MUTYH Associated Polyposis (MAP)

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Abstract: MUTYH Associated Polyposis (MAP), a Polyposis predisposition caused by biallelic mutations in the Base Excision Repair (BER) gene MUTYH, confers a marked risk of colorectal cancer (CRC). The MAP phenotype is difficult to distinguish from other hereditary CRC syndromes. Especially from Familial Adenomatous Polyposis (FAP) and to a lesser extend Lynch Syndrome, which are caused by germline mutations in the APC andMismatch Repair (MMR) genes, respectively.

Here we review research findings regarding MUTYH interactions, genotypic and phenotypic characteristics of MAP, as well as surveillance and treatment of the disease. The applied papers, published between 1/1 2002- 1/2 2008, were found through PubMed.

The exact role of MUTYH in CRC tumorgenesis is still uncertain, although MAP tumors show distinct molecular features, including somatic G:C>T:A transversions in the APC gene. Furthermore, cooperation between the BER and the MMR systems exists, as MUTYH interacts with MMR gene-products. Possibly, monoallelic defects in both pathways are of significance to CRC development.

Specific MUTYH variants are found to be characteristic in distinct ethnic populations, which could facilitate future genetic screening. Knowledge concerning functional consequences of many MUTYH germline mutations remains sparse. Most thoroughly investigated are the two most common MUTYH variants, Y179C and G396D, both generating dysfunctional gene products.

Phenotypic features of MAP include: development of 10-100 colorectal adenomas, debutting at 46-47 years, often CRC at time of clinical diagnosis, and in some, development of extracolonic manifestations.

Key Words: Colorectal cancer, MUTYH associated polyposis, The MUTYH gene, base excision repair, (Attenuated) familial adenomatous polyposis, Lynch syndrome.

INTRODUCTION

Colorectal Cancer (CRC) is the second most prevalent cancer worldwide [1]. In 35% of CRC patients, statistically significant effects of hereditary factors have been found [2]. For some of these patients the genetic background is known; major CRC syndromes being: Lynch Syndrome, Familial Adenomatous Polyposis (FAP) and MUTYH Associated Polyposis (MAP), which will be focused on in this review. Fig. (1) shows a delimitation of the groups of patients, which are referred to in this paper.

Lynch Syndrome is characterized by the development of particularly CRC and endometrial cancer at a young age. Lynch Syndrome is an autosomal dominant disease often caused by germline mutations in one of the Mismatch Repair (MMR) genes [3, 5-7]. The clinical and genetic features of the syndrome have previously been thoroughly reviewed in [5-9].

Another autosomal dominant disease, Familial Adenomatous Polyposis (FAP), is caused by a germline mutation in the APC gene, and confers a near 100% risk of developing CRC. FAP has been shown to account for less than 0.1% of all CRC cases [10]. The characterization of the APC gene and protein-product has been repeatedly reviewed, among others in [11] and [12]. Phenotypic characteristics of FAP include: early development of more than 100 and up to thousands of colorectal adenomas, as well as extracolonic manifestations such as gastric and duodenal adenomas, desmoid tumors and congenital hypertrophy of the retinal pigment epithelium (reviewed in [8, 13-16]). In FAP, genotype-phenotype correlations have been identified, specific APC gene mutations being associated with particular manifestations reviewed in [11] and [17]. Of particular clinical interest is the milder phenotypical FAP variant, Attenuated Familial Adenomatous Polyposis (AFAP), which is associated with APC mutations in the extreme ends of, or in the alternatively spliced region of exon 9 [11, 18]. AFAP is distinguished from FAP by the development of less than 100 colorectal adenomas, fewer extracolonic manifestations and the later development of CRC [11, 13-16, 19]. One study showed that about 8% of registered FAP families present with an AFAP phenotype [19].

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The following groups of patients are defined on the basis of both clinical and genetic characteristics, and will be referred to throughout the text.

**On a clinical basis:**

**Polyposis patients:** Patients presenting with adenomas in the colon and/or rectum.

**CRC patients:** Patients presenting with carcinomas in the colon and/or rectum, with or without previous/synchronous occurrence of Polyposis.

**MAP patients:** (OMIM 608456)

Polyposis patients with detected biallelic germline mutations in the **MUTYH** gene.

**On a mixture of clinical and genetic basis:**

**FAP patients:** (OMIM 175100)

Polyposis patients presenting with > 100 adenomas in the colon and/or rectum and – in this paper - with a detected germline mutation in the **APC** gene.

**AFAP patients:** (OMIM 175100)

Polyposis patients presenting with < 100 adenomas in the colon and/or rectum. (Possibly with a detected germline mutation in the **APC** gene).

**Lynch Syndrome patients**

(Previously known as HNPCC (Hereditary Non-Polyposis Colorectal Cancer) patients): (OMIM: HNPCC1: 120435, HNPCC2: 609310)

Several guidelines for the clinical diagnosis of these patients exist (e.g. The Amsterdam criteria I and II [3] and the Bethesda criteria [4]). Familial disposition for CRC, possibly with a detected germline mutation in a Mismatch Repair (MMR) gene.

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**Fig. (1).** Delimitation of the groups of patients.

Prophylactic screening of Lynch Syndrome patients, FAP patients and their families is shown to reduce the development of CRC and CRC-associated mortality markedly [5, 9, 10, 13, 16, 20]. For Lynch Syndrome patients, the recommendation is colonoscopy from about 20-25 years of age, in intervals of 1-3 years [5, 7, 9, 20]. The benefit of screening for endometrial cancer and other cancers associated with Lynch Syndrome is still controversial, and recommendations should be adjusted according to the individual patient’s wishes, family history and possibly genotype [7, 9, 20].

