Molecular pathology of colorectal cancer predisposing syndromes

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CHAPTER 6

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The natural history of a combined defect in *MSH6* and *MUTYH* in a HNPCC family

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Abstract In the inherited syndromes, *MUTYH*-associated polyposis (MAP) and hereditary nonpolyposis colorectal cancer (HNPCC), somatic mutations occur due to loss of the caretaker function that base-repair (BER) and mismatch repair (MMR) genes have, respectively. Recently, we identified a large branch from a *MSH6* HNPCC family in which 19 family members are heterozygous or compound heterozygous for *MUTYH* germ line mutations. *MSH6/MUTYH* heterozygote mutation carriers display a predominant HNPCC molecular tumour phenotype, with microsatellite instability and underrepresentation of G>T transversions. A single unique patient is carrier of the *MSH6* germline mutation and is compound heterozygote for *MUTYH*. Unexpectedly, this patient has an extremely mild clinical phenotype with sofar only few adenomas at age 56. Four out of five adenomas show characteristic G>T transversions in *APC* and/or *KRAS2*, as seen in *MUTYH* associated polyposis. No second hit of *MSH6* is apparent in any of the adenomas, due to retained MSH6 nuclear expression and a lack of microsatellite instability. Although this concerns only one case, we argue that the chance to find an additional one is extremely small and currently a mouse model with this genotype combination is not available. Moreover, the patients brother who is also compound heterozygous for *MUTYH* but lacks the *MSH6* germline mutation presented with a full blown polyposis coli. In conclusion, these data would support the notion that abrogation of both MSH6 DNA mismatch repair and base repair might be mutually exclusive in humans.

Keywords Base excision repair · Colorectal cancer · HNPCC · Mismatch repair · *MUTYH* · Urinary tract

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Abbreviations

BER	Base excision repair
MMR	Mismatch repair
MAP	*MUTYH*-associated polyposis
HNPCC	Hereditary nonpolyposis colorectal cancer
8-oxoG	8-oxo-guanine
CRC	Colorectal cancer
MCR	Mutation cluster region
MSI	Microsatellite instability
LOH	Loss of heterozygosity
IHC	Immunohistochemistry
MSS	Microsatellite stable

Introduction

Somatic genetic alterations direct the development of colorectal malignancies. In the majority of cases, such mutations occur in an apparently sporadic context.

In a group of distinct inherited syndromes however, many somatic mutations occur as a consequence of the loss of caretaker function of the base-repair (BER) or mismatch repair (MMR) systems in, *MUTYH*-associated polyposis (MAP) and hereditary nonpolyposis colorectal cancer (HNPCC), respectively [1, 2]. Loss of MMR function is also seen in 15% of sporadic colorectal cancer (CRC) due to promoter methylation [3].

BER is a multi-step process that repairs frequently occurring 8-oxo-guanine (8-oxoG) DNA lesions [4]. Until recently inherited deficiencies in the BER pathway had not been causally linked with any human genetic disorder. However, in 2002 it was discovered that biallelic mutations in *MUTYH* (formerly *MYH*) lead to the autosomal recessive syndrome exerting adenomatous colorectal polyposis and CRC [1]. The MMR pathway consists of a highly conserved set of proteins in humans, which are primarily responsible for the post-replicative correction of nucleotide mispairs and extra-helical loops. The MMR system includes *hMLH1* and *hPMS2*, which form a heterodimer (hMutLα) and *hMSH2* and *hMSH6*, forming the hMutSα-heterodimer. hMutSα has been shown to bind specifically to G*T DNA mismatches, other base–base DNA mismatches and to 1-, 2- or 3 nucleotide insertion–deletion loops [5]. Germline mutations in one of the MMR genes underlie the autosomal dominant HNPCC syndrome.

Due to the reduced ability of mutant *MUTYH* to recognize and repair A/8-oxoG mismatches, in tumours of MAP patients specific G:C>T:A somatic transversions can be found in genes such as *APC* and *KRAS2* with an incidence of up to 40 and 60%, respectively [6]. In *APC* the G>T transversions appear to have a preference for G bases in GAA sequences whereas in *KRAS2* a preferential GGT>TGT [c.34G>T, p.Gly12Cys] transition of codon 12 can be found [1, 7].

