EUROPEAN JOURNAL OF CANCER 47 (2011) 1046–1055

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First evidence for digenic inheritance in hereditary colorectal cancer by mutations in the base excision repair genes

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ARTICLE INFO

Article history:
Received 8 July 2010
Accepted 23 November 2010
Available online 30 December 2010

Keywords:
OGG1
MUTYH
Mutations
Base excision repair
HNPCC phenotype
Digenic inheritance

ABSTRACT

Biallelic mutations in the base excision repair gene Mut Y homologue (*MUTYH*) are responsible for variable recessively inherited phenotypes of polyposis. Beside *MUTYH*, the proteins 8-oxo-guanine DNA glycosylase (*OGG1*) and *MTH1* (or *NUDT1*) are also involved in the repair of 7,8-dihydro-8-oxoguanine (8-oxo-G), previous studies, however, only found missense mutations of unclear pathogenicity in either *MTH1* or *OGG1*. To investigate the role of a defective 8-oxo-G repair we performed a germline mutation screening in the genes *OGG1*, *MTH1* and *MUTYH*, in 81 patients with a clinical phenotype ranging from attenuated or atypical adenomatous polyposis coli including hyperplastic polyps to hereditary non-polyposis colorectal cancer (HNPCC) type X syndrome without mono- or biallelic mutations in either *APC*, *MUTYH* or the DNA mismatch repair genes.

We describe here the first pathogenic germline mutation in *OGG1*, a splice site mutation affecting exon 1, which was inherited from the father, in combination with a maternal *MUTYH* missense mutation p.Ile223Val in a female patient with advanced synchronous colon cancer and adenomas at the age of 36 years pointing towards digenic inheritance for colorectal cancer (CRC) predisposition.

Monoallelic missense mutations in *MTH1* (3x), *OGG1* (2x), or *MUTYH* (3x) were identified in 10 patients (12%), three of them were novel.

Our findings indicate that mutations in other genes of the 8-oxo-G repair beside *MUTYH* are involved in CRC predisposition. Oligogenic inheritance affecting genes of a certain repair pathway might therefore be the missing link between monogenic and polygenic traits.

1. Introduction

The known genes predisposing for colorectal cancer (CRC) or adenomatous polyposis coli can so far not explain the genetic basis of neither highly suspicious hereditary CRC-patients without microsatellite instable tumours (MSS-CRC), categorised as hereditary non-polyposis colorectal cancer (HNPCC) type X nor attenuated or atypical forms of familial adenomatous polyposis coli (aFAP or atFAP). Recessive biallelic mutations in the base excision repair (BER) gene *Mut Y homologue* (*MUTYH*; OMIM 604933) are responsible for variable phenotypes of polyposis and CRC predisposition.[1–3] The gene

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.11.016

products of *MUTYH*, *OGG1* (8-oxo-guanine DNA glycosylase; OMIM 601982) and *MTH1* (or *NUDT1*; nucleoside di-phosphate-linked moiety X motif 1; OMIM 600312) act synergistic in the prevention and repair of DNA defects induced by 7,8-dihydro-8-oxoguanine (8-oxo-G) mutagenesis, i.e. G:C ⩾ T:A transversions. These somatic transversions affect *APC* and/or *KRAS* which results in adenomatous and hyperplastic polyps.[4] This allows the hypothesis that compound heterozygote mutations in the corresponding genes involved in BER might predispose for CRC or polyposis coli. All preceding studies failed to detect pathogenic germline mutations in *MTH1* or *OGG1* in patients with sporadic CRC, HNPCC-suspected CRC, and different polyposis/FAP cohorts, but revealed single heterozygote missense mutations of unclear pathogenicity in *MTH1*: p.Val83Met, p.Arg31Gln in *OGG1*: p.Ser85Ala, p.Arg154His, p.Ala288Val, p.Ile321Thr; p.Asp322Asn.[1,3,5–11]

Interestingly, two possibly pathogenic missense mutations of either *MTH1* (c.92G>A; p.Arg31Gln) or *OGG1* (c.589A>T; p.Arg197Trp) were reported in combination with *MUTYH* mutations, either pathogenic or unclassified, in four patients with CRC.[12] Both missense mutations, p.Arg31Gln in *MTH1* and p.Arg197Trp in *OGG1* change the character of the amino acid (aa), are predicted as 'possibly damaging' or 'probably damaging' in PolyPhen (scores: 1.529 and 3.227) and are located in a functional domain (Nudix or HhH). However, the heterozygote *MTH1* p.Arg31Gln did not co-segregate with the multiple adenoma phenotype in the family reported.[3] In the context of CRC or polyposis, truncating germline mutations have so far not been reported for *MTH1* or *OGG1*. A pathogenic frameshift *OGG1* germline mutation was reported in correlation with Alzheimer's disease.[13] Other somatic pathogenic mutations were detected in tumours of lung and kidney which also show frequent LOH of *OGG1*.[14–16]

By mutation screening of *MTH1* and *OGG1* in addition to *MUTYH*, in 81 patients with HNPCC type X, atFAP or aFAP we set out to investigate the role of these repair genes in generating intestinal neoplasias. We detected the first pathogenic germline mutation in *OGG1* in combination with a mutation in *MUTYH* for a patient with multiple adenomas and early onset synchronous CRC. Additionally we identified monoallelic heterozygous missense mutations in one of the genes in 10 patients i.e. 12% of our cohort, three of them are novel. Our results indicate a possible cooperative role of mutations in *MUTYH*, *OGG1*, and *MTH1* in patients and families with CRC.

