Constitutional mismatch repair deficiency and childhood leukemia/lymphoma – report on a novel biallelic *MSH6* mutation

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ABSTRACT

Biallelic mutations of mismatch repair genes cause constitutional mismatch repair deficiency associated with an increased risk for childhood leukemia/lymphoma. We report on a case with constitutional mismatch repair deficiency caused by a novel *MSH6* mutation leading to a T-cell lymphoma and colonic adenocarcinoma at six and 13 years of age, respectively. A review of the literature on hematologic malignancies in constitutional mismatch repair deficiency showed that in almost half of the 47 known constitutional mismatch repair deficiency families, at least one individual is affected by a hematologic malignancy, predominantly T-cell lymphomas. However, diagnosing constitutional mismatch repair deficiency may be difficult when the first child is affected by leukemia/lymphoma, but identification of the causative germline mutation is of vital importance: (i) to identify relatives at risk and exclude an increased risk in non-mutation carriers; (ii) to prevent hematopoietic stem cell transplantation from sibling donors also carrying a biallelic germline mutation; and (iii) to implement effective surveillance programs for mutation carriers, that may reduce constitutional mismatch repair deficiency-associated mortality.

Key words: constitutional mismatch repair deficiency, hereditary cancer, *MSH6*, childhood T-cell lymphoma, leukemia.

Citation: Ripperger T, Beger C, Rahner N, Sykora KW, Bockmeyer CL, Lehmann U, Kreipe HH, and Schlegelberger B. Constitutional mismatch repair deficiency and childhood leukemia/lymphoma – report on a novel biallelic MSH6 mutation. Haematologica 2010;95:841-844 doi:10.3324/haematol.2009.015503

Introduction

Following DNA replication and DNA damage, the DNA mismatch repair (MMR) machinery recognizes and directs repair of base-base mismatches and insertion/deletion loops.[1] Monoallelic germline mutations in the MMR genes MutL homolog 1 (*MLH1*), MutS homolog 2 (*MSH2*), *MSH6*, or post-meiotic segregation increased 2 (*PMS2*), are known to cause Lynch syndrome (LS), characterized by an early onset of non-polyposis colorectal cancer and extra colonic malignancies, e.g. carcinomas of the endometrium, stomach, or upper uroepithelial tract, while leukemias or lymphomas are not common in LS.[2,3] In contrast, biallelic germline mutations lead to a more severe disease designated as constitutional mismatch repair deficiency (CMMRD).[4,5] CMMRD is characterized by: (i) childhood onset of leukemia/ lymphoma, brain tumors, and other rare malignancies, e.g. rhabdomyosarcoma;[6] (ii) early onset of colorectal cancer or other LS-associated malignancies; and (iii) phenotypic features reminiscent of neurofibromatosis type 1 (NF1), mainly café-au-lait spots (CLS).[5,7,8]

Here we report on a novel biallelic germline mutation in *MSH6* causing CMMRD with childhood T-cell non-Hodgkin's lymphoma (T-NHL) and metastasized colorectal cancer by the age of 13. In addition, we review CMMRD families with known MMR gene mutations with at least one affected individual with a hematologic malignancy.

Design and Methods

Immunohistochemistry was performed in accordance with standard protocols. For the analysis of microsatellite instability (MSI), ten markers (Bethesda panel and five additional markers[9]) were investigated.

Mutation analysis of *MSH6* (NM_000179) was performed by PCR amplification and direct sequencing. Mutation nomenclature follows the recommendation of the Human Genome Variation Society.

Written informed consent was obtained from all subjects before immunohistochemistry, microsatellite analysis, and blood or tissue sample collection for mutation analysis.

Clinical case

A non-resectable mediastinal T-NHL was diagnosed in our female patient at six years of age (V:10, Figure 1). Six months

Acknowledgments: the authors would like to thank the study subjects and their attending doctors for their participation in this study, Bernd Haermeyer for his expert technical assistance, and Gillian Teicke for her help in editing the manuscript.
Funding: TR was supported by a grant from Hannover Biomedical Research School, Graduate School of Excellence, PhD Program Molecular Medicine, Hannover Medical School, Hannover, Germany, and by the EU COST initiative EUGESMA.
Manuscript received on August 10, 2009. Revised version arrived on October 9, 2009. Manuscript accepted on October 13, 2009.
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The Online version of this article has a Supplementary Appendix.

after her initial chemotherapy following the NHL BFM 95 protocol arm MR, she relapsed. The second regimen followed the ALL REZ BFM 2002 pilot 02 protocol arm S2, and she received hematopoietic stem cell transplantation (HSCT) from a matched sibling donor as consolidation.