Recommendations for FAP surveillance was recently reviewed in [13] and [16]. Sigmoidoscopy is advised to FAP patients commencing in the early teens, typically in intervals of 1-3 years, according to the clinical manifestations [13, 15, 16]. Furthermore, individual assessment is especially necessary in FAP families displaying a more severe phenotype [16]. Additionally, FAP patients should be offered endoscopy of the upper gastrointestinal tract from the age of about 25-30 years, in intervals of 1-5 years, according to the severity of duodenal Polyposis [16]. Moreover, prophylactic colectomy is often advisable for FAP patients. Especially in patients with an early first appearance of the disease, the surgical procedure recommended varies between the individual FAP patients [13, 15, 16]. For AFAP patients, colonoscopy in intervals of 2 years is advised starting from 18-20 years of age, due to the later CRC development and the typically more distal location of adenomas in AFAP patients compared to FAP patients [16, 19].

In as many as 30% of patients with a FAP-like phenotype, no germline mutations in the **APC** gene can be found [15]. Similarly, one study of patients with an AFAP-like phenotype (3-100 adenomas), found that merely about 10% of these patients had inherited germline **APC** mutations [18]. However, another study (N=59) showed that almost 70% of patients with an AFAP-like phenotype (10-100 adenomas) had a germline **APC** mutation [19].

In 2002, the significance of mutations in the **MUTYH** gene regarding the development of the Polyposis predisposition syndrome **MUTYH** Associated Polyposis (MAP) was discovered [21]. Since then, many aspects of MAP have been investigated, and the important question of how, these new discoveries can be used in the genetic counseling and screening of individuals at risk of developing MAP, now stands to be answered.

Here we review the main genetic aspects of MAP, including analysis of functional consequences and the outlining of specific ethnic allelic frequencies of **MUTYH** variants. In addition, we review the clinical aspects of the syndrome and introduce a new nomenclature for **MUTYH** germline mutations, which is likely to replace the nomenclature, which is currently used.
We believe that this review provides a broad, up-to-date overview of existing findings regarding MAP. We hope to provide perspective of the significance of MUTYH, as well as of which issues regarding MAP call for future investigation.

PATHOGENESIS OF MAP: THE MUTYH GENE AND BASE EXCISION REPAIR

Base Excision Repair

The MUTYH gene product is part of the Base Excision Repair (BER) system, which serves as an important part of cells’ defense against oxidative damage to the DNA. The BER system and the functional role of MUTYH have previously been reviewed in detail by [22-31].

Oxidative DNA damage, caused by reactive oxygen species (ROS), which are produced during aerobic metabolism, exposure to certain chemicals or radiation, constantly threatens the integrity of cellular DNA. The oxidized base 7,8-dihydroxy-8-oxoguanine (8-oxo-G) is one of the most stable and mutagenic products of oxidative DNA damage. 8-oxo-G is often mistakenly paired with adenine (A), resulting in the appearance of Guanine:Cytosine > Thymidine:Adenine (G:C > T:A) transversions at the next round of DNA replication, as the detection of stable 8-oxo-G:A base-pairs is missed by the replicative DNA polymerases [22-31].

The human DNA-damage-specific glycosylases OGG1, MUTYH and MTH1, which are central enzymes in the BER pathway, function by specifically recognizing and facilitating the removal of 8-oxo-G [23-31]. The specific mechanism of recognition of the DNA-damage-specific glycosylases is characterized as “base-flipping”, involving the outwards rotation of nucleotides from the DNA helix. This allows the incorporated bases to be assessed by fitting into base-specific pockets of the glycosylases [22, 23, 26, 29-32]. The repair process at the damaged site is subsequently completed by the synthesis and incorporation of newly replicated DNA, involving several repair steps, which are facilitated by a sequence of DNA repair enzymes [23, 25, 26, 28-30]. The MUTYH glycosylase acts as the third level of BER, as it postreplicatively excises the misincorporated A opposite 8-oxo-G [23-25, 29-31]. For this reason, defective MUTYH function is associated with an increased frequency of G:C > T:A transversions [29]. Inactivation of MUTYH has accordingly been associated with various cancer forms [29], including lung cancer, gastric cancer, CRC [25, 33-36] and recently also endometrial cancer [37].

A number of studies of the human MUTYH glycosylase, both in vitro and in vivo, have demonstrated that the MUTYH glycosylase directly interacts with various proteins involved in other DNA repair pathways (reviewed by [29] and [31]).

Several studies have also screened the OGG1 and MHT1 genes in Polyposis patients without any significant findings of association with Polyposis or CRC phenotypes. [21, 38-41]. However, OGG1 variants have recently been demonstrated to be significantly associated with a multiple adenoma phenotype [42] and the development of sporadic CRC [43], although the latter association was of borderline significance and should be further investigated [43]. This review focuses exclusively on the significance of MUTYH mutations in relation to MAP and CRC.

THE MUTYH GENE

The MUTYH glycosylase is encoded by the MUTYH gene, located on the short arm of chromosome 1 (1p32.1-p34.3). The gene consists of 16 exons and encodes a protein of 535 amino acids, the MUTYH glycosylase. Characterization of the MUTYH gene and functional variants has been reviewed in [22-26, 29].

Exon 3 in MUTYH is alternatively spliced in various ways, generating different MUTYH transcripts [25, 29, 44]. In accordance with HGVS nomenclature rules, the longest MUTYH transcript existing (NM_012222.2 extended at the 5’ of exon 3) will be used as coding DNA reference sequence, as outlined by Leiden Open Variation Database (LOVD) at http://www.LOVD.nl/MUTYH [44]. The use of the longest existing MUTYH transcript as coding DNA reference sequence is predicted to replace the previous commonly used MUTYH transcript (NM_001048171), although many authors still refer to this. Also, experts in this field (J. Sampson, F. Hes and S. Aretz) have recently discussed the use of MUTYH reference sequence. They agree that NM_012222.2 extended at 5’ of exon 3 is the best option to use as reference sequence in the future [45].

Consequently, the amino acid numbering in this paper differs from the one used in many previous papers. An overview of the new and old terms regarding some of the most common MUTYH mutations mentioned in this paper can be found in Table 1.

DEVELOPMENT OF COLORECTAL ADENOMAS AND CRC TUMORGENSES IN MAP

Somatic mutations in the APC gene are important in CRC tumorigenesis due to the gatekeeper role of the APC tumor suppressor gene, which is involved in many cellular processes. (Reviewed in [11] and [12].) The development of colorectal adenomas is likely to be initiated by an APC gene left dysfunctional as a result of germline or somatic mutations [11].