2. Materials and methods

2.1. Mutation screening

The clinical phenotypes of the 81 patients analysed are subclassified and summarised in Table 1: 28 meet the HNPCC 'Amsterdam' or 'Bethesda' criteria[17–19] without a polyposis phenotype, 24 overlap between HNPCC and forms of familial adenomatous polyposis coli (FAP),[2,3] and 29 patients were classified with atypical and 24 with attenuated FAP. Within the FAP-group, patients with and without hyperplastic polyps beside adenomatous polyps were subdivided. The criteria for classification in Amsterdam, attenuated and atypical FAP are described in detail in the legend of Table 1. Mutation analysis for *APC*, *MLH1*, *MSH2*, and *MSH6* was negative, biallelic *MUTYH* mutations were ruled out for all patients. The patients CRCs were microsatellite stable (MSS) and showed positive immunohistochemical staining of DNA mismatch repair proteins MLH1, MSH2 and MSH6. Patients gave informed consent to participate in the study. DNA extraction from EDTA blood, standard PCR, exonuclease 1 and shrimp alkaline phosphatase digestion (Amersham Biosciences) and sequencing on ABI PRISM 3100 Avant (Big Dye v1.1) were performed following standard procedures. All exons and exon–intron boundaries of *MUTYH*, *NUDT1*/*MTH1* and *OGG1* were screened for mutations by sequencing (for details see supplemental material). Primer sequences are available upon request. For mutation description we applied the predominant transcripts 1a for *OGG1* (NM_002542) and p18 of *MTH1* (NM_198953) and the longest transcript NM_001128425 for *MUTYH*. Deletions were investigated only for *MUTYH* exons 1, 2, 3, 14, and 16 by MLPA kit P008 (MRC Holland).

70 DNA probes of patients aged over 80 years served as control cohort for mutations and variants. Additional 70 healthy controls were analysed for *OGG1* p.Ser326Cys and 430 for *MUTYH* p.Arg182Cys and p.Ile223Val.

2.2. cDNA expression analyses

From total RNA extracted from peripheral blood by the PAX Gene Blood RNA and Preparation kit (PreAnalytix) cDNA was generated with the First-strand cDNA-Synthesis kit (Amersham Biosciences). Expression analysis of a genomically heterozygous mutation in *OGG1* exon 1 was performed with cDNA primers located in exon 1 forward and exon 3 reverse,

Table 1 – Clinical phenotypes of 81 patients of the study cohort: Amsterdam positive with 3 CRC cases in two generations of the family, one under 50 years of age; Amsterdam old without age restriction; Bethesda positive[17–19]; and alleviated forms of familial adenomatous polyposis coli also including hyperplastic polyps i.e. attenuated familial adenomatous polyposis coli (aFAP) with 10–100 adenomas after 25 years of age; atypical familial adenomatous polyposis coli (atFAP) with up to 10 adenomas and/or CRC.[2,3]

	No polyposis	atFAP	aFAP	Number of patients
Amsterdam positive	12	5	2	19
Amsterdam old	3	5	–	8
Bethesda positive	13	7	5	25
Hyperplastic polyps and adenomas reported	–	4	10	14
Adenomas reported	–	8	7	15
Number of patients	28	29	24	81

amplifying a fragment of 528 bp representing all transcripts 1a–c and 2a–e[20] followed by sequencing. Elongated extension time allowed amplification of cDNA fragments including intron 1 in case of read-through due to a splice defect in exon 1. Expression of *MUTYH* missense mutation in exon 8 c.667A>G; p.Ile223Val was analysed with primers located in the exon–exon junctions of exons 6/7 forward and exons 13/14 reverse. For amplification and sequencing of *MTH1* missense mutation c.247G>A; p.Val83Met in exon 4 in all alternative *MTH1* transcripts we used primers in exon 1 forward, exon 2 forward or exon 3 forward, respectively, in combination with reverse primer in exon 5.

2.3. Tumour screening

Paraffin extracted DNA of the sigmoid colon cancer of patient FG and of an adenoma of her aunt underwent sequencing in *BRAF* exon 15, *KRAS* exon 2, 3, and *APC* exon 15.