At the age of 13, the patient presented with episodes of rectal bleeding and abdominal colic. In a colonoscopy, a bifocal ulcerative carcinoma was observed in the transversal colon and numerous tubulovillous polyps with high-grade intraepithelial atypia were seen throughout the colon. Colectomy with end ileostomy was performed. Histopathological investigations showed a bifocal colonic adenocarcinoma with local lymph node metastases. Adjuvant chemotherapy was performed according to the FOLFOX4 regimen. One year later, a rectal adenoma with low grade intraepithelial atypia was detected. Proctectomy with endoanal mucosectomy and ileal pouch-anal anastomosis was performed.

Due to her medical history of cancer, the patient was referred for genetic counseling. She presented with several CLS, and lateral conjunctival melanosis of the left eye. Her consanguineous parents (2nd degree cousins; IV:8 and IV:9) reported a colorectal cancer in a paternal uncle (IV:7) married to a sister of the mother (IV:10). At the age of 42, he was diagnosed with synchronous adenocarcinomas in the sigmoid and descending colon.

Results and Discussion

A novel mutation in MSH6

A medical history of a T-NHL at the age of six, a relapse at the age of eight, and the diagnosis of colorectal cancer at the age of 13 in a young girl is rare. The combination of: (i) cancer history; (ii) CLS; (iii) consanguinity of the parents; and (iv) early onset of synchronous colorectal cancer in a paternal uncle led us to speculate that an MMR defect might be the reason for the family history of cancer.

In our patient, MSI analysis and immunohistochemical analysis of MMR proteins displayed high-grade MSI and a loss of MSH6 in non-malignant and malignant cells of the colonic mucosa. In contrast, following HSCT, reactive lymphocytes infiltrating the colonic mucosa displayed nuclear staining for MSH6 (Figure 2A). In addition, MSI analysis and immunohistochemistry of colorectal cancer specimens from the diseased paternal uncle were performed. Both specimens displayed high-grade MSI. However, in contrast to our patient, loss of MSH6 was seen only in malignant cells.

Sequence analysis of *MSH6* in the index patient displayed a homozygous single nucleotide deletion in exon 4 (c.691delG) leading to a frameshift and premature termination (p.Val231TyrfsX15) (Figure 2B). Subsequently, the diseased uncle, the father, and the mother were shown to be

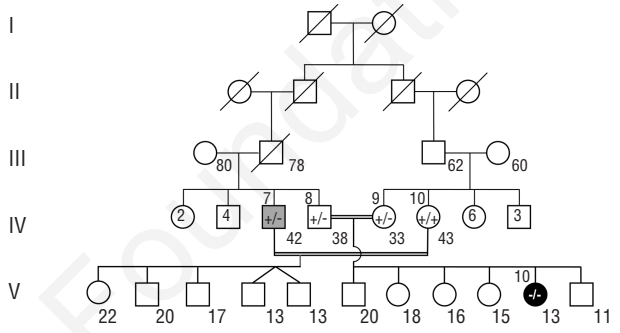

Figure 1. Pedigree of the family investigated. Roman numerals (I-V), generations; circles, females; squares, males; numbers above the symbol, individual identifier; numbers below the symbol, age at diagnosis of colorectal cancer (diseased individuals) or age (healthy individuals); black symbol, patient with childhood T-NHL at the age of six, T-NHL relapse at the age of eight, and colorectal cancer at the age of 13; gray symbol, diseased uncle with synchronous colorectal cancer; +/+, wild-type; +/-, heterozygous carrier, -/-, homozygous carrier of the identified frameshift mutation in *MSH6* (c.691delG, p.Val231TyrfsX15).

