European Journal of Human Genetics (2014) 22, 1334–1337

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SHORT REPORT

Biallelic *MUTYH* mutations can mimic Lynch syndrome

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The hallmarks of Lynch syndrome (LS) include a positive family history of colorectal cancer (CRC), germline mutations in the DNA mismatch repair (MMR) genes, tumours with high microsatellite instability (MSI-H) and loss of MMR protein expression. However, in ~10–15% of clinically suspected LS cases, MMR mutation analyses cannot explain MSI-H and abnormal immunohistochemistry (IHC) results. The highly variable phenotype of *MUTYH*-associated polyposis (MAP) can overlap with the LS phenotype, but is inherited recessively. We analysed the *MUTYH* gene in 85 'unresolved' patients with tumours showing IHC MMR-deficiency without detectable germline mutation. Biallelic p.(Tyr179Cys) *MUTYH* germline mutations were found in one patient (frequency 1.18%) with CRC, urothelial carcinoma and a sebaceous gland carcinoma. LS was suspected due to a positive family history of CRC and because of MSI-H and MSH2–MSH6 deficiency on IHC in the sebaceous gland carcinoma. Sequencing of this tumour revealed two somatic *MSH2* mutations, thus explaining MSI-H and IHC results, and mimicking LS-like histopathology. This is the first report of two somatic *MSH2* mutations leading to an MSI-H tumour lacking MSH2–MSH6 protein expression in a patient with MAP. In addition to typical transversion mutations in *KRAS* and *APC*, MAP can also induce tumourigenesis via the MSI-pathway.
European Journal of Human Genetics (2014) **22,** 1334–1337; doi:10.1038/ejhg.2014.15; published online 12 February 2014

Keywords: *MUTYH*; base excision repair; somatic mutations; Lynch syndrome; immunohistochemistry; DNA mismatch repair

INTRODUCTION

Lynch syndrome (LS) is the most frequent autosomal dominant predisposition for early-onset colorectal cancer (CRC) (MIM# 114500) and associated tumours,[1] and is diagnosed by the detection of a germline mutation in one of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2*. In tumours, the typical clues for LS include high microsatellite instability (MSI-H) and the loss of MMR protein expression in immunohistochemistry (IHC). However, in 10–15% of our cases clinically suspected of LS, the MSI-H and abnormal IHC results in the tumours cannot be explained by MMR germline mutation analyses.[2,3]

Germline mutations in the human Mut Y homolog (*MUTYH;* MIM# 604933) also lead to a predisposition to CRC and adenomas but with an autosomal recessive inheritance. The clinical picture of *MUTYH*-associated polyposis (MAP, MIM' 608456) is extremely variable and ranges from severe polyposis coli to attenuated forms with late age of onset or few adenomas or CRC, which creates phenotypic overlap with LS.[4–9]

So far, three MAP patients have been described in the literature with an IHC loss of MLH1 protein staining and an MSI-H tumour: one male with a biallelic *MUTYH* germline mutation c.1227_28dupGG; p.(Glu410Glyfs*43) affecting the base excision repair (BER), and one somatic mutation in the *MLH1* gene detected in his left-sided CRC at age of 49 years,[10] the second with two heterozygous *MUTYH* mutations c.536A>G; p.(Tyr179Cys) and c.1187G>A; p.(Gly396Asp) and biallelic methylation of the *MLH1* promoter in her caecal carcinoma at the age of 50 years[11] and the third with homozygous *MUTYH* mutation c.536A>G; p.(Tyr179Cys) and no analyses for methylation or somatic mutation in *MLH1* performed in the right-sided colon carcinoma diagnosed at age 50 years.[12] In some publications CRC patients with MSI-H tumours were excluded for *MUTYH* analyses or MSI was not analysed.[13,14] Three further MAP patients were identified with MSI-H tumours but no information on IHC for MMR proteins was given,[15,16] whereas other studies detected no biallelic *MUTYH* mutation carrier in patients with MSI-H tumours.[17–20] To investigate the role of MAP as a possible cause of somatic mutations in MMR genes we analysed *MUTYH* in blood DNA of 85 'unresolved' patients suspected of having LS due to IHC loss of MMR protein expression.