In MMR deficiency apart from the frameshift mutations in repetitive DNA stretches, under representation of G>T transversions and possibly preferential G>A somatic alterations in *APC* and *KRAS2* are found, this in contrast to the G>T transversions in BER deficiency [8, 9].

Although *MUTYH* is the most important cellular player in the removal of adenine in an A/8-oxoG mismatch, also MMR has been shown to play a role since *MSH2* and *MSH6* are activated upon recognition of 8-oxoG [10, 11]. Moreover, it was recently demonstrated that amino acid residues 232–254 of *MUTYH* interact with MutsSα via *MSH6* and this interaction stimulates the glycosylase activities of *MUTYH* [12].

In order to determine the effect of different combinations of BER and MMR defects we studied the branch of a HNPCC family in which *MSH6* and *MUTYH* germline mutations co-segregate [13]. Nineteen family members are heterozygous or compound heterozygous for [c.494A>G, p.Tyr165Cys] and/or [c.1145G>A, p.Gly382Asp] in *MUTYH*, 11 also carry a pathogenic *MSH6* [c.1784del T, p.Leu595fs] germline mutation. We analysed the somatic mutation spectrum of *APC* and *KRAS2*, microsatellite instability including *MUTYH/OGG1* repeats, MSH2/MSH6 protein expression and studied the clinical phenotype.

Materials and methods

Patients

We studied a branch of a Dutch HNPCC family in which *MSH6* and *MUTYH* germline mutations co-segregate (Fig. 1, Table 1) [12]. Cases were analysed following the medical ethical guidelines described in the Code Proper Secondary Use of Human Tissue established by the Dutch Federation of Medical Sciences; http://www.fmwv.nl/gedragscode/goedgebruik/code.

Germline mutation analysis

Mutation analysis was performed as described for *MSH6* and *MUTYH* [13, 14]. For further details see http://www.lumc.nl/4080/DNA/MSH6.html and http://www.lumc.nl/4080/DNA/MUTYH.html.

DNA isolation

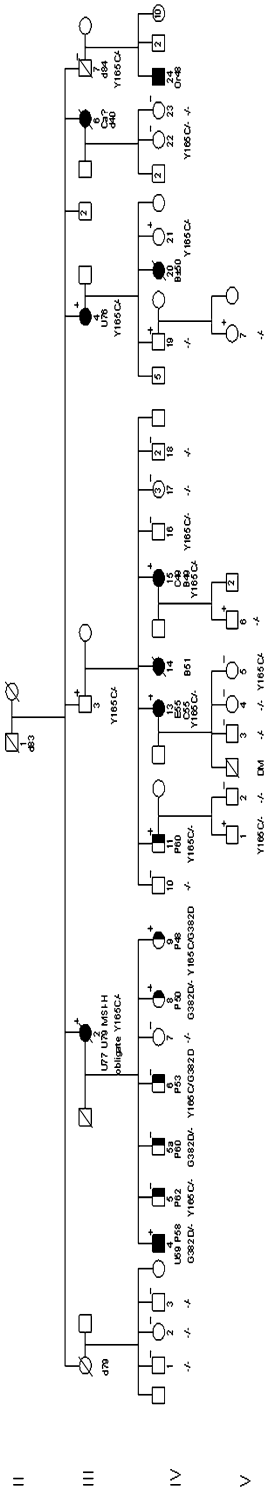
From nine patients 18 tumours were collected. Genomic DNA of normal colon and colorectal tumour tissue was extracted from paraffin embedded material as described [15].

Microsatellite instability (MSI) analysis

Microsatellite analysis was performed as described [15].