3. Results

3.1. Mutation screening and segregation analysis

The results of the mutation analysis of *MUTYH*, *MTH1* and *OGG1* (NM_001128425; NM_002542.4; and NM_002452) in 81 patients with phenotypes between MSS–CRC suspicious of HNPCC type X, atFAP or aFAP are summarised in Table 2.

In *OGG1*, we identified a heterozygous splice site mutation c.137G>A in patient FG in the last nucleotide of exon 1 disrupting a splice donor sequence with a high sequence conservation of 73%. In case of protein expression of p.Arg46Gln PolyPhen prediction is 'probably damaging' (score 2.327). The mutation in *OGG1* exon 1 was not found on 140 control chromosomes. The same patient was also found heterozygote for *MUTYH* c.667A>G; p.Ile223Val, a missense mutation with low evolutionary conservation but localised in the helix–hairpin–helix domain. PolyPhen prediction (score: 0.748) was 'benign'. This missense change was absent in 860 control chromosomes and not found in routine testing of *MUTYH* for more than 500 patients in our laboratory. Segregation analysis in the family of FG revealed that the patient's mother and two aunts (diagnosed with adenomas at ages of 66 and 67 years, or CRC at 56 years of age) carry the *MUTYH* p.Ile223Val missense mutation heterozygous whilst the *OGG1* splice site mutation was derived from the father with only one polyp at age of 65 years (pedigree in Fig. 1). Further family members of the maternal pedigree with CRC at 55 or 70 years of age or adenomas were not available for genetic testing.

Furthermore, in *OGG1* the novel missense mutation p.Arg10Leu was found heterozygote in one patient and the *OGG1* missense mutation p.Gly308Glu was found for the first time as a heterozygous germline mutation in another patient (Table 2). Missense mutation c.29G>T; p.Arg10Leu in *OGG1* changes a hydrophilic polar basic into an aliphatic hydrophobic non-polar neutral amino acid of very low evolutionary conservation. The mutation is not located in a known functional domain, PolyPhen prediction is 'possibly damaging' (score: 1.955). This mutation in patient MG with CRC at age 54 years segregates with adenomas and hyperplastic polyps in the proximal colon at age of 50 years in two siblings and was absent in a sibling healthy at age 80 years (colonoscopy), 140 control chromosomes were negative as well. The *OGG1* c.923G>A; p.Gly308Glu mutation causes an exchange from an aliphatic non-polar neutral to an acid hydrophilic polar amino acid with very high evolutionary conservation, which is described as region V with a suggested structural role[21] but no predicted protein domain, PolyPhen prediction is 'probably damaging' (score: 2.739). *OGG1* p.Gly308Glu was found in an Amsterdam-positive family for patient FU diagnosed with endometrial cancer at age of 51 years and CRC at age of 54 years and her affected son with multiple adenomas and hyperplastic polyps at age of 22 years. The mutation was absent in the 41 years old healthy daughter, and was found once in 140 control chromosomes. The common and probably polymorphic *OGG1* c.977C>G; p.Ser326Cys missense change was detected to be heterozygous in 24 and to be homozygous in 6 patients i.e. 37% of our patients and in 50 controls i.e. 36% of our control cohort. *OGG1* p.Ser326Cys has no evolutionary conservation and is not located in a known functional domain, PolyPhen prediction is 'benign' (score: 1.205). The intronic variations in *OGG1* c.386-25T>C, c.566-4G>A, and c.748-15C>G (rs2072668) are not located in conserved branch or splice sites, and c.1585*66A>G in the 3′UTR of variant NM_016821, which is located before the polyadenylation site were found with different frequencies in patients and controls and were classified as polymorphisms,[15,22] detailed information is given in Table 2.

We identified two novel heterozygote germline missense mutations in *MTH1* p.Asp99Asn and p.Met116Leu of unclear pathogenicity in one patient each and the known p.Val83Met missense mutation in two patients (Table 2): *MTH1* c.295G>A; p.Asp99Asn represents an exchange from a negative charged to a neutral amino acid with low evolutionary conservation located in the Nudix domain, PolyPhen prediction 'possibly damaging' (score: 1.549), not found in the control cohort. *MTH1* p.Asp99Asn was found in CRC patient KA deceased at age 37 years, the family history is Amsterdam-positive but no family members were available for genetic testing. Patient KA was also heterozygous for a silent polymorphic single nucleotide polymorphism (SNP) c.1449C>T; p.Thr483Thr in *MUTYH*.[23] *MTH1* c.346A>T; p.Met116Leu results in an exchange of amino acids of similar chemical character with low evolutionary conservation located in the Nudix domain, PolyPhen prediction 'benign' (score: 1.442), absent in the control cohort. The mutation p.Met116Leu in *MTH1* was found in patient BR diagnosed with 80 adenomas and hyperplastic polyps at age of 46 years and a negative family history. The c.247G>A; p.Val83Met mutation in *MTH1* is a conservative amino acid exchange with high evolutionary conservation located in the Nudix domain, PolyPhen prediction 'benign' (score: 1.430). The mutation p.Val83Met was found once in our control cohort, was detected in 16% of controls by,[10] and was reported with an allelic frequency of 0.05–0.09 in normal population.[3,7,24] The missense mutation p.Val83Met in *MTH1* was detected in two patients, AE diagnosed with multiple adenomas (10–100) since the age of 26 years, her mother died at age of 68 years and had CRC with coinciding adenomas. The second patient QA was diagnosed with CRC at age 32 years and mucinous endometrial cancer at age of 34 years without a family history. In patient PH three rare *MTH1*