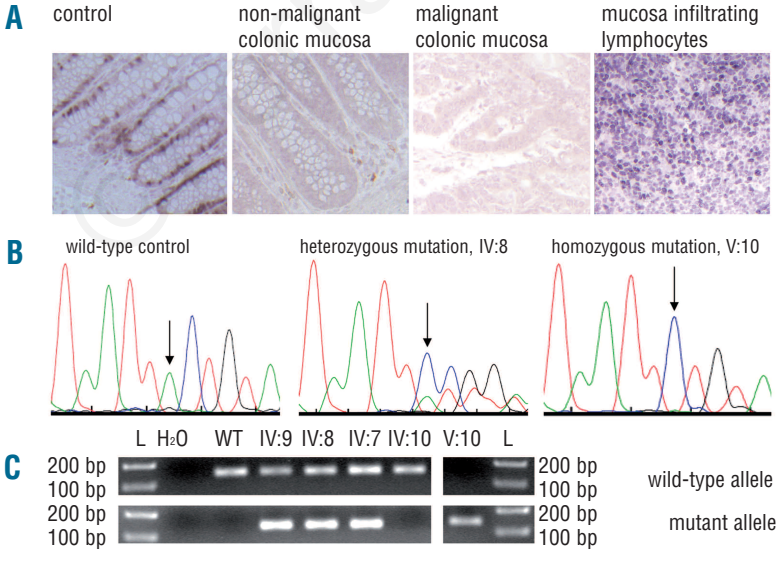

Figure 2. Immunohistochemistry and molecular analyses in family members. (A) Immunohistochemical analysis of colorectal cancer of our patient (V:10). While MSH2, MLH1 and PMS2 staining were inconspicuous (*data not shown*), a loss of MSH6 was seen in malignant and non-malignant colonic mucosa in comparison to a control. In contrast, following HSCT, lymphocytes infiltrating the colonic mucosa show nuclear staining for *MSH6* (NBT/BCIP staining, 100x magnification). (B) Direct sequencing of *MSH6* (NM_000179) identified a novel frameshift mutation in exon 4: c.691delG, p.Val231TyrfsX15. The electropherograms represent an example of two independent sequence analyses. Black arrows indicate the G in the wild-type sequence that is deleted in one or both alleles in the case of a monoallelic or biallelic mutation, respectively. (C) Allele-specific PCR. Using a specific forward primer for the wild-type or mutant allele, results of direct sequencing were confirmed by allele-specific PCR. DNA was extracted from peripheral blood cells of the parents (IV:9, IV:8) and their siblings (IV:7, IV:10). To access DNA of our patient prior to HSCT, DNA was extracted from a formalin-fixed paraffin-embedded lymph node without any evidence of malignancy. WT, wild-type control; L, molecular marker.

heterozygous carriers of the identified mutation in *MSH6*. The maternal aunt married to the diseased uncle does not carry the familial mutation. Sequencing results were confirmed by allele-specific PCRs (Figure 2C).

In summary, the molecular findings of a highly microsatellite unstable colorectal cancer with a loss of MSH6 confirm the consideration of an MMR defect. While the diseased uncle carries one mutated allele, our patient suffering from a more severe cancer phenotype has a biallelic mutation leading to a constitutional deficiency of MSH6.

CMMRD and hematologic malignancies – predominance of T-cell lymphomas

CMMRD has been reported in 47 families affecting 77 individuals (*Online Supplementary Table S1*). In almost half of these families (20 out of 47, 43%), at least one individual was affected by a hematologic malignancy. Approximately one-third of the affected individuals (26 of 77, 34%) suffered from hematologic malignancies, namely non-Hodgkin's lymphoma (NHL; n=17), acute lymphoblastic leukemia (ALL; n=6), acute myeloid leukemia (AML; n=3), acute leukemia (n=1), or atypical chronic myelogenous leukemia (n=1). While the disease-causing mutation can be found in *MLH1* and *MSH2* in up to 90% of patients with LS,[10] CMMRD is mainly caused by biallelic mutations in *PMS2* and *MSH6* (Figure 3).[5] Biallelic mutations of *MSH6*, either homozygous or compound heterozygous, were found in 10 of the 47 families (21%) comprising 14 affected individuals (*Online Supplementary Figure S1*). Even though hematologic malignancies tend to be more frequent in families with biallelic mutations in *MLH1* or *MSH2*,[5] we report here on the second CMMRD family with an *MSH6* mutation and a lymphoma as the primary malignancy. In general, a predominance of NHL, especially T-NHL, can be seen in CMMRD patients with hematologic malignancies (*Online Supplementary Table S1*). Lymphomas reported so far frequently occurred as a sole malignancy (n=8) or in combination with colorectal cancer (n=7). Notably, the combination of an early onset of lymphomas, mainly T-NHL, followed by gastrointestinal adenomas and adenocarcinomas completely reflects the phenotype seen in biallelic MMR gene knock-out mice that may serve as a good model for CMMRD.[11]

Acute myeloid leukemia in CMMRD

Despite the clear predominance of lymphoid malignancies, 3 patients with CMMRD developed AML (*Online Supplementary Table S1*). Even though relatively rare, there are two interesting aspects concerning AML and CMMRD:

(i) 2 out of 3 AMLs were secondary AMLs following treatment of medulloblastomas, leading to the hypothesis that these AML cases might be therapy related.[12] In contrast, the third case of AML occurred as a primary neoplasm one year before the diagnosis of medulloblastoma, indicating a potential link between medulloblastoma and AML that are both rather infrequent malignancies in CMMRD;[5]