APC and *KRAS2* somatic mutation analysis

Samples were screened for the presence of mutations in the Mutation Cluster Region (MCR) codons 1286–1513 of *APC* and for mutations in codon 12 and 13



◀ **Fig. 1** Pedigree of a HNPCC family in which *MSH6* and *MUTYH* germline mutations co-segregate. *Abbreviations*: C, colorectal cancer; E, endometrial cancer; U, urinary tract cancer; P, polyp; B, breast cancer; Or, Oral squamous cell carcinoma; DM, diabetes mellitus; +, carrier of *MSH6* [c.1784delT, p.Leu595fs] mutation, –, wt *MSH6*, –/–, *MUTYH* mutation negative. *Note*: The pedigree is slightly different depicted than the one previously published because of some minor intentional changes in the latter (i.e. the number of unaffected siblings and one patient with C32 belonging to the other branch) for privacy reasons. For further questions the corresponding author can be contacted [12]

of *KRAS2*, by sequencing analysis as described [16]. For detection of known HNPCC associated somatic mutations outside the MCR of *APC*, eight different primersets for eleven target sequences were used (Table 2) [9]. PCR is performed under standard conditions (33 cycles with an annealing temperature of 60°C) PCR products were sequenced at the Leiden Genome Technology Center (LGTC; http://www.lgtc.nl) and analysed with the Mutation Surveyor software package (Softgenetics, State College, PA).

Loss of heterozygosity (LOH)

Analysis was done by direct sequencing as described [17]. PCR was performed on DNA from paired tumour and normal tissue under standard conditions with primer sets for [Tyr165Cys] and [Gly382Asp] as described in Table 2.

Microsatellite analysis of *MUTYH/OGG1*

Analysis of repeats in *MUTYH* and *OGG1* was done by direct sequencing. PCR was performed under standard conditions with primer sets for 2 (A)5 repeats in the coding region of *MUTYH* of which one is known to be located in the binding site of *PCNA* [18]. In the coding region of *OGG1*, two repeats were tested; a (C)5 and a (T)5 repeat, primers described in Table 2.

Immunohistochemistry (IHC) of MSH6 and MSH2

Staining of the MMR proteins was done as described [15].

Results

The clinical phenotype of the HNPCC family (Fig. 1) in which *MSH6* and *MUTYH* germline mutations co-segregate is described in Table 1 [12]. The molecular characteristics are summarized in Table 3.

Table 1 (Pre) malignant tumours in the extended HNPCC family in which *MSH6* and *MUTYH* germline mutations co-segregate

Patient	Tumour	Age at diagnosis	Age 12-2005	*MSH6* mutation	*MUTYH* mutation
III.2	Transitional cell carcinoma right renal pelvis and transitional cell carcinoma left ureter	77	d89	+[a]	[Tyr165Cys]+[=][a]
III.3	None	79	FU ends at 86	+	[Tyr165Cys]+[=]
III.4	Transitional cell carcinoma renal pelvis	76	93	+	[Tyr165Cys]+[=]
III.6	Anamnestic carcinoma	40	d40	na	na
III.7	Unknown		d84	wt	[Tyr165Cys]+[=]
IV.4	Transitional cell carcinoma ureter and anamnestic 1 polyp of the colon (adenomatous)	59	66	+	[–]+[Gly382Asp]
IV.5	4 Polyps left-sided (adenomatous and hyperplastic)	62	69	wt	[Tyr165Cys]+[=]
IV.5a	1 Hyperplastic polyp	60	68	wt	[=]+[Gly382Asp]
IV.6	Polyposis coli; > 100 adenomatous polyps	53	61	wt	[Tyr165Cys] + [Gly382Asp]
IV.8	2 Polyps (adenomatous and hyperplastic polyp)	50	58	+	[–]+[Gly382Asp]
IV.9	5 Adenomas	48	56	+	[Tyr165Cys]+[Gly382Asp]
IV.11	Tubulovillous adenoma	60	66	+	[Tyr165Cys]+[=]
IV.13	Endometrial carcinoma and rectal carcinoma	55	65	+	[Tyr165Cys]+[=]
IV.14	Breast carcinoma (ductal, invasive)	51	d52 (±)	na	na
IV.15	Breast carcinoma and colon carcinoma	49	55	+	[Tyr165Cys]+[=]
IV.16	None		61	wt	[Tyr165Cys]+[=]
IV.19	None		59	+	wt
IV.20	Breast carcinoma	±50	d50 (±)	na	na
IV.21	None		58	+	[Tyr165Cys]+[=]
IV.22	None		48	wt	[Tyr165Cys]+[=]
IV.24	Oral squamous cell carcinoma	48	FU ends at 48	na	na
V.1	None		34	+	[Tyr165Cys]+[=]
V.5	None		32	wt	[Tyr165Cys]+[=]
V.6	None		30	+	wt
V.7	None		30	+	wt