Defects in the MUTYH gene were first shown to be associated with a Polyposis predisposition by Al Tassan et al. in 2002 [21]. Defects in the BER genes were suspected, when 11 tumors from 3 related Polyposis patients showed somatic mutations in the APC gene, consistent with a defective BER system, while no germline APC mutations were found [21].

The cause as to why, mutations in the MUTYH gene predispose to the development of colorectal adenomas in particular, has not yet been fully determined. However, the number of spontaneous somatic C:G > T:A transversions in the APC gene is significantly greater in tumor cells with biallelic MUTYH germline mutations compared to tumor cells without MUTYH mutations [21, 38, 40, 46, 47].

The DNA sequence adjacent to the sites of C:G > T:A transversions in the 3’ end of the APC gene appears to be of significance to the specificity of the MUTYH glycosylase. A significantly higher occurrence of GAA sites has repeatedly been demonstrated 3’ to the C:G > T:A transversions, thus creating stop codons, and resultanty a truncated APC pro-
tein. These results are seen even though the G:C > T:A transversions theoretically could occur at any other G:C site in the APC gene [21, 38, 48]. Also, in vitro experiments with *E. coli* MutY have revealed a pronounced sequence preference for MutY to GAA [49]. However, the significance of these findings and the question as to why GAA sites seem to be prone to G:C > T:A transversions, remain unclear and call for further investigations.

Both the high number of GAA sites in the APC gene compared to other key tumorgenesis genes frequently involved in other cancers [23], as well as the considerable exposure to ROS in the gastrointestinal tract, could be part of the explanation of why germline MUTYH mutations, and subsequently somatic APC mutations, are associated with development of particularly CRC [23].

Characteristic molecular profiles of colorectal tumors, both adenomas and carcinomas, taken from MAP patients have been found [46, 50]. Distinct features of MAP tumors include: C>G > T:A transversions in the APC gene and the proto-oncogene K-Ras [46, 50]. Comparable with somatic APC mutations, statistically significant numbers of somatic mutations in the K-Ras gene have been found in MAP tumors, compared to tumors without MUTYH mutations [47, 50]. The described K-Ras mutations have all been identical G>C transversions in codon 12, G12C [46, 47].

### Table 1. Overview of Nomenclature for MUTYH Germline Mutations

| Nomenclature from Reference Sequence NM_001048171 | Nomenclature from Reference Sequence NM_012222.2 (Extended at the 5' end of exon 3)* |
|--------------------------------------------------|--------------------------------------------------------------------------------------|
| p.V22M                                          | p.V22M                                                                               |
| p.Y90X                                          | p.Y104X                                                                              |
| p.Y114H                                         | p.Y128H                                                                              |
| p.Y165C                                         | p.Y179C                                                                              |
| p.R168H                                         | p.R182H                                                                              |
| p.R168C                                         | p.R182C                                                                              |
| p.R171Q                                         | p.R185Q                                                                              |
| p.G175E                                         | p.G189E                                                                              |
| p.R227W                                         | p.R241W                                                                              |
| p.R231C                                         | p.R245C                                                                              |
| p.V232F                                         | p.V246F                                                                              |
| p.Q324H                                         | p.Q338H                                                                              |
| p.E369fsX437                                     | p.E383fsX451                                                                         |
| p.G382D                                         | p.G396D                                                                              |
| p.P391L                                         | p.P405L                                                                              |
| p.A459D                                         | p.A473D                                                                              |
| p.E466X                                         | p.E480X                                                                              |
| p.S501F                                         | p.S515F                                                                              |
| c.934-2A>G                                       | c.976-2A>G                                                                            |
| c.1391delAGG                                     | c.1433delAGG                                                                         |
| c.1103delGGA                                     | c.1145delGGA                                                                         |
| c.1395delGGA                                     | c.1437delGGA                                                                         |
| c.1103delC                                       | c.1145delC                                                                           |
| c.1186_1187insGG                                  | c.1228_1229dupGG                                                                    |
| c.IVS10-2A>G                                     | c.IVS10-2A>G                                                                         |

*The new nomenclature for MUTYH mutations is based on referral to the longest existing MUTYH transcript and were found at http://www.LOVD.nl/MUTYH
Molecular features in MAP tumors are characteristic to these, compared to carcinomas from Sporadic CRC, FAP or Lynch Syndrome tumors [46], which can potentially be used in classification of CRCs [50]. These molecular characteristics of MAP carcinomas include: low MSI (Microsatellite instability), low frequencies of APC, β-catenin mutations and LOH (Loss of Heterozygosity) of 18q, harboring the SMAD4 gene, and the karyotype of tumor cells typically being near-diploid [46, 50].

The issue of a possible significance of the MUTYH gene in Sporadic CRC has been only sparsely addressed, and with contradictory outcomes. Halford et al. found no indications of MUTYH involvement in Sporadic CRC [39]. In contrast, somatic mutations in the MUTYH gene have recently been demonstrated in Sporadic CRC, indicating a role of the MUTYH gene in Sporadic CRC tumorgenesis [51].

**INTERACTION BETWEEN THE MUTYH GENE AND THE MISMATCH REPAIR GENES**

The Mismatch Repair (MMR) system functions postreplicatively correcting DNA errors, which occur during DNA replication. The normal MMR function and characterization of defects in the MMR system have previously been reviewed in [7, 29] and [52]. Key proteins in the human MMR system include MutL homologs (MTH1 and Pms2) and MutS homologs (MSH2, MSH3, MSH6), the latter group forming two heterodimeric complexes [7, 29, 52].

Presumably cooperation between the BER and the MMR systems exists, since in vitro experiments have shown that MUTYH physically interacts with the MSH2/MSH6 heterodimeric complex via a MSH6-binding domain [53, 54]. These two studies have further demonstrated, that the MSH2/MSH6 complex stimulates the activity of the MUTYH glycosylase by enhancing the affinity of MUTYH for 8-oxo-G:A mismatched base pairs in the DNA [53, 54].