Table 2 – Results of the mutation screening and classification in pathogenic (P) and unclassified variant (UV) or polymorphic single nucleotide polymorphism (SNP) in patients with colorectal cancer (CRC), endometrial cancer (EC) or other diagnoses: attenuated FAP (aFAP) or atypical FAP (atFAP). Positive family history of CRC is designated as (FA+), fulfilling the Amsterdam criteria as (A+) and Bethesda criteria (B+). Missense mutations were novel or described[15,22–24,51] and with evolutionary conservation (EvCons) of amino acid (aa) in *Saccharomyces cerevisiae* (S.c.), *Escherichia coli* (E.c.), *Arabidopsis thaliana* (A.th.), *Xenopus tropicalis* (X.tr.), *Mus musculus* (M.m.), *Danio rerio* (D.r.), *Tetraodon nigroviridis* (T.n.), chemical characters of amino acid substitutions were specified, localisation in Pfam-predicted protein domain (http://pfam.sanger.ac.uk/) and PolyPhen prediction (http://genetics.bwh.harvard.edu/pph/) with PSIC (position-specific independent counts) score were listed.

Clinical data	Mutations in MUTYH	Mutations in OGG1	Mutations in MTH1	Evaluation
36y synchronous CRC (A+) FG	c.667A>G; p.Ile223Val (UV) both aa hydrophobic non-polar PolyPhen prediction benign: 0.748 low EvCons (in M.m., X.tr., A.th., E.c.), in HhH domain, segregates with adenoma/CRC	c.137G>A; (p.Arg46Gln) (P) splice site mutation with 73% sequence conservation, verified in cDNA, novel in germline		Double heterozygote: pathogenic *OGG1* splice site mutation, not present in 70 controls and possibly pathogenic *MUTYH* missense mutation affecting a functional domain, absent in 430 controls and >500 routine testings
54y CRC+2 adenomas (atFAP, FA+) MG		c.29G>T; p.Arg10Leu (UV) novel, changes hydrophilic polar basic into aliphatic hydrophobic non-polar neutral amino acid, low EvCons (X.tr.), no functional domain, PolyPhen prediction possibly damaging: 1.955, segregates with adenomas/ hyperplastic polyps		Possibly pathogenic *OGG1* missense mutation, not present in 70 controls
51y EC, 54y CRC (A+) FU		c.923G>A; p.Gly308Glu (UV) changes aliphatic non-polar neutral into hydrophilic polar acid aa, novel in germline, PolyPhen prediction probably damaging 2.739; very high EvCons (in M.m., D.r., T.n., X.l., S.c.), no predicted functional domain, segregates with polyposis		Possibly pathogenic *OGG1* missense mutation, 1/70 controls
30x, 24 heterozygous, 6 homozygous, different phenotypes		c.977C>G; p.Ser326Cys (SNP) both aa hydrophilic neutral polar, PolyPhen prediction benign: 1.205, no EvCons, not located in a known functional domain		No pathogenicity assumed: also present in 36% of controls (50 of 140, 38 heterozygous, 12 homozygous)
6x different phenotypes		c.566-4G>A (SNP) no conserved splice site		No pathogenicity assumed: also in 25% of our controls and published controls
19x		c.748-15C>G (SNP) (rs2072668) no conserved branch site		No pathogenicity assumed: high allele frequencies in controls
4x		c.1585*66A>G (SNP) in variant NM_016821 located before poly-adenylation-site of alternative exon 8		No pathogenicity assumed: high allele frequencies in controls

(*continued on next page*)

Table 2 – (*continued*)