Abbreviations: d, death; +, carrier of *MSH6* [c.1784delT, p.Leu595fs] mutation; FU, follow up; na, not analysed; wt, wild type

[a] Obligate carrier

Table 2 Primers used for HNPCC related *APC* mutation screening, *MUTYH* LOH analysis and MSI analysis in *MUTYH* and *OGG1*

Primer	*APC* nucleotide	5′–3′ forward	5′–3′ reverse	Annealing temperature
Ca6 and Ca18	731–786	gcaaataggcctgcgaagta	gatgagatgccttgggactt	58
Co8/K39 and Cx7	780–860	cccaaggcatctcatcgtag	tagaccaattccgcgttctc	58
K10	877–930	tttgcagatctccaccactg	tatgggcagcagagcttctt	58
Co86 and Co39	923–986	aagaagctctgctgcccata	ggattcaatcgagggtttca	58
Cx10	1901–1966	acctccaaccaacaatcagc	tgagaaaagcaaaccggagt	58
22–18	1525–1585	atgcctccagttcaggaaaa	tgttggcatggcagaaataa	58
Co88	1768–1828	gaaaaagaaaccaacttcacca	tgggagcttatcattgaagacc	58
Co10	1093–1160	tggacagcaggaatgtgttt	ttggtctctcttcttcttcatgc	58
MUTYH [Tyr165Cys]		cccacaggaggtgaatcaact	gttcctaccctctgccatc	60
MUTYH [Gly328Asp]		ggcagtggcatgagtaacaag	cttgcgctgaagctgctct	60
MUTYH (A)5 repeat (*PCNA* binding site)		ctacaaggcctccctccttc	ctgcactgttgaggctgtgt	60
MUTYH (A)5 repeat		aagtatatgggctggccttg	caacaaagacaacaaaggtagtgc	60
OGG1 (C)5 repeat		aaaggtggctgactgcatct	tttcctcacccagttccttg	60
OGG1 (T)5 repeat		gggtcagataacttagtctcatcactt	aggaaacctagggaggacacc	60

Heterozygous *MUTYH* [Tyr165Cys] mutation carriers with a wild type *MSH6* germline status

Patient IV.5 developed four colon polyps, whereas three other family members; IV.16, IV.22 and V.5 show no abnormalities. From patient III.7 the tumour status is unknown. Two polyps (one hyperplastic and one adenoma) from patient (IV.5), displayed a microsatellite stable (MSS) phenotype and expressed MSH6 and MSH2. The adenoma showed a [c.35G>A,

Table 3 Clinical information and molecular characteristics

Category	Patient number	Age of diagnosis	Age 12-2005	Gender	*MSH6* germline mutation[a]	*MUTYH* germline amino acid change	LOH MUTYH	MSI	MSI repeat *MUTYH/ OGG1*	*APC* somatic mutation	*APC* amino acid change	*KRAS2* somatic mutation	*KRAS2* amino acid change	MSH2 staining	MSH6 staining	Tumour
A	IV.5	62	69	M	wt	[p.Tyr165Cys] +[=]	no	S	no	wt	wt	wt	wt	+	+	Sigmoid HP
A	IV.5	62				[p.Tyr165Cys] +[=]	no	S	no	wt	wt	[c.35G>A] +[=]	[p.Gly12Asp] +[=]	+	+	Rectal, tub. vill. ad. LG
B	IV.13	56	65	F	+	[p.Tyr165Cys] +[=]	no	H	no	wt[b]	wt	wt	wt	0	na	Rectal ca.
B	IV.13	56					no	H	no	wt[b]	wt	wt	wt	+	na	Endometrial ca.
B	IV.15	49	55	F	+	[p.Tyr165Cys] +[=]	no	L	no	[c.4487_4499del CTCCAGA-TGGATT]+[=][c]	[p.Thr1496fs] +[=]	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	0	Colon ca. left
B	IV.15	49				[p.Tyr165Cys] +[=]	no	S	no	[c.4487_4499del CTCCAGA-TGGATT]+[=][c]	[p.Thr1496fs] +[=]	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	0	Colon ad. left[d]
B	IV.15	49					no	H	no	wt[c]	wt	wt	wt	+	0	Breast ca. left
B	III.4	76	93	F	+	[p.Tyr165Cys] +[=]	no	L	no	na	na	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	0	Renal pelvis, pap. transitional cell ca., Gr III
B	III.2	77	d89	F	+[e]	[p.Tyr165Cys] +[=][e]	nma	H	nma	nma	nma	wt	wt	+	0	Ureter left, pap. transitional cell ca., GrII
B	III.2	79				[p.Tyr165Cys] +[=][e]	nma	H	nma	wt	wt	wt	wt	na	na	Renal pelvis right, transitional cell ca. GrIII