Several MUTYH germline mutations are shown to influence the interaction between MUTYH and MSH6, followed by a massive decrease in activity of the MUTYH protein [54, 55]. Mutations in one or more of the genes involved in the two systems possibly affect the repair of DNA damage caused by 8-oxo-G. The importance of this in regard to CRC tumorgenesis is still uncertain, although there have been indications, that interaction between a defect MUTYH gene and a defect MMR gene is of significance in regard to CRC risk. In a study by Niessen et al. (N=210), a significantly higher frequency of carriers of monoallelic MUTYH mutations was found among CRC patients who also had a specific MMR mutation (5/36=14%), in comparison to groups of CRC patients with other MMR mutations (1/40= 2.5%) or without MMR mutations (1/134 =0.7%) [56]. In this study a particularly strong association between monoallelic MUTYH germline mutations and en missense variant of the MSH6 gene was found (4/20 = 20%) [56], consistent with the before mentioned interaction between the two corresponding proteins.

In contrast, studies of CRC patients have indicated that the BER and MMR pathways may be mutually exclusive, although none have found significant results [48, 57]. Furthermore, Van Puijenbroek et al. found a remarkably mild Polyposis phenotype in a patient both compound heterozygote for MUTYH mutations, as well as being a carrier of a MSH6 germline mutation, supporting this notion [58]. However, these studies do not provide substantial data for any conclusions, for which reason further investigations need to be carried out.

**ALLELIC FREQUENCIES OF MUTYH GERMLINE MUTATIONS IN DIFFERING POPULATIONS**

An overview of the most commonly identified germline mutations in the MUTYH gene to date can be found at the LOVD at http://www.LOVD.nl/MUTYH.

Specific mutations in the MUTYH gene are found in different populations, see Fig. (2). In European populations the two missense mutations Y179C and G396D are most frequently seen, and have solely been found in Caucasians. The allelic frequencies of Y179C and G396D found among MAP patients are much higher compared to those found in background populations, see Fig. (3).

In Asian populations, Y179C and G396D do not seem to be of significance with regard to the development of Polyposis, since neither has been found in Asian Polyposis patients or in the corresponding background populations [42, 43, 55, 59-61]. In studies of Korean and Japanese Polyposis patients (N=97), 7.2% were biallelic carriers of other germline mutations in the MUTYH gene [55, 61]. Other studies of Korean and Singaporean Polyposis patients (N= 63) failed to find any MUTYH mutations after screening of coding regions in the entire gene, although these results may be biased due to small sample size [43, 59, 60]. Characteristic mutations found in Japanese Polyposis patients include the missense mutation R245C and the splice-site mutation IYS10-2A>G, neither of which were found in the corresponding background population [55].

To date, five unrelated Indian MAP patients have been identified, all were homozygote for the missense mutation E480X [38, 62, 63]. However, in a case-control study of Indian Polyposis patients (cases: N=120 and controls: N=100), merely one case and one control were found to be heterozygote for E480X, while no other MUTYH mutations were found [64]. These results suggest that MUTYH mutations are unlikely to be of significant importance to development of Polyposis among Indian individuals. Other MUTYH variants found in noteworthy allelic frequencies in characteristic populations are: c.1145delC (found in Italian MAP patients in allelic frequencies of 0.07-0.11 [65, 66]), A473D (found in Finnish Polyposis and CRC patients, in the latter group with an allelic frequency of 0.01 [67]) and E383fsX451 (found in Portuguese MAP patients with allelic frequencies of 0.15-0.19 [68, 69]).

In addition, other MUTYH polymorphisms without any apparent pathogenic importance have been found. The most frequent of these are V22M, Q388H and S515F, the allelic frequencies of which in healthy control groups are found to be equivalent to those found among MAP patients [21, 39-41, 48, 56, 67, 69-78].
Fig. (2). Mean Allelic Frequencies among Carriers of MUTYH Germline Mutations.

N= Number of biallelic and monoallelic MUTYH germline mutation carriers. Included are mutations which are believed to be of pathogenic significance, and which are found with an allelic frequency of > 0.03 in mutation carriers from the respective countries.

The figure is based on data collected from the following studies: Al-Tassan et al., 2002; Jones et al., 2002; Sieber et al., 2003; Sampson et al., 2003; Halford et al., 2004; Fleischmann et al., 2004; Gismondi et al., 2004; Isidro et al., 2004; Venesio et al., 2004; Wang et al., 2004; Aceto et al., 2005; Kairupan et al., 2005; Miyaki et al., 2005; Leite et al., 2005; Aretz et al., 2006; Kanter-Smoler et al., 2006; Niessen et al., 2006; Russel et al., 2006, Ajith Kumar et al., 2007.

Fig. (3). Mean Allelic Frequencies of p. Y179C and p. G396D in Background Populations.

N= Number of tested individuals. The individuals tested belong to control groups without Polyposis.

The figure is based on data collected from the following studies: Al-Tassan et al., 2002; Enholm et al., 2003; Sieber et al., 2003; Croitoru et al., 2004; Isidro et al., 2004; Leite et al., Miyaki et al., 2005; Peterlongo et al., 2005; Zhou et al., 2005; Aretz et al., 2006; Kairupan et al., 2005; Kanter-Smoler et al., 2006; Niessen et al., 2006; Russel et al., 2006.
FUNCTIONAL CONSEQUENCES OF SPECIFIC GERMLINE MUTYH MUTATIONS

The two most common MUTYH mutations, the Y179C and G396D, are situated in the catalytic and C terminal domains of MUTYH, respectively. Both of these MUTYH residues have important roles in the recognition of 8-oxo-G in A:8-oxo-G mispairs [31, 32, 49, 79, 80]. Accordingly, functional studies of murine variants corresponding to Y179C and G396D have indicated compromised substrate recognition as a consequence of these mutations [81].