Clinical data	Mutations in MUTYH	Mutations in OGG1	Mutations in MTH1	Evaluation
42y 2x CRC (B+MSS) KU		c.386–25T>C (SNP) not in branch site		No pathogenicity assumed: rare intronic sequence variant
37y CRC (A+) KA	c.1449C>T; p.Thr483Thr (SNP), no splice site predicted		c.295G>A; p.Asp99Asn (UV) novel, from hydrophilic acid to neutral polar aa, PolyPhen prediction possibly damaging: 1.549, low EvCons (D.r.), located in Nudix domain	Possible pathogenic unclassified *MTH1* missense mutation in a function al domain, not present in 70 controls In combination with a silent *MUTYH* SNP, no pathogenicity assumed
46y multipe adenomas and hyperplastic polyps (aFAP, FA-) BR			c.346A>T; p.Met116Leu (UV) novel, aa of similar chemical character, PolyPhen prediction benign: 1.442, low EvCons (X.tr.), located in Nudix domain	Possible pathogenic unclassified *MTH1* missense mutation in a function al domain, not present in 70 controls
26y multiple adenomas (B+, aFAP, FA+) AE 32y CRC, 34y EC (B+, FA-) QA			c.247G>A; p.Val83Met (UV) both aa hydrophobic non-polar PolyPhen prediction benign: 1.430; high EvCons (X.tr., A.th., D.r., M.m), located in Nudix domain	Unclear pathogenicity of a *MTH1* missense mutation in a function al domain, in 1 of our 70 controls, allelic frequency of 0.05–0.09 in normal population
52y polyposis+16 hyperplastic polyps (aFAP) PH			c.152+11G>A (SNP) and c.102C>T; p.Gly34Gly (SNP) and c.111C>T; p.Gly37Gly (SNP)	Rare intronic variation, not located in a predicted branch site, not in 70 controls, rare silent SNPs, not in 70 controls
25x (22x heterozygous, 3x homozygous)			c.357C>T; Asp119Asp (SNP) (rs1799832)	Polymorphic silent SNP, present in 27 of 70 controls, no pathogenicity assumed, allelic frequency 0.2 in dbSNP
39x (33x heterozygous, 6x homozygous)			c.299-31C>T (SNP) not located in predicted branch site	Frequent intronic polymorphism, no pathogenicity assumed
24x (19x heterozygous, 5x homozygous)			c.471*49>G in 3′ UTR (SNP) not located in polyadenylation site	Frequent polymorphism in the 3′ UTR, no pathogenicity assumed
5x heterozygous			c.471*31C>T in 3′ UTR (SNP) not located in polyadenylation site	Polymorphism in the 3′ UTR, no pathogenicity assumed
62y CRC+2 adenomas (A+) HH	c.544C>T; p.Arg182Cys (UV), high EvCons (M.m., X.tr., A.th., E.c.), in Endo_3c domain			Possible pathogenic unclassified *MUTYH* missense mutation in a functional domain, absent in 430 controls
56y CRC (A+) KW	c.536A>G; p.Tyr179Cys (P)			Monoallelic pathogenic *MUTYH* missense mutation
68y adenomas+ hyperplastic polyps (atFAP) PE	c.1187G>A; p.Gly396Asp (P)			Monoallelic pathogenic *MUTYH* missense mutation
63y CRC+2 polyps (A+) KS	c.1187G>A; p.Gly396Asp (P) segregates with adenomas 36y (son)			Monoallelic pathogenic *MUTYH* missense mutation

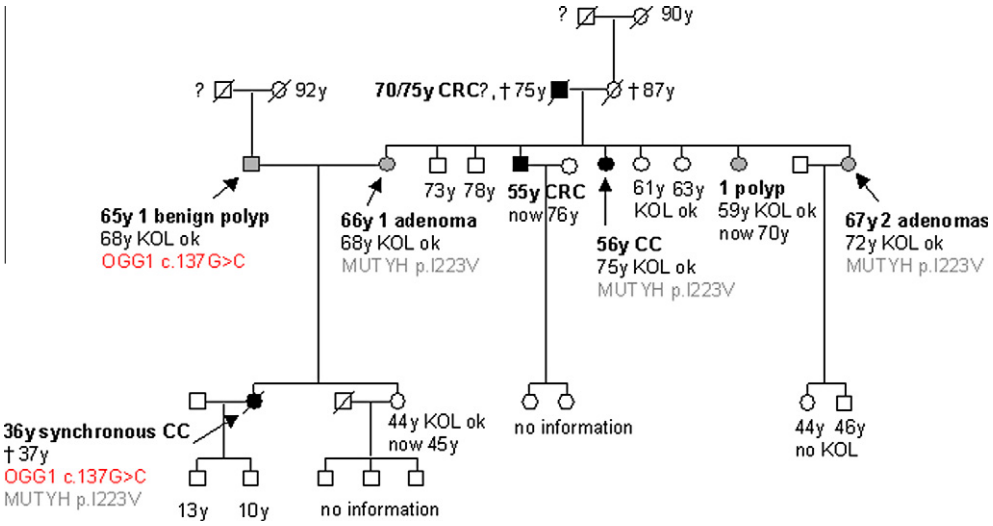

Fig. 1 – Pedigree of the patient with two heterozygote mutations, a splice site mutation in *OGG1* exon 1 c.137G>A and an unclassified missense mutation p.Ile223Val in *MUTYH* and segregation within the family members. (KOL ok = colonoscopy without finding.)

sequence variants c.102C>T; p.Gly34Gly, c.111C>T; p.Gly37Gly and the intronic variation c.152+11G>A not located in a predicted branch site were found, which were not present in 140 control chromosomes. The exonic *MTH1* sequence variant c.357C>T; p.Asp119Asp (rs179832),[10] intronic variation c.299-31C>T, not located in predicted branch site,[10] and c.471*31C>T and c.471*49C>T in 3′UTR, not located in the polyadenylation site, were detected frequently in patients and controls and were classified as polymorphisms, for details see Table 2.