Table 3 continued

Category	Patient number	Age of diagnosis	Age 12-2005	Gender	*MSH6* germline mutation[a]	*MUTYH* germline amino acid change	LOH MUTYH	MSI	MSI repeat *MUTYH/OGG1*	*APC* somatic mutation	*APC* amino acid change	*KRAS2* somatic mutation	*KRAS2* amino acid change	MSH2 staining	MSH6 staining	Tumour
C	IV.4	59	66	M	+	[=]+[p.Gly382Asp]	no	H	no	wt	wt	wt	wt	+	0	Distal ureter right, transitional cell ca. GRII
C	IV.8	50	58	F	+	[=]+[p.Gly382Asp]	no	S	no	[c.4475_4476-delCC]+[=]	[p.Ala1492fs] +[=]	wt	wt	+	+	Colon tub. ad. LG
D	IV.6	53	61	M	wt	[p.Tyr165Cys] +[p.Gly382Asp]	no	S	no	wt	wt	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	+	Polyposis coli with HG
E	IV.9	48	56	F	+	[p.Tyr165Cys] +[p.Gly382Asp]	no	S	no	wt	wt	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	+	Sigmoid ad. LG
E	IV.9	54					no	S	no	[c.4612G>T] +[=]	[p.Glu1538X] +[=]	wt	wt	+	+	Rectal villous ad. HG
E	IV.9	54					no	S	no	[c.4618G>T] +[=]	[p.Glu1540X] +[=]	[c.34G>T] +[=]	[p.Gly12Cys] +[=]	+	+	Caecum villous ad. LG
E	IV.9	54					no	S	no	[c.4612G>T] +[=]	[p.Glu1538X] +[=]	wt	wt	+	+	Rectal villous ad. LG
E	IV.9	54					no	S	no	wt	wt	[c.38G>A] +[=]	[p.Gly13Asp] +[=]	+	+	Caecum villous ad. LG

Abbreviations: M, male; F, female; na, not analysed; nma, no material available; wt, wild type; ad, adenoma; ca, carcinoma; HP, hyperplastic; HG, high grade dysplastic; LG, low grade dysplastic

Note: Tumours were categorized based different on germline mutation combinations. Category A; heterozygous *MUTYH* [Tyr165Cys] mutation carrier with wild type *MSH6* germline status. Category B; heterozygous *MUTYH* [Tyr165Cys] mutation carriers with *MSH6* [c.1784delT, p.Leu596fs] germline mutation. Category C; heterozygous *MUTYH* [Gly382Asp] mutation carriers with *MSH6* [c.1784delT, p.Leu596fs] germline mutation. Category D; compound heterozygous *MUTYH* [Tyr165Cys, Gly382Asp] mutation carrier with wild type *MSH6* germline status. Category E; compound heterozygous *MUTYH* [Tyr165Cys, Gly382Asp] mutation carrier with *MSH6* [c.1784delT, p.Leu596fs] germline mutation

[a] *MSH6* [c.1784delT, p.Leu595fs] mutation

[b] SNP rs 41115 heterozygote [c.4479G>A]
+[=]

[c] SNP rs 41115 homozygote [c.4479G>A]+[c.4479G>A]

[d] Precursor adenoma next to carcinoma

[e] Obligate carrier

p.Gly12Asp] *KRAS2* mutation. No *APC* somatic mutations were detected (Table 3, category A).

