenable for complete resection. At a mean follow-up of 61 months all patients enrolled in this surveillance protocol are alive.

4. Discussion

This is the first report describing an international consortium established to focus on the biology, genetics, clinical spectrum and possible interventions for children with CMMRD. We suggest a robust molecular approach for the screening and molecular diagnosis of CMMRD and report encouraging early clinical outcomes using this surveillance protocol.

In contrast to Lynch syndrome, for which carriers have no clinical manifestations other than cancers in adults [2], CMMRD predisposes children to a distinct though complex array of clinical manifestations and a wide tumour spectrum [24]. Skin lesions resembling those in NF-1 were observed in all individuals with CMMRD. Therefore, in order to distinguish CMMRD from other phakomatoses, one must consider other variables such as a history of consanguinity and a family history of young relatives with malignant brain tumours, lymphomas or gastrointestinal malignancies.

Since CMMRD is highly penetrant, the paucity of cancers in the parents and lack of Lynch syndrome related tumours in other family members are surprising. Indeed, a recent literature review [25] reported that Lynch syndrome associated malignancies are absent in up to 85% of CMMRD pedigrees. Furthermore, many of the germline mutations observed in our study were not reported previously in Lynch syndrome families. Similarly, MSH2 mutations which are most commonly found in Lynch syndrome are less common to absent in CMMRD, while PMS2 and MSH6 are more commonly observed in the syndrome. A possible explanation for this phenomenon is low penetrance of heterozygous mutations in MSH6 and PMS2 found in CMMRD and the deleterious functional consequence of homozygous mutations. Highly penetrant mutations in MSH2, may be embryonically lethal when homozygous leading to the lower prevalence observed in CMMRD.

Taken together, these findings suggest that family history of consanguinity and a history of lymphomas, GI and brain tumours combined with presence of café-au-lait spots should raise a high index of suspicion for CMMRD while lack of history of Lynch syndrome related tumours does not exclude the diagnosis of the syndrome.
The tumour spectrum of CMMRD in children was previously reported to include mostly malignant neoplasms. We observed both low-grade CNS tumours and premalignant GI lesions. This suggests that some cancers in CMMRD, may evolve from low grade tumours. Therefore, a surveillance protocol may detect these early lesions and be beneficial for these individuals (Fig. 3).

MSI is considered the gold standard diagnostic test for MMR deficiency in Lynch syndrome [26]. Our observations suggest that for CMMRD related tumours, MSI is neither sensitive nor specific particularly in non-gastrointestinal malignancies and low-grade intestinal polyps (Fig. 1). These finding are consistent and add statistical strength to previous reports of smaller cohorts [27].

Since several patients had cancers exhibiting MSI-stable and other cancers with MSI-high genotypes, it is reasonable to assume that in the context of CMMRD, MSI is tissue specific and not related to the individual’s specific mutation.

In contrast, IHC of the corresponding MMR proteins was 100% sensitive in all tumour types, for the detection of the MMR gene mutation in our cohort (Fig. 1).

Therefore, IHC of either normal colonic or skin tissues could be used to screen for CMMRD and to guide genetic testing. This fast and simple screening tool...
would also allow for early implementation of surveillance protocol. These molecular tools can only guide the physician and not replace genetic testing of every patient suspected to have CMMRD.

Results of the surveillance protocol developed by our group suggest efficiency in early diagnosis of premalignant and malignant tumours of brain and colon [23]. Especially since all tumours were amenable for complete resection. For both cancers, resection is the most important prognostic factor to date. The finding of low grade and premalignant lesions further supports the role of early detection and intervention in these devastating tumours. Although the mean follow-up of 61 months is relatively short, the fact that all patients who underwent the surveillance protocol are still alive is encouraging especially in light of the large burden of tumours (n = 40) diagnosed during the surveillance period. Since early detection and monitoring of leukaemia and lymphoma has not yet shown to improve survival, the role of surveillance for these cancers is still unclear. It is therefore important to note that our early findings are not sufficient for clear evidence based recommendations for implementation of a surveillance protocol for all CMMRD patients.

Finally, based on our clinical and molecular observations, we suggest an approach to individuals and families with suspected CMMRD (Fig. 4). In a child (<18 y) with either: (1) café au lait spots and a history of consanguineous parents, or (2) café au lait spots and a family member with CMMRD related tumours, we recommend to stain biopsy from a tumour or normal tissue for four MMR proteins. Negative staining should prompt genetic testing to initiate surveillance protocol and further counselling to family members.

We conclude, in this first report from our international consortium that, we were able to define clinical risk factors, molecular tools and an approach for detection and possible intervention for children with CMMRD. Further studies will shed light on the biological mechanisms of tumour formation, and the design of specific treatment options for children and family members with this intriguing syndrome.

Conflict of interest statement

None declared.

Acknowledgment

b.r.a.i.n. child organization.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.12.005.

References