Five heterozygous mutations were identified in our cohort (Table 2), in four patients without a second mutation in *MUTYH*, *OGG1*, or *MTH1* detectable. The *MUTYH* missense mutation p.Ile223Val was found in combination with a heterozygote *OGG1* splice site mutation in patient FG and was described above. Missense mutation c.544C>T; p.Arg182Cys was identified in patient HH[25] changing a basic, strongly polar amino acid with high evolutionary conservation located in the Endo_3c domain, to a weakly polar semiacid one. PolyPhen prediction was 'probably damaging' (score: 2.792) for p.Arg182Cys, which was absent in 860 control chromosomes. The common pathogenic *MUTYH* hotspot mutations (previously reported as p.Tyr165Cys and p.Gly382Asp, correspond to p.Tyr176Cys and p.Gly393Asp in another transcript) were present as heterozygote in three patients: p.Gly396Asp in KS and PE, p.Tyr179Cys in KW. Of the four heterozygous *MUTYH* mutation carriers, three patients (KS, KW, HH) display a highly positive family history fulfilling the Amsterdam criteria.

3.2. *cDNA analyses*

The cDNA analysis of the patient FG with the predicted splice site mutation c.137G>A in the splice donor of *OGG1* exon 1 revealed monoallelic expression of the wild type allele in the normally spliced mRNA fragment. No aberrant splicing products were detectable. The *MUTYH* missense mutation c.667A>G; p.Ile223Val in exon 8 showed biallelic expression on cDNA level and no aberrant splicing. In cDNA available of patient AE with the heterozygote *MTH1* missense mutation c.247G>A; p.Val83Met all known alternative transcripts of *MTH1* (for details see supplemental material) were expressed from both alleles so that there is no evidence of aberrant splicing.

3.3. *Tumour analyses*

BER deficiency is frequently accompanied by an excess of somatic G:C-⩾T:A transversions and KRAS hotspot mutations in 33–64% of *MUTYH*-associated tumours.[1] For further validation of a defective 8-oxoG repair in our patient, we searched for somatic mutations in the colon cancer of patient FG (*OGG1* c.137G>A+*MUTYH* p.Ile223Val). No mutation was detected in the hotspot regions in *BRAF* exon 15, *KRAS* exon 2 or 3 and *APC* exon 15.

4. Discussion

MUTYH, *OGG1* and *MTH1* are involved in the prevention and repair of DNA defects induced by 8-oxo-G mutagenesis. We hypothesise that the additive effect of double heterozygote mutations in genes involved in BER might predispose for CRC or alleviated forms of polyposis coli. So far, only biallelic mutations in the *MUTYH* gene are confirmed to predispose for polyposis coli and CRC, this with a variable age of onset, clinical phenotype and histopathology.[4] In the literature, monoallelic germline missense mutations without explicit pathogenicity in *MTH1* or *OGG1* were found in patients with sporadic CRC, HNPCC-suspected CRC, or different FAP cohorts.[1,3,5,6,11]

4.1. *Combined heterozygous mutations*

One study on 45 unselected CRC patients with one heterozygous *MUTYH* aberration found additional missense mutations of unknown pathogenicity in either *MTH1* p.Arg31Gln or *OGG1*