Heterozygous *MUTYH* [Tyr165Cys] mutation carriers with a *MSH6* [c.1784del T, p.Leu595fs] germline status

Five of eight mutation carriers, showed a diverse spectrum of tumour types (Table 3) including colon adenomas (IV.15, IV.11), a colon and a breast carcinoma (IV.15), a rectum and a endometrium carcinoma (IV.13), two papillary transitional cell carcinomas of the renal pelvis (III.4, III.2) and one of the ureter (III.2). Three family members V.1, IV.21, and III.3 did so far not present with any HNPCC or MAP associated lesion. Five tumours (a rectum, endometrium, breast renal pelvis papillary transitional cell and ureter papillary transitional cell carcinoma) of three patients (IV.13, IV.15, III.2) are MSI-High with diminished or abrogated MSH2 staining or abrogation of MSH6 staining if tested. No *KRAS2* and *APC* somatic mutation was identified in three of the five tumours. Two tumours however, of patients IV.15 and III.4; a colon carcinoma including its precursor adenoma and a papillary transitional cell carcinoma, showed limited or no instability, with minor shifts of *BAT25* and *BAT40*. Nonetheless MSH6 staining was abrogated. Surprisingly only in these latter tumours the typical, MAP associated [c.34G>T, p.Gly12Cys] *KRAS2* mutation was found. In both the colon carcinoma and its precursor adenoma, a somatic deletion of 13 nucleotides in *APC* was identified (Table 3, category B).

Heterozygous *MUTYH* [Gly382Asp] mutation carrier with a wild type *MSH6* germline status

One patient (IV.5a) presented with one hyperplastic polyp, not further molecular characterized.

Heterozygous *MUTYH* [Gly382Asp] mutation carriers with a *MSH6* [c.1784del T, p.Leu595fs] germline status

Patient IV.4 showed a transitional cell carcinoma, patient IV.8 showed one low-grade dysplastic adenoma. The papillary transitional cell carcinoma of IV.4 tested MSI-High with abrogation of MSH6 expression. No mutations in *KRAS2* or *APC* were identified. A low-grade dysplastic adenoma from IV.8 showed a MSS phenotype with retained MSH6 staining. No somatic mutation in *KRAS2* was identified. In *APC* a [c.4475_4476delCC, p.Ala1492fs] mutation was found (Table 3, category C).

Compound heterozygous *MUTYH* [Tyr165Cys] + [Gly382Asp] mutation carrier with a wild type *MSH6* germline status

Patient IV.6 showed a full-blown polyposis phenotype of colorectal adenomas. In one adenoma the MAP characteristic *KRAS2* mutation; [c.34G>T, p.Gly12Cys] was identified. No somatic mutations were identified in the tested areas of *APC*. As expected, the specimen had a MSS phenotype and showed normal protein expression of MSH2 and MSH6 (Table 3, category D).

Compound heterozygous *MUTYH* [Tyr165Cys,Gly382Asp] mutation carrier with a *MSH6* [c.1784del T, p.Leu595fs] germline status

The phenotype of patient IV.9 with the triple mutations is remarkably mild. The patient to date developed five pathologically verified colon adenomas (Table 3) only one with high-grade dysplasia, the other four are low-grade dysplastic (minimal mucosal changes have been coagulated during endoscopy). All five tumours from patient (IV.9) showed a MSS phenotype and retained nuclear expression of MSH6, suggesting the absence of a second hit in *MSH6*. Two rectum adenomas lack *KRAS2* mutations but carry an *APC* [c.4612G>T, p.Glu1538X] somatic mutation (Table 3, category E). One caecum adenoma carried the *MUTYH* associated somatic *KRAS2* [c.34G>T, p.Gly12Cys] mutation. This specimen also showed a [c.4618G>T, p.Glu1540X] mutation in *APC*. A second caecum adenoma showed a *KRAS2* [c.38G>A, p.Gly13Asp] mutation and no *APC* somatic mutations (Table 3, category E). Although the [Gly13Asp] alteration is found in a low frequency in our *MUTYH* family cohort (data not shown), this mutation represents the most frequent somatic mutation found in *KRAS2* in HNPCC patients with a MMR mutation [8].