(ii) CMMRD patients have phenotypic similarities with NF1 (*Online Supplementary Table S1*).[5] NF1, an autosomal dominant disorder caused by germline *NF1* mutations occurring *de novo* in up to 50% of the cases, predisposes to a variety of benign and malignant neoplasms in childhood,[13] including juvenile myelomonocytic leukemia.[14-16] Generally, NF1 is diagnosed on the basis of clinical criteria.[17] Mutation analysis of *NF1* is not usually performed. Like other disorders, CMMRD is an important differential diagnosis of NF1,[17] and should be considered in the presence of medulloblastoma and AML in children or adolescents, as well as in the case of LS-associated malignancies.[5] In contrast to extensive molecular analysis of the very large *NF1* gene, CMMRD can be investigated by a relatively easy screening for MMR deficiency using immunohistochemistry. If there are any doubts in the diagnosis of NF1, CMMRD deficiency should be ruled out.[5]

Chemotherapy in CMMRD

Little is known about the efficiency of standard protocols used to treat childhood lymphoma and leukemia in CMMRD. *In vitro* analyses showed that certain drugs, e.g. monofunctional methylating agents, are less effective in MMR-deficient cell lines.[1,10,18] One can speculate that treatment response might differ between lymphomas with acquired MMR deficiency surrounded by MMR-competent cells, including a competent immune system, and lymphomas in CMMRD patients with MMR deficiency in all cells. Further investigations are needed to assess the frequency of MSI and/or MMR gene mutations in hematologic malignancies and to test their influence on the outcome.

Recognizing CMMRD

In CMMRD, the median age of onset is 4.5 years (range 0.42-14) for T-NHL, six years (range 2-15) for ALL and six years (range 0.4-17) for hematologic malignancies in general. In comparison to brain tumors (median 8 years, range 2-35 years),[5] LS-associated malignancies (median 16 years, range 8-41 years), and other malignancies (median 13 years, range 1-65 years) in CMMRD,[5] hematologic malignancies have the earliest median age of onset. This early disease onset may render the diagnosis of CMMRD difficult since, in 15 out of the 20 families (75%), no other family members were affected by LS-associated malignancies when the first child became affected (*Online Supplementary Table S1*). Moreover, lymphoblastic lymphomas and ALL are frequent

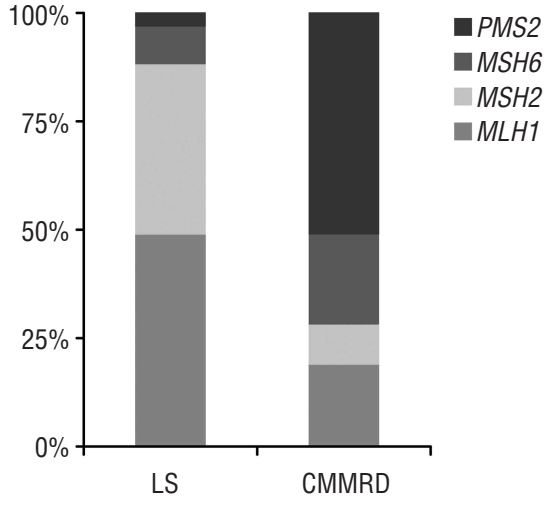

Figure 3. Molecular cause of LS and CMMRD. Relative distribution of causative mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2* in LS[10] and CMMRD[5] indicating the predominance of mutations *MLH1* and *MSH2*, and *MSH6* and *PMS2* in LS and CMMRD, respectively.

hematologic malignancies in childhood. Obviously, no germline mutations will be found in most of them, but awareness of CMMRD in children with NHL, ALL and other rare tumors in CMMRD may allow for an earlier diagnosis, at least in some affected families. This clearly requires careful evaluation and re-evaluation of the family history of cancer at the time of diagnosis and during follow-up of the patient.

In the family reported here, the mediastinal T-NHL was the primary neoplasm diagnosed. While at that point, no family history indicative of an MMR deficiency was present, synchronous colorectal cancer in a 42-year old paternal uncle (IV:7, Figure 1) was diagnosed one year later, i.e. one year prior to the lymphoma relapse and six years before the diagnosis of metastasized colorectal cancer in our patient (V:10). Due to the diagnosis of synchronous colorectal cancer before the age of 50, revised Bethesda guidelines[19] were fulfilled and LS could have been considered in the paternal uncle, opening up the possibility of identifying the underlying germline mutation.[19]