The Y179C mutation is located in the N-terminal end of the MUTYH, and is part of the pseudo-HhH (helix-hairpin-helix) motif in the catalytic region of the MUTYH. This region is thought to promote the base-flipping mechanism in substrate recognition, participate in maintaining stability during this process, as well as being involved in DNA binding [30, 49, 79, 82] (thoroughly reviewed in [31]). Studies of biallelic Y179C mutations in human cell lines illustrate, that defective MUTYH function results from both significantly reduced levels of MUTYH protein (Protein levels of 5-10% compared to wild-type MUTYH levels) as well as from reduced binding and cleavage ability towards the mispaired substrates [83].

G396D is located in the C-terminal domain of the MUTYH, which is thought to be responsible for 8-oxo-G recognition and binding, as well as mediating the base-flipping mechanism [31, 32, 49, 79, 80]. Human cell lines with biallelic G396D mutations show defective MUTYH function as a result of the production of a dysfunctional protein. The protein shows both reduced binding activity of mispaired substrates (about 50% of the wild-type MUTYH activity), as well as lower rates of repair, compared to the wild type MUTYH [83]. However, the MUTYH protein levels in these cell lines were found to be equivalent to the levels of wild type protein, indicating that protein instability is not a consequence of G396D mutations [83].

These results are consistent with previous studies of the corresponding E. coli MutY variants, which have been shown to severely compromise the activity of the glycosylase [21, 49]. In one study, the Y179C and G396D variants showed a 98% and a 86% reduction in adenine removal from a G:A substrate, compared to the wild-type protein, respectively [21]. Likewise, both MutY variants exhibit significantly reduced rates of adenine removal compared to wild-type MutY. The variants corresponding to G396D and Y179C, showing a 6-fold and 80-fold slower rate, respectively [49]. Also, considerably reduced binding affinities for G and 8-oxo-G substrates were observed in the cases of both variants [49].

Functional studies of additional MUTYH mutations have also been conducted, although none as comprehensive as for Y179C or G396D, for which reason merely a short overview will be given in the present review: R182C, R182H, R185Q and G189E, all are located in the pseudo-HhH motif of the MUTYH catalytic domain, are considered to induce functional MUTYH changes comparable to those observed in cell lines with Y179C variants [57, 69, 74, 75]. The missense variants P405L and A473D, both located in the C-terminal domain, are both supposed to have functional significance [67, 74].

Functional analyses of two MUTYH mutations, which lie close to or within the MSH6-binding domain of the MUTYH gene, have been preformed: The variants R241W and V246F have preserved their ability to physically interact with MSH6, but both show reduced MUTYH function [54]. R245C, also located near the MSH6 binding domain, is likewise assumed to compromise the interaction between the MUTYH and MSH6 [55].

THE SIGNIFICANCE OF THE MUTYH GENE IN RELATION TO THE DEVELOPMENT OF POLYPOSIS

Results from several studies indicate, that mutations in the MUTYH gene, more often than previously assumed, are the disease causing factor in Polyposis patients. Sieber and colleagues found, that an AFAP-like phenotype is more often caused by germline mutations in the MUTYH gene than in the APC gene [40]. Another study of patients with AFAP phenotypes, found APC germline mutations and MUTYH biallelic mutations in equal numbers of families [19]. Consistent with these results, a study of CRC patients by Enholm et al. has suggested, that the contributions of germline mutations in the APC gene and the MUTYH gene are fairly equal [84].

In studies of Polyposis patients (N=995), it has been found that 5-22% of the patients with 3-100 adenomas and 7.5-17% of those with over 100 adenomas had biallelic mutations in the MUTYH gene [19, 40, 85]. Furthermore, several studies have found that none of the patients, who had biallelic germline MUTYH mutations (N=63), presented with a phenotype consistent with severe classical FAP, the criteria being: more than 100 polyps or early-onset CRC (before the age of 50 years), and the development of more than 100 polyps before the age of 35 years, respectively [40, 85, 86]. These results indicate that a higher proportion of AFAP-like phenotypes are caused by MUTYH mutations compared to FAP-like phenotypes.

CLINICAL FEATURES OF MAP

Table 2 provides an overview of phenotypic characteristics of MAP, using information gathered from different studies of MAP patients.

MODE OF INHERITANCE

MAP is an autosomal recessive disease, caused by biallelic mutations in the MUTYH gene. The majority of family histories of MAP patients were found to be consistent with a recessive inheritance, typically with affected siblings, but unaffected parents [19, 21, 38-40, 62, 65, 66, 68, 69, 74, 75, 85-87]. Furthermore, Russel et al. found no MUTYH germ-line mutations among Polyposis patients, who were negative for APC germline mutations, and had a family anamnese consistent with a dominant mode of inheritance [75]. In contrast, other studies have found family histories appearing to follow a dominant mode of inheritance among MAP patients in about 15-30% of the studied cases [68, 69, 74, 78, 85]. However, it is possible, that a recessive trait as MAP, due to a relatively high frequency of heterozygote mutation carriers in some populations, can mimic dominant inheritance, dis-
playing a pseudo-dominant mode of inheritance, especially in cases of parental consanguinity. On the other hand, some have proposed a co-dominant model for mode of inheritance, suggesting an increased CRC risk for monoallelic MUTYH germline mutation carriers compared to non-carriers, as will be discussed further [48, 57, 70, 71, 84, 88-92].

In studies of healthy controls, no unaffected individuals, who were homozygote for germline MUTYH mutations, have been found [57, 70, 71, 84, 90, 91] and Table 3. This indicates that biallelic germline MUTYH mutations are highly penetrant. Accordingly, in a case-control study of CRC patients (N= 2,239), all homozygote MUTYH carriers (N=12) developed CRC before the age of 60 years [92].

**AGE AT TIME OF CLINICAL DIAGNOSIS OF MAP**

The average age at time of clinical diagnosis among MAP patients is typically around 47 years (range: 13-72 years, N=106) [19, 57, 62, 76, 77, 85-87] and Table 2.