In all tested specimens neither LOH of *MUTYH* nor microsatellite instability, in the tested repeats in *MUTYH* and *OGG1*, was detected (Table 3).

Discussion

We identified a branch from a previously described Dutch HNPCC family where *MSH6* and *MUTYH* germline mutations co-segregate. In order to determine the effect of different combinations of BER and MMR defects we analysed somatic mutation spectra of *APC* and *KRAS2*, microsatellite instability including *MUTYH/OGG1* repeats, MSH2/MSH6 protein expression and studied the clinical phenotype.

In this family of the 34 *MSH6* [c.1784del T, p.Leu595fs] mutation carriers 11 also carry a *MUTYH* mutation, of which one bi-allelic [11]. The remaining 23 individuals lack *MUTYH* mutations, either tested or obligatory negative (not taking in account the possibility of a "new" *MUTYH* mutation in this branch, as *MUTYH* mutations are found in 1–2% of the general population) [1, 19].

In individuals with a combined defect in *MSH6* and *MUTYH* (heterozygous) a higher incidence of urothelial cancers was found compared to a *MSH6* defect alone (three out of 10 versus none out of 23, $P = 0.022$ Fisher exact), suggesting that a single *MUTYH* mutation modifies the risk for developing for urothelial cancers in *MSH6* mutation carriers.

A predominant HNPCC molecular phenotype was observed in tumours from patients heterozygous for *MUTYH* and *MSH6* defects, which suggest that a second inactivating somatic hit on *MSH6* took place and MMR deficiency is the leading cause of tumourigenesis in these patients, although in two out of nine tumours the *MUTYH* characteristic [c.34G>T] somatic transversion in *KRAS2* was observed. Microsatellite instability seemed less extensive in the latter cases, with MSH6 expression abrogated. Remarkable is that in one of these two (including the precursor adenoma) a genomic 13 bp *APC* deletion was found not typical for HNPCC. In cases where no *APC* alteration was identified it should be noted that only the major cluster region for somatic mutations in *APC* was screened including published hot spots for specific somatic HNPCC mutations.

Out of eight *MSH6* and *MUTYH* (heterozygous [Tyr165Cys]) mutation carriers two present with late onset tumours (III.2, III.4). The age of onset in three other cases (IV.15, IV.13, IV.11) is lower with five different tumours (three colon tumours) at an age range of 49–60, the remaining three cases did so far not present with tumours (III.3, IV.21, V.1). Croitoru et al. [19] concluded that heterozygote mutation carriers for [Tyr165Cys] have an increased risk (although not significant) for colorectal cancer (CRC) with an odds ratio of 2.1.

The relative mild clinical phenotype of patient IV.9, who is compound heterozygous for *MUTYH* [Tyr165Cys] and [Gly382Asp] and also carrying the *MSH6* germline mutation might be explained, at least in part, by a selection against MSH6 mismatch repair deficient cells. Such is in line with Kambara et al. [20] who suggested that BER and DNA MMR pathways are mutually exclusive implying that cells with abrogation of both pathways are not viable and undergo apoptosis.

The molecular phenotype of the tumours of this patient occur most likely as a result of *MUTYH* dysfunction, while no mismatch repair deficiency seems evident despite the presence of a germline *MSH6* defect. These results are remarkable in view with the natural mutation rate in cells, estimated at 1×10^{-6} cells per gene, per cell division. There are 1×10^{10} epithelial cells in the colon of which potentially one percent is dividing. That would imply that every cell division 10^2 intestinal cells are at risk for a second hit in *MSH6.* In *MUTYH* compound heterozygotes the mutation rate is increased by a factor 100 (10^4 cells are then at risk for a second mutational hit in *MSH6*). So far this does not appear to be the case in the triple mutation case (IV.9). Unfortunately a mouse model with this genotype combination is not available.