p.Arg197Trp in four patients.[12] We report here the first explicit pathogenic *OGG1* germline mutation in combination with a possibly pathogenic *MUTYH* mutation in patient FG presenting with advanced synchronous CRC at age of 36 years and additional adenomas. The heterozygous *OGG1* germline missense mutation c.137G>A; (p.Arg46Gln) hits the last nucleotide of *OGG1* exon 1 with 73% sequence conservation and affects the splicing process (Table 2). The patient showed monoallelic expression of the wild type allele in cDNA analyses suggesting a loss of function for the mutated OGG1 allele p.Arg46Gln. No aberrant splicing products were detectable, we therefore suspect non sense-mediated mRNA decay (NMD) of the mutant allele due to a premature stop codon in codon 47 in a read-through mRNA including intron 1. This pathogenic *OGG1* mutation was absent in 140 control chromosomes and was previously reported as a homozygous somatic mutation in a lung cancer cell line,[15] and heterozygously in a patient with lung cancer[16] and one kidney tumour.[14] Patient FG was in addition heterozygote for *MUTYH* p.Ile223Val, a missense mutation located in the helix–hairpin–helix domain, which we classified as possibly pathogenic. Expression of *MUTYH* p.Ile223Val was biallelic in cDNA without evidence of aberrant splicing, but a functional consequence can be assumed on protein level. In the literature, *MUTYH* p.Ile223Val was found heterozygote in a patient diagnosed with 100–1000 adenomas at age of 54 years without a family history for CRC.[26] This is the first report of a pathogenic germline *OGG1* mutation detected in double heterozygote status with a possible pathogenic *MUTYH* missense mutation in a patient diagnosed with synchronous CRC and adenomas at 36 years of age. The family history was positive for late onset CRC and adenomas in the maternal line for family members carrying the *MUTYH* mutation and negative for the father's side, where the *OGG1* mutation came from. This lets us assume an additive effect of di- or oligogenic inheritance of monoallelic mutations in different genes of the same repair pathway, and widens the spectrum of possible oligogenic cancer predispositions which might be the missing link between monogenic cancer predisposition and polygenic traits. For the children and siblings of patient FG, the formal genetic risk for the inheritance of both mutations is 25%. Whether or not the monoallelic *MUTYH* or *OGG1* mutation alone already leads to an increased risk of gastrointestinal neoplasias, consultation and screening recommendations have to be worked out carefully. Several studies demonstrated a moderately increased risk for CRC in heterozygous *MUTYH* mutation carriers[12,27–29] and postulated unrecognised low-risk alleles contributing to a polygenic disease mechanism. Due to the extremely heterogeneous clinical picture of MAP, colonoscopy and endoscopic surveillance of the gastrointestinal tract are recommended in intervals of 2–3 years starting in the teenage years.[2,30] The clinical appearance of the patients with possible BER-deficiency due to double heterozygote mutation in either *MTH1* or *OGG1* in combination with a *MUTYH* mutation was not described in detail.[12]

It has been proposed that a biallelic *MUTYH* deficiency in a tumour is accompanied by an excess of somatic G:C⩾T:A transversions in the *APC* gene in ~43%,[1] and is associated with *KRAS* hotspot mutations in 33–64%.[31] This might also apply for a combined defect of *MUTYH* and *OGG1*, however, no somatic mutation was detected in the tumour of patient FG in *KRAS* exon 2 and 3 and parts of *APC* exon 15. This does not rule out a defective 8-oxo-G repair in our patient as a combined defect of *OGG1* and *MUTYH* in patient FG might not in the same extent lead to a defective 8-oxo-G repair, as both defects only enhance transversion additively but might be bypassed at least to some extent by other repair mechanisms of redundant function.[32–34]

In the context of CRC or polyposis, truncating germline mutations have so far not been reported for *MTH1* or *OGG1*, but the missense mutations detected in patients with sporadic CRC, HNPCC-suspected CRC, or FAP might be pathogenic as well.[6–9,12,35] So far, truncating and therefore proven pathogenic *OGG1* germline mutations were only reported for a patient with Alzheimer's disease[13] or lung cancer,[16] as homozygous somatic mutation in a lung cancer cell line,[15] and heterozygote somatic in one kidney tumour.[14]

By mutation screening of *MUTYH*, *MTH1* and *OGG1* in 81 patients with HNPCC type X, atFAP or aFAP we identified monoallelic, possibly pathogenic heterozygous missense mutations in one of the genes in 10 patients (12%), of those, p.Arg10Leu in *OGG1* and p.Asp99Asn and p.Met116Leu in *MTH1* are novel. The two heterozygous *OGG1* germline missense mutations p.Arg10Leu and p.Gly308Glu in one patient each both change the character of the amino acid, segregate with adenomas in the affected families and were predicted as 'probably damaging' (Table 2). *OGG1* p.Gly308Glu is located in a highly conserved region V,[21] and was previously reported as a somatic mutation in one kidney cancer[14] and a head and neck cancer.[36] *OGG1* p.Ser326Cys is contrarily discussed as a possible predisposition for diverse cancer types including CRC due to a reduced glycosylase activity[37–42] but protective to breast cancer.[43] *OGG1* p.Ser326Cys was present in 30 patients (37%). Due to the overrepresentation in 36% of our controls and 44.5% in Japanese population,[44] benign PolyPhen prediction and lacking evolutionary conservation, we regard p.Ser326Cys as a common SNP not increasing CRC risk in a significant manner.