MATERIALS AND METHODS

The study cohort includes 85 patients suspected of having LS based on the MSI-H and pathological IHC results in their tumours: 23 had IHC loss of MLH1 and PMS2, 35 had loss of MSH2 and MSH6, 19 showed loss of MSH6 only, and 8 lacked expression of PMS2 only. All patients met at least one of the Bethesda criteria[21] and were tested negative for germline mutations and deletions or duplications in the *MLH1*, *MSH2*, *MSH6*, *EPCAM* and *PMS2* genes before this study. MLH1-deficient cases were included if *MLH1* promoter methylation was absent in tumour DNA. Sixty nine patients were from Germany and sixteen from the USA. All patients provided their informed consent for cancer genetics research.

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Received 26 September 2013; revised 20 December 2013; accepted 16 January 2014; published online 12 February 2014

Control cohort: 403 CRC patients with either confirmed LS by pathogenic MMR germline mutation ($N=326$) or sporadic tumourigenesis by *BRAF* mutation and *MLH1* promoter methylation in tumour DNA ($N=77$).

DNA extraction from EDTA blood, standard PCR and direct sequencing with ABI PRISM 3100 Avant (Big Dye v1.1) was performed according to standard protocols. For computer analysis of the sequences the software Mutation surveyor 3.1 (SoftGenetics, State College, PA, USA) was used.

Mutation pre-screening of *MUTYH* was performed by direct sequencing of exon 6–7 and 12–13 including the hotspot mutations p.(Tyr179Cys) and p.(Gly396Asp) after PCR amplification using Ampli-Taq Gold (Life Technologies, Carlsbad, CA, USA) and digestion with Exo-SAP kit (Affymetrix, Santa Clara, CA, USA). At least one of these two hotspot mutations is found in 82–98% of all MAP cases in Caucasian, Canadian, American and Australian populations[12,22] and in our own unpublished cohort. In a further step, sequencing has been completed for all 16 coding exons and flanking regions of *MUTYH* (GenBank: NM_001128425.1; NG_008189.1) for 73 patients, 12 patients could not be analysed completely due to lack of DNA. In addition, deletion/duplication screening of all *MUTYH* exons was carried out by multiplex ligation-dependent probe amplification (MLPA) with MLPA kit P378-A1 Lot0910 (MRC Holland, Amsterdam, The Netherlands) and analysed with GeneScan 3.7 software (Life Technologies).

Owing to the accepted nomenclature, which uses the longest *MUTYH*-transcript (NM_001128425.1) as a reference, nucleotide and amino acid numbering after nucleotide position 157 (amino acid 53) may differ from other publications by up to 42 nucleotides (14 amino acids). The frequent mutations p.(Tyr179Cys) in exon 7 and p.(Gly396Asp) in exon 13, previously reported as p.(Tyr165Cys) and p.(Gly382Asp) correspond to p.(Tyr176Cys) and p.(Gly393Asp) using other transcripts of *MUTYH*.

Germline *MUTYH* mutations were submitted to the LOVD database (http://chromium.liacs.nl/LOVD2/colon_cancer/home.php?select_db=MUTYH).

Tumour DNAs from formalin-fixed paraffin-embedded tissues available from the biallelic mutation carrier were sequenced for somatic MAP-specific G:C>T:A transversions in *MSH2* (NM_000251.2; NG_007110.2), *KRAS* exon 2 and 3 (NM_004985.3; NG_007524.1), and *BRAF* exon 15 (NM_004333.3).

RESULTS

Biallelic *MUTYH* germline mutations lead to biallelic somatic transversions in the MMR gene *MSH2*

In one patient displaying loss of MSH2 and MSH6 on IHC of his sebaceous gland carcinoma, we detected homozygosity for the pathogenic *MUTYH* germline mutation c.536A>G; p.(Tyr179Cys) in exon 7. A deletion of the second allele was excluded by MLPA. The patient was diagnosed with two synchronous rectal adenocarcinomas T2 N0 G2 and several tubulo-villous adenomas at age 55, an urothelial bladder carcinoma at age 65, a sebaceous gland carcinoma and a sebaceous gland hypertrophy at age 66 years. The rectal cancer and the urothelial bladder cancer were microsatellite stable and had normal MMR protein staining by IHC.