Characteristically, the age at time of clinical diagnosis is higher among patients with biallelic MUTYH germline mutations compared to Polyposis patients without MUTYH mutations. This applies both to studied Polyposis patients with <100 adenomas [57, 65, 74] as well as to Polyposis patients with >100 adenomas, who are also negative for APC germline mutations [40, 93]. In one of these studies (N= 58) this result was statistically significantly [74].

Because of the recessive mode of inheritance, the identification of MAP patients is complicated, as MAP patients often seem to be sporadic cases with no family history of the disease at clinical presentation. Consequently, most MAP patients are diagnosed due to symptoms and not as a result of prophylactic screening, unlike many FAP and AFAP patients [85]. In accordance to this, many MAP patients are also typically discovered at a later time in the course of their disease than other groups of Polyposis patients. For example, this can be illustrated by the higher proportion of MAP patients, who have already developed CRC at the time of their clinical diagnosis as compared to FAP patients (see later).

**DEVELOPMENT OF ADENOMAS**

In several studies of Polyposis patients, the phenotypes in regard to the number of adenomas are generally seen to be more severe in MAP patients compared to the phenotypes of AFAP patients, but milder compared to those of classic FAP patients [40, 61, 68, 74, 85].

Characteristically, MAP patients develop between 10-100 adenomas (Table 2), which is a smaller number than seen in the classic FAP phenotype. Several studies of Polyposis patients have all found the highest incidence of bialle-
lic MUTYH mutations in groups of patients with between 15-100 adenomas [40, 57, 61, 69, 85]. In the applied studies, the incidences of biallelic MUTYH mutations in the groups of patients having 15-100 adenomas, were found to be between 16-47% (N=835). However, none of the results were statistically significant.

In a recent study of AFAP patients (N=140), comparing the clinical features of patients with APC (N=93) and biallelic MUTYH germline mutations (N=26), no significant differences between the two groups were found [19]. However, this result might be biased due to the smaller sample size of the MAP patients compared to the patients with germline APC mutations. When compared to Polyposis patients without mutations in neither the MUTYH nor the APC gene, MAP patients seemed to develop the lowest number of adenomas, although no statistically significant results have been found [40, 77].

The morphology of the adenomas appears to be similar regardless of whether their occurrence is caused by germline mutations in the MUTYH or in the APC gene [40, 65]. Likewise, microadenomas have been found in patients with both genotypes [40, 46, 62].

DEVELOPMENT OF CRC

In several studies of CRC patients (N=3,320), biallelic germline MUTYH mutations were found in 0.4-1.9% of all cases (Table 3). Based on these results, the contribution of biallelic MUTYH mutations to CRC seems to correspond to,
or even be greater than that of FAP, FAP accounting for less than 0.1% of all CRC cases, as found by Bülow [10].

In two other studies of CRC patients (N= 2,268), a significant association between biallelic germline mutations in the MUTYH gene and the development of CRC, has been determined [75, 92]. In one of these studies (N=2,239), MAP patients were found to have a 93-fold increased risk of developing CRC compared to a group of unaffected controls from the general population [92].

Among MAP patients, the average age of CRC onset is found to be 47 years (range: 29-72 years) [19, 62, 68, 75, 85, 87]. The frequency of patients with a synchronous CRC at time of diagnosis is greater among MAP patients compared to among FAP patients [69, 74, 93]. These results comply with the fact that FAP patients generally are diagnosed earlier than MAP patients, facilitated by the dominant mode of inheritance of FAP and the use of Polyposis Registers in many countries. Therefore, the prophylactic treatment of FAP prevents the development of CRC in a higher number of patients. As both probands and call-ups were included in the applied studies, and as none of the results were statistically significant, more specific studies examining only probands are needed.

There have been many inconsistent results regarding the typical location of carcinoma in MAP patients. Over-representation of both right and left sided CRC has been demonstrated [19, 39, 46, 71, 74, 75, 87]. Presumably, the location of CRC among MAP patients should not be considered important, as the prognosis seems to be independent of the CRC location [74].

**ASSOCIATED CANCERS AND EXTRACOLONIC MANIFESTATIONS**

The manifestation of other primary cancers than CRC or other extracolonic manifestations are less frequent among MAP patients compared to among FAP patients [74]. Several studies have failed to report other cancers than CRC or any extracolonic features among the examined MAP patients [38, 40, 57, 61, 65, 66, 69, 71, 93]. The methods of clinical examination were not specified in the applied studies. For this reason, bias could be suspected, as patient information is often gathered from several different databases without assurance that all patients were systematically examined.

Conversely, several studies have described the occurrence of extracolonic features in MAP patients, mostly upper gastrointestinal lesions [37, 40, 62, 63, 74, 75, 78, 84-87, 94-96], see Table 4.

However, these results should be regarded with reservations, as the numbers of examined MAP patients in the majority of the studies were very small. In addition, bias could result from a difference in the methods of investigation used in the individual studies, as these are only sparsely describe in most of the studies. Also, some of the reported extracolonic manifestations were reported in very low frequencies among the examined MAP patients. They are therefore more likely to be present by chance, rather than being associated with biallelic MUTYH mutations.

The described findings suggest that extracolonic manifestations are generally not a part of the characteristic MAP phenotype, but can occur. However, further studies with more systematic and thorough investigation of MAP patients are needed to address this issue.

**HETEROZYGOTE AND CRC RISK**

At present, no conclusive evidence has been found, that monoallelic carriers of MUTYH germline mutations have an increased CRC risk compared to the general population. However, as seen in Table 3, percentages of carriers of monoallelic germline MUTYH mutations are generally larger among CRC patients, than the same percentages among the corresponding background populations. Furthermore, several studies have shown a tendency for a slightly elevated CRC risk [40, 41, 57, 62, 66, 70, 71, 85, 88, 90, 93, 97], especially in those over 55 years of age [41, 89, 92, 98]. However, reservations towards these studies should be taken, as results from the mentioned studies have failed to be convincing, with merely one study achieving a slight statistical significance [97]. Also, some of the mentioned studies have been criticized for the statistical methods used [98, 99], and meta-analyses of the different studies have found inconsistent results [89, 98].