Although the number of cases is low, a striking potentiating effect of a combined heterozygote *MSH6* and *MUTYH* mutation status is not evident except perhaps for urothelial tumours. However, recently, a *MUTYH* mutation combined with non-pathogenic (or low penetrant) *MSH6* missense mutation is reported to be associated with an increased cancer risk for colorectal cancer [21]. Other combined defects of *APC* and *MLH1 or MSH2* have been reported to accelerate tumourigenesis (summarized in [22]). The finding of an unexpectedly mild clinical phenotype in an individual with combined *MUTYH* deficiency and a heterozygote pathogenic *MSH6* germline mutation should be seen with caution considering the variable expression of MAP and HNPCC in general. The molecular characteristics of the tumours of this patient studied, however, point to selection against MSH6 abrogation.

References

1. Al Tassan N, Chmiel NH, Maynard J et al (2002) Inherited variants of MYH associated with somatic G:C → T:A mutations in colorectal tumours. Nat Genet 30:227–232
2. Lynch HT, Smyrk T (1996) Hereditary nonpolyposis colorectal cancer; an updated review. Cancer 78:1149–1167
3. Cunningham JM, Christensen ER, Tester DJ (1998) Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 58:3455–3460
4. Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362:709–715
5. Peltomaki P (2001) Deficient DNA mismatch repair; a common etiologic factor for colon cancer. Hum Mol Genet 10:735–740
6. Lipton L, Halford SE, Johnson V et al (2003) Carcinogenesis in MYH-associated polyposis follows a distinct genetic pathway. Cancer Res 63:7595–7599
7. Jones S, Emmerson P, Maynard J et al (2002) Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C → T:A mutations. Hum Mol Genet 11:2961–2967

8. Oliveira C, Westra JL, Arango D et al (2004) Distinct patterns of KRAS mutations in colorectal carcinomas according to germline mismatch repair defects and hMLH1 methylation status. Hum Mol Genet 13:2303–2311
9. Huang J, Papadopoulos N, McKinley AJ et al (1996) APC mutations in colorectal tumours with mismatch repair deficiency. Proc Natl Acad Sci USA 93:9049–9054
10. Mazurek A, Berardini M, Fishel R (2002) Activation of human MutS homologs by 8-oxo-guanine DNA damage. J Biol Chem 277:8260–8266
11. Gu YS, Parker A, Wilson TM, Bai HB, Chang DY, Lu AL (2002) Human MutY homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/human MutS homolog 6. J Biol Chem 277:11135–11142
12. Wagner A, Hendriks Y, Meijers-Heijboer EJ et al (2001) Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree. J Med Genet 38:318–322
13. Wijnen J, de Leeuw W, Vasen H et al (1999) Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 23:142–144
14. Nielsen M, Franken PF, Reinards THCM et al (2005) Multiplicity in polyp count and extracolonic manifestations in 40 Dutch patients with MYH associated polyposis coli (MAP). J Med Genet 42:e54
15. De Jong AE, van Puijenbroek M, Hendriks Y et al (2004) Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. Clin Cancer Res 10:972–980
16. Nielsen M, Poley JW, Verhoef S et al (2006) Duodenal carcinoma in MUTYH-associated polyposis coli. J Clin Pathol (in press)
17. Van Puijenbroek M, Dierssen JW, Stanssens P et al (2005) Mass spectrometry-based loss of heterozygosity analysis of single-nucleotide polymorphism loci in paraffin embedded tumours using the MassEXTEND assay: single-nucleotide polymorphism loss of heterozygosity analysis of the protein tyrosine phosphatase receptor type J in familial colorectal cancer. J Mol Diagn 7:623–630
18. Parker A, Gu Y, Mahoney W, Lee SH, Singh KK, Lu AL (2001) Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J Biol Chem 276:5547–5555
19. Croitoru ME, Cleary SP, Di Nicola N et al (2004) Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. J Natl Cancer Inst 96:1631–1634
20. Kambara T, Whitehall VL, Spring KJ et al (2004) Role of inherited defects of MYH in the development of sporadic colorectal cancer. Genes Chromosomes Cancer 40:1–9
21. Niessen RC, Sijmons RH, Ou J et al (2006) MUTYH and the mismatch repair system: partners in crime? Hum Genet 119:206–211
22. Soravia C, DeLozier CD, Dobbie Z et al (2005) Double frameshift mutations in APC and MSH2 in the same individual. Int J Colorectal Dis 20:466–470