LS was suspected originally due to the patient's personal history in addition to his family history of a brother with caecal CRC T3 N2 G3 at age 56 years and two affected cousins (CRC at age 50; breast cancer at age 41 years) (Figure 1). Biallelic copies of the *MUTYH* mutation c.536A>G; p.(Tyr179Cys) were also found in DNA extracted from the paraffin-embedded normal tissue of the deceased brother. DNA of further family members was not available for genetic testing to assess the *MUTYH* mutation status.

Chronic lymphadenitis as a typical histological feature of LS was described in the CRC of the index patient and his brother. Information on Crohn's-like lymphocytic response and tumour-infiltrating lymphocytes reported in BER- and MMR-deficient tumours[16,23] was not given.

Tumour analyses

Tumour sequencing of the index patient's sebaceous gland carcinoma identified two somatic pathogenic transversion mutations in the *MSH2* gene (c.2101G>T; p.(Glu701*) in exon 13 and c.2554G>T; p.(Glu852*) in exon 15, Supplementary Figure 1A) explaining the development of MSI-H in tumourigenes is and loss of the MSH2 and MSH6 proteins in this tumour. The phase of the *MSH2* mutations in exon 13 and 15 in tumour DNA could not be determined, as we did not find heterozygous SNPs in the neighbouring genomic regions in blood DNA of the patient and the amplification of 4.6 kb from exon 13 to 15 in tumour DNA for subcloning was not successful due to the poor DNA quality. The other two tumours, a rectal carcinoma and an urothelial carcinoma that were microsatellite stable, both had a heterozygous somatic *KRAS* hotspot mutation c.34G>T; p.(Gly12Cys) transversion in exon 2 (Supplementary Figure 1B).