A suggested explanatory model for a possible association between monoallelic MUTYH mutations and a co-dominant mode of inheritance of CRC, is LOH of chromosome 1p, where the MUTYH gene in located, possibly representing an early event in CRC tumorgenesis [48, 70]. According to this model, loss of the wild-type MUTYH gene on 1p in monoallelic MUTYH mutation carriers is likely to contribute to an increased CRC risk, as 1p LOH has been found in tumors from monoallelic MUTYH mutation carriers [48, 77]. In contrast, other studies that have investigated 1p LOH in tumors from monoallelic MUTYH mutation carriers have failed to find results of sufficient significance to support this theory [40, 84].

On the other hand, Peterlongo et al. combined results from 9 case-control studies of CRC patients (Cases: N= 2,707 and controls: N= 2,321), and were not able to demonstrate a significant association between monoallelic carriers of MUTYH germline mutations and the development of CRC [91]. Consequently, as it—in the worst of cases—can only be a matter of a minimally increased CRC risk compared to the risk of the general population, it seems unlikely, that the tendency for an increased CRC risk in heterozygote individuals is powerful enough to be of diagnostic or prophylactic importance.

The exact role of monoallelic MUTYH germline mutations in CRC tumorgenesis is still uncertain, but as mentioned earlier, interactions with other genes, for example a MMR gene, are possibly of significance.

In addition, several large studies of Polyposis patients have found frequencies of monoallelic MUTYH mutation carriers that correspond fairly well to those of biallelic carriers, see Table 5.

This indicates that monoallelic MUTYH germline mutations may be associated with a Polyposis phenotype. However, as seen in Table 5, the reported frequencies of mono- and biallelic carriers vary considerably among studies. This is likely to be a result of differing inclusion criteria, as these are not thoroughly described in all of the applied studies.
The significance of monoallelic \textit{MUTYH} mutations needs to be assessed further in comparable studies on the subject.

**GENETIC COUNSELING AND PROPHYLAXIS**

Frequently, MAP patients have already developed CRC at the time of clinical diagnosis, before prophylactic treatment can be initiated. Genetic testing and counseling of individuals at risk of developing MAP, is essential for the future prospect of MAP patients, so that prophylactic screening can be initiated. In this context it is important that knowledge about the disease and mode of inheritance is continuously searched for and utilized for the organization of guidelines, which we believe will assure the best treatment for these patients.

**DETERMINATION OF THE GENOTYPE**

Based on the recessive mode of inheritance most commonly seen in MAP families, siblings to an affected individual have a 25% a priori risk of disease. Consequently, determination of the genotype is especially important in these individuals [62, 75, 92]. In some MAP families, the mode of inheritance is pseudo-dominant, i.e. appears to be dominant although in reality recessive [19, 74, 87].

In practice, it is important to search for germline mutations in both the \textit{APC} and the \textit{MUTYH} gene. This applies to both individuals having a familiar disposition for multiple adenomas and/or CRC as well as in apparently sporadic CRC cases, if the clinical presentation gives hints of a Polyposis syndrome. In cases with a positive family anamnesis, the most probable mode of inheritance can guide the assessment of which gene to start with, i.e. the \textit{APC} or the \textit{MUTYH} gene when a dominant mode or recessive mode is seen, respectively [19].

Furthermore, the characteristic mutations in specific populations makes it possible to target the MAP screening in accordance with ethnic background, thereby making the screening more efficient, when the background of the patient is known [57, 64].

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### Table 4. Extracolonic Manifestations Reported in MAP Patients

| Extracolonic Manifestation | Number of MAP Patients (with Biallelic \textit{MUTYH} Mutations) Examined | Overall Percentage of MAP Patients with Extracolonic Manifestations | Reported by |
|---------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------|-------------|
| Duodenal lesions (Adenomas and/or cancer) | N= 154                                                                 | 13%                                                                          | [40, 62, 74, 75, 77, 85, 86, 87, 88, 94] |
| Gastric lesions (Fundic gland polyps or stomach cancer) | N= 133                                                                 | 8%                                                                           | [62, 85, 86, 87, 88] |
| CHRPE (Congenital hypertrophy of the retinal pigment epithelium) | N= 22                                                                 | 18%                                                                          | [40, 93] |
| Osteomas                  | N=14                                                                  | 14%                                                                          | [93]        |
| Desmiod cysts            | N=14                                                                  | 7%                                                                           | [93]        |
| Oesophageal cancer        | N=16                                                                  | 6%                                                                           | [87]        |
| Thyroid carcinoma         | N= 57                                                                 | 4%                                                                           | [85, 95]    |
| Breast cancer (N=22 (female MAP patients)*1) | 18%                                                                  | [87]                                                                          |
| Dental cysts             | N=14                                                                  | 7%                                                                           | [93]        |
| Tooth agenesis           | N=7                                                                   | 14%                                                                          | [78]        |
| Lipoma                   | N=56                                                                  | 4%                                                                           | [85]        |
| Multiple sebaceous adenomas (N=2 (case reports)) | 100%                                                                 | [63, 95]                                                                      |
| Sebaceous carcinoma (N=1*2 (case reports)) | 100%                                                                 | [37]                                                                          |
| Pilomatricomas           | N=2 (case reports)                                                   | 100%                                                                         | [96]        |
| Melanoma                 | N=4                                                                   | 25%                                                                          | [84]        |
| Basocellular carcinoma of the skin | N=49                                                                  | 2%                                                                           | [88]        |
| CNS carcinoma            | N=55                                                                  | 4%                                                                           | [77, 88]    |
| Leukemia                 | N=6                                                                   | 17%                                                                          | [77]        |
| Uterus cancer            | N=49                                                                  | 2%                                                                           | [88]        |

*1 An additional case of breast cancer in a biallelic \textit{MUTYH} mutation carrier was reported by Olschwang et al. 2007, however no information on sex proportions in the examined MAP patients was reported [88].