Two novel *MTH1* germline missense mutations p.Asp 99Asn and p.Met116Leu both located in the Nudix domain were absent in controls and were regarded as possibly pathogenic: The carrier of p.Asp99Asn mutation deceased at age 37 years from CRC and displayed an Amsterdam-positive family history for CRC, unfortunately none of the family members were available for genetic testing. The mutation p.Met116Leu, was found in a patient with 80 adenomas and hyperplastic polyps at age of 46 years. The prevalence of hyperplastic polyps has been described in the setting of BER-deficiency, as this might result in transversions and therefore in somatic *KRAS* mutations.[4] Two patients are carriers of *MTH1* p.Val83Met, AE with multiple adenomas (10–100) since the age of 26 years, and QA with diagnoses of CRC and endometrial cancer at ages of 32 and 34 years. The p.Val83Met mutation is controversially discussed with respect to pathogenicity and aberrant splicing.[3,5,7] The cDNA of patient AE revealed biallelic expression in all expected alternative cDNA transcripts of *MTH1* so that there is no evidence of aberrant splicing due to p.Val83Met at least in leucocytes. Despite equal protein production level and hydrolysation efficiency of 8-oxo-G[45] a pathogenic effect due to a higher

thermo-liability of Met83 was described.[46] Mutation p.Val83-Met was found once in our control cohort (i.e. 1.4%), was detected in 16% of controls by[10] and was reported with an allelic frequency of 0.05–0.09 in normal population.[3,7,24] As a functional effect of p.Val83Met can not be ruled out, we designate this as a mutation with unclear pathogenicity despite occurrence in controls.

The incidence of *MUTYH* mutations was approximately 6% for either monoallelic, clearly pathogenic hotspot mutations or presumably pathogenic missense mutations (Table 2). One of these, the probably pathogenic missense mutation p.Arg182Cys in *MUTYH* changes the Endo_3c domain and is predicted as 'probably damaging'.[25] The mutation was found in a patient with CRC at 62 years of age, coinciding adenomas and an Amsterdam-positive family history. The incidence rate of the common *MUTYH* hotspot mutations p.Tyr179Cys and p.Gly396Asp in 3.7% of our patients is slightly above the general population frequency of up to 2.1%.[47] Furthermore, three of the four monoallelic *MUTYH* mutation carriers show a positive family history of CRC indicating a dominantly inherited genetic predisposition disparate from the recessive MAP inheritance. Possible explanations might be the functional combination of a monoallelic *MUTYH* mutation with mutations in other genes[32] or incidentially inheritance of another CRC predisposing gene beside the *MUTYH* alteration.

On the other hand, *MUTYH* mutations may exert a dominant-negative effect. A monoallelic missense mutation with a dominant-negative effect might generate an autosomal dominant pattern of inheritance: *MUTYH* missense mutations could be gain of function mutations which still enable the recognition of a A:8-oxoG mismatch, but (1) not the repair by stuck DNA-binding which protects the mismatch from being repaired by a functional MUTYH protein, or (2) looses the ability to determine the daughter strand and perform changes on the wrong strand leading to higher number of A:T to C:G transversions.[48] The catalytically inactive variant of *OGG1* p.Lys249Gln e.g. retains high-affinity binding to an 8-oxoG:C-containing substrate but cannot excise 8-oxoG.[49] The same might be true for *OGG1* mutations: the missense mutations p.Gly308Gln and p.Arg10Leu in *OGG1* seemed to follow a dominant pattern of inheritance, explainable by a 'gain of function' effect.

As familial clustering of polyposis could only in case of a dominant-negative effect be explained by a single heterozygous mutation in *MUTYH*, *OGG1* or *MTH1* we assume additional oligogenic influences of mutations in other genes, e.g. also involved in the BER. Mutations in *NEIL2*, *TDG* and *UNG* were found in patients with familial CRC whilst *NTHL1*, *NEIL1*, *MPG* and *SMUG1* seem to play a limited role.[50] Our results give new insights in BER involved in CRC susceptibility and indicate a cooperative role of *OGG1* and *MTH1* aberrations in addition to *MUTYH* mutations in CRC patients.

Our study reveals a possible contribution especially of *OGG1* mutations to familial CRC and polyposis but necessitates further studies, in regard to inheritance and/or functional consequences for the proteins. There is functional redundancy in the DNA repair mechanisms, and for 8-oxo-G repair e.g. mismatch repair proteins MSH2 and MSH6 (involved in HNPCC predisposition), the CSB protein and BRCA 1 and 2 have also been implicated.[21,33] Beside results of frequently performed high throughput association studies for large numbers of patients with familial CRC, the important information gained by targeted mutation analysis of genes within one functional unit (i.e. BER) in molecular preselected patient cohorts to work out the importance of oligogenic traits should not be overlooked.

Funding Source

German Cancer Aid – Mildred Scheel Foundation and the Wilhelm-Sander Foundation (2005.054.1).

Conflict of interest statement

None declared.

Acknowledgements

This work was supported by the German Cancer Aid – Mildred Scheel Foundation and the Wilhelm-Sander Foundation (2005.054.1). We would like to thank all patients and families for their participation in this study, as well as their respective doctors for contributing clinical information.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.11.016.

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