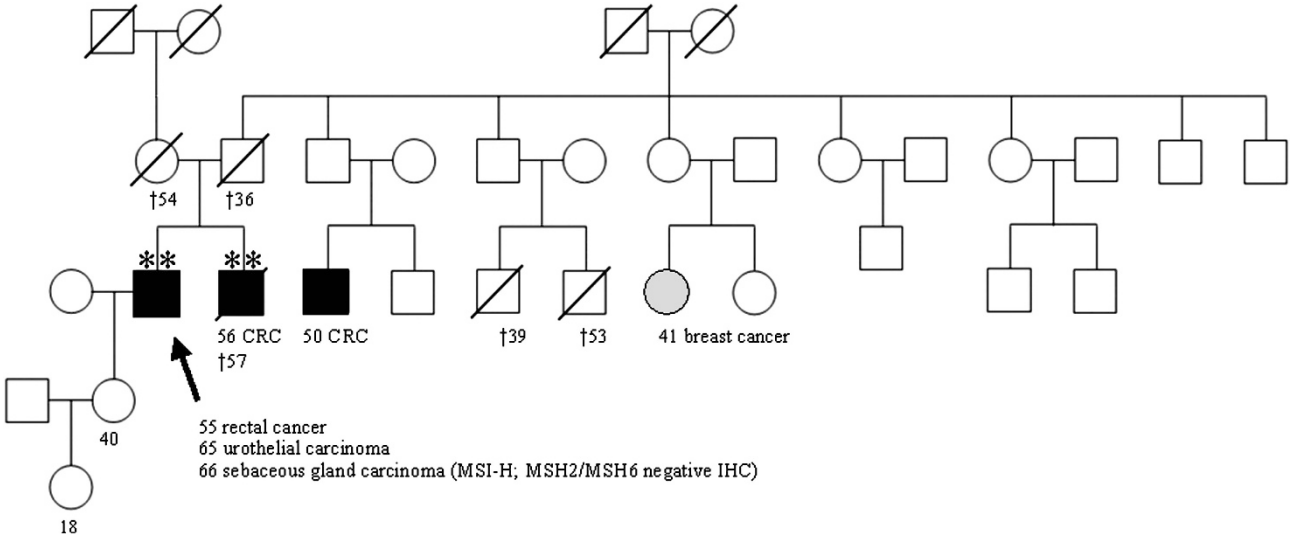

Figure 1 Pedigree of the family with the affected individual III:2 and his affected brother III:3 both bearing a homozygous *MUTYH* p.(Tyr179Cys) mutation (**). The sebaceous gland carcinoma revealed an MSI-H phenotype with loss of MSH2/MSH6 expression. Germline mutation analysis of MMR genes was negative. No genomic DNA was available from other affected family members with CRC as well as no detailed information concerning the parents.

Monoallelic *MUTYH* germline mutations in LS suspected individuals and controls

None of the remaining patients analysed were found to have *MUTYH* mutations. The control cohort of 403 CRC patients revealed five patients with monoallelic heterozygous hotspot mutations (two in exon 7 and three in exon 13). No second *MUTYH* mutation was found in these patients.

DISCUSSION

Biallelic *MUTYH* mutations impair the BER process and can result in somatic mutational inactivation of both MMR-alleles mimicking LS by displaying a MMR-deficient tumour. To our knowledge, in the literature six MAP patients with MSI-H tumours were described,[15,16] three of them with an IHC MLH1-deficiency in their MSI-H CRC.[10–12] The loss of MLH1 staining in those three MAP tumours with MSI-H was caused by a somatic MAP-specific mutation in *MLH1* together with a second undetectable defect,[10] whereas biallelic methylation of the *MLH1* promoter was causing the MLH1-deficiency in the other MAP patient.[11]

Here we present a MAP-patient, in which BER deficiency was associated with the development of two somatic MAP-specific G>T transversion mutations in the *MSH2* gene in the tumour resulting in MMR deficiency, MSI-H and a loss of MSH2 and MSH6 expression in the patient's sebaceous gland carcinoma. This patient's CRC and urinary bladder carcinoma revealed the MAP-specific *KRAS* G>T transversion mutations.[14] The somatic G>T transversions in *MSH2* or *KRAS*, respectively, in three different tumours of the patient are very likely to be due to the germline BER defect.[7,8]

Interestingly, three further cancer diagnoses were reported in this family including CRC and breast cancer, which are compatible with a recessive mode of inheritance and the clinical phenotype of MAP.[23–25] Biallelic *MUTYH* mutations were detected in the brother and might also have induced the tumourigenesis in the cousins (not investigated). Heterozygous *MUTYH* mutations were not identified in the remaining 84 index patients, for which somatic mutations or further germline pathomechanisms, for example, large genomic rearrangements such as inversions in the MMR genes have to be considered.[3,26–28] In the control cohort the *MUTYH* mutation frequency of 1.24% was comparable to the frequencies of 0%–4.8% reported in the general population.[12,22,29]

As MAP can manifest in an extremely variable phenotype, *MUTYH* mutation analysis should also be considered in patients with a low number of adenomas, early-onset or synchronous or metachronous CRC and in patients with MSS tumours.[30,31] With an incidence of only 1.18% in MMR-deficient CRC patients with typical histological features of LS and several adenomas MAP can mimic LS. These patients need different surveillance and due to the different pattern of inheritance a different risk annotation for family members.[32] We suggest to perform *MUTYH* genetic testing in these unresolved MSI-H cases with additional adenomas either in the two fragments harbouring the common mutations or in the course of next-generation sequencing cancer kits including the MMR genes, *APC* and *MUTYH* besides others.[18]

We reported for the first time a MAP patient initially suspected of LS, in which two MAP-specific somatic *MSH2* mutations explained the IHC loss of MSH2/MSH6 in his MSI-H tumour. MAP might be a differential diagnosis for CRC patients with MSI-H tumours and several adenomas.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the German Cancer Aid (Deutsche Krebshilfe grant 110780) and the Wilhelm Sander-Stiftung grant 2012.081.1 as well as grants P30 16058 and P01 CA124570 from the National Cancer Institute, USA, for support of this work. We also thank all patients for their participation in this study, as well as their respective doctors for contributing clinical information.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)