*2 This Patient was found to have biallelic \textit{MUTYH} mutations, although no colorectal adenomas were found at the age of 53 years old. However, one case of be early-onset CRC (before 50 years of age) was seen in the patient’s family. This patient had both endometrial cancer as well as sebaceous carcinoma [37].
As Y179C and G396D at present are the most frequently found mutations in Caucasian MAP patients, an obvious possibility would be to screen specifically for these in Caucasian individuals [65]. A disadvantage of such a selective screening is, that MAP patients, who are compound heterozygote for just one of these variants, or homozygote for other MUTYH variants, would be missed [57, 77, 86, 100]. Eliason et al. demonstrated an increased clinical sensitivity for the detection of MUTYH mutations in their study, when all exons and intron-exon boundaries of the MUTYH gene were screened, compared to the sole testing for Y179C and G396D [100]. Accordingly, all coding regions of the MUTYH gene should be screened in individuals found to be heterozygote for Y179C or G396D to establish their true genetic status [85, 86, 100]. Recently, Piccioli et al. have designed specific assays for detecting the 6 most frequently found MUTYH mutations using a multiplex T-ARMS-PCR method [101]. This method has been shown to be both accurate and inexpensive, and can furthermore be adapted according to the specific frequencies of MUTYH mutations in different population groups [101]. In patients presenting with an atypical MAP phenotype, i.e. <10 colorectal adenomas or familial mismatch repair proficient CRCs, van Puijenbroek et al. have proposed a prescreening method also considered to be cost-effective [102]. This method consists of the screening of tumors for KRAS2 c.34G > T, a somatic mutation in the KRAS2 gene shown to be more common in MAP patients compared to sporadic CRC cases. This should be followed by screening for population specific MUTYH mutations in cases positive for the aforementioned KRAS2 mutation [102].

The frequencies of Y179C and G396D in the general population are low compared to the occurrence among MAP patients (Fig. 2 and 3), and for this reason, there is at present no indications for MAP screening of the general population [75, 84, 85]. However, genetic testing of spouses of MUTYH mutation carriers to assess the genotype and corresponding disease risk of offspring, has been recommended [77].

**PROPHYLAXIS AND TREATMENT**

The prophylactic surveillance of MAP patients is recently reviewed by Vasen et al. in [16]. Here a surveillance protocol in accordance with the recommendations for AFAP patients is suggested [16]. However, some recommend beginning at the age of 20-25 years, which is later compared to AFAP recommendations [19]. The surveillance of MAP pa-
tients should consist of colonoscopy in two-yearly intervals as opposed to sigmoidoscopy in FAP patients, due to the often more attenuated phenotype and distal polyp location of MAP compared to FAP [16, 19]. Furthermore, upper gastrointestinal endoscopy starting from the age of 25-30 years is advised in MAP patients [16], even though the question of upper gastrointestinal endoscopy ought to be further investigated in studies, more specifically researching extracolonic manifestations in MAP patients.

Naturally, the outlined recommendations should be adjusted according to the number, size and degree of dysplasia of the adenomas of the individual patient [57].

Since MAP patients typically develop less adenomas than FAP patients, the prophylactic treatment of MAP patients should as a starting point be aimed at colonoscopy with polypectomy. It could however be appropriate to apply colectomy in MAP patients developing a particularly large number or advanced adenomas [16, 19, 68].

GENETIC COUNSELING OF HETEROZYGOTE CARRIERS OF MUTYH GERMLINE MUTATIONS

In our judgment, there is no indication for prophylactic surveillance in heterozygote carriers of MUTYH germline mutations at the present time. However, we believe that relatives at risk of developing MAP should be searched for on the basis of family anamnesis, if MUTYH germline mutations are discovered, in order for appropriate measures to be made on this basis.

CONCLUSION AND FUTURE PERSPECTIVES

The full extent of the significance of MUTYH in the development of CRC is yet to be resolved. Biallelic germline mutations in the MUTYH gene are found to be associated with a markedly increased risk of developing Polyposis and CRC. The interactions between MUTYH and the MMR system could play a role in the CRC tumorgenesis in MAP patients.

At times, it can be difficult to distinguish between the phenotypes of FAP, AFAP, Lynch syndrome and MAP. Aspects regarding phenotypic differences between MAP patients and other Polyposis patients form the base of the recommendations for counseling and prophylactic treatment of MAP patients, which is stated here. Germline mutations found in the MUTYH gene have shown a great ethnic variability, and further knowledge about this could be used to target the genetic screening of Polyposis patients towards specific population groups. Genetic screening for germline mutations in the MUTYH gene as well as in the APC gene should be performed on equal terms, perhaps guided by the most probable mode of inheritance. Prophylactic surveillance of MAP patients could be colonoscopy with polypectomy in mind from 20-25 years of age.

In the future, more MAP patients could be identified before developing CRC by establishing MAP registers and finding call-up patients based on family anamnesis, as it is currently done for FAP patients in many countries. In this way the future prospects of MAP patients could be improved markedly.

SEARCH CRITERIA

The applied papers were all found in the PubMed database using the following terms: MYH Associated Polyposis /MUTYH Associated Polyposis /MutYH Associated Polyposis. The search was only for material published in English and was made without time-limitation. All papers published between 01/01 2002 and 01/02 2008. We also searched reference lists of relevant papers.

ABBREVIATIONS

| A          | Adenine          |
|------------|------------------|
| AFAP       | Attenuated Familial Adenomatous Polyposis |
| APC        | Adenomatous Polyposis Coli |
| BER        | Base Excision Repair |
| C          | Cytosine         |
| CRC        | Colorectal Cancer |
| FAP        | Familial Adenomatous Polyposis |
| G          | Guanine          |
| HhH        | Helix-Hairpin-Helix |
| HNPCC      | Hereditary Non-Polyposis Colorectal Cancer |
| LOH        | Loss of Heterozygosity |
| LOVD       | Leiden Open Variation Database |
| MAP        | MUTYH Associated Polyposis |
| MMR        | Mismatch Repair  |
| MSI        | Microsatellite Instability |
| MUTYH gene | MYH gene = MutY Human Homolog gene |
| ROS        | Reactive Oxygen Species |
| 8-oxoG     | 8-oxo-7,8-dihydroxy-2'-deoxyguanosine |

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