Conclusions

Diagnosing CMMRD has important implications for the entire family. Healthy parents, as in our case, will learn that they carry a monoallelic mutation in an MMR gene and may thus suffer from LS. In addition, they have to cope with the knowledge that their other children have an *a priori* risk of 25% and 50% to carry a biallelic and monoallelic mutation, respectively. Healthy siblings of the parents are confronted with a 50% *a priori* risk of being a carrier of a heterozygous mutation that can be inherited by their own children. Considering the wide-reaching clinical relevance of this rare syndrome, genetic counseling should be offered to all families at risk. Identification of the causative germline mutation would have three main clinical consequences: (i) following genetic counseling of individuals at risk, molecular genetic testing allows the identification of those carrying the mutation and exclusion of an increased risk in non-mutation carriers; (ii) prevention of HSCT from sibling donors also carrying a biallelic germline mutation, that may have an adverse clinical outcome, as was recently reported in another cancer syndrome caused by a heterozygous runt-related transcription factor 1 (*RUNX1*) germline mutation;[20] and (iii) surveillance programs for mutation carriers, e.g. regular colonoscopy, enabling early diagnosis and treatment of LS and CMMRD-associated neoplasms. Besides the improvements required for the therapy of diseased CMMRD patients, efficient surveillance programs have to be developed to reduce mortality, as has been demonstrated for regular colonoscopy[21] in patients with LS.

Authorship and Disclosures

TR and BS co-ordinated the study. TR, CB, and KWS recruited the patients. NR participated in the evaluation of the family. TR, CLB, UL, and HHK performed the histopathological and molecular genetic analyses. TR and BS wrote the paper.

The authors reported no potential conflicts of interest.

References

1. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006;7(5):335-46.
2. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med. 2003;348(10):919-32.
3. Lynch HT, Lynch JF, Lynch PM, Attard T. Hereditary colorectal cancer syndromes: molecular genetics, genetic counseling, diagnosis and management. Fam Cancer. 2008;7(1):27-39.
4. Scott RH, Mansour S, Pritchard-Jones K, Kumar D, MacSweeney F, Rahman N. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic MSH6 mutations. Nat Clin Pract Oncol. 2007;4(2):130-4.
5. 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.
6. Kratz CP, Holter S, Etzler J, Lauten M, Pollett A, Niemeyer CM, et al. Rhabdomyosarcoma in patients with constitutional mismatch-repair-deficiency syndrome. J Med Genet. 2009;46(6):418-20.
7. Bandipalliam P. Syndrome of early onset colon cancers, hematologic malignancies & 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: is it time for Lynch III? Clin Genet. 2007;71(6):499-500.
9. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997;57(21):4749-56.
10. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol 2003;21(6):1174-9.
11. Edelmann L, Edelmann W. Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. Am J Med Genet C Semin Med Genet 2004;129C(1):91-9.
12. Etzler J, Peyrl A, Zatkova A, Schildhaus HU, Ficek A, Merkelbach-Bruse S, et al. RNA-based mutation analysis identifies an unusual MSH6 splicing defect and circumvents PMS2 pseudogene interference. Hum Mutat. 2008;29(2):299-305.
13. O'Marcaigh AS, Shannon KM. Role of the NF1 gene in leukemogenesis and myeloid growth control. J Pediatr Hematol Oncol 1997;19(6):551-4.
14. Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 2005;105(1):410-9.
15. Flotho C, Steinemann D, Mullighan CG, Neale G, Mayer K, Kratz CP, et al. Genome-wide single-nucleotide polymorphism analysis in juvenile myelomonocytic leukemia identifies uniparental disomy surrounding the NF1 locus in cases associated with neurofibromatosis but not in cases with mutant RAS or PTPN11. Oncogene. 2007;26(39):5816-21.
16. Steinemann D, Arning L, Praulich I, Stuhrmann M, Hasle H, Star J et al. Mitotic recombination and compound-heterozygous mutations are predominant NF1-inactivating mechanisms in children with juvenile myelomonocytic leukemia (JMML) and neurofibromatosis type 1. Haematologica. 2010;95(2):320-3.
17. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet. 2007;44(2):81-8.
18. Levati L, Marra G, Lettieri T, D'Atri S, Vernole P, Tentori L, et al. Mutation of the mismatch repair gene hMSH2 and hMSH6 in a human T-cell leukemia line tolerant to methylating agents. Genes Chromosomes Cancer. 1998;23(2):159-66.
19. Umar A, Boland CR, Terdiman JP, Syngal S, de la CA, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-8.
20. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia--a review. Br J Haematol. 2008;140(2):123-32.
21. Vasen HF, Moslein G, Alonso A, Bernstein I, Bertario L, Blanco I, et al. Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). J Med Genet. 2007;44(6):353-62.