MutYH (MYH) and colorectal cancer

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Abstract
MAP (MutYH-associated polyposis) is a recently described colorectal adenoma and carcinoma predisposition syndrome that is associated with biallelic-inherited mutations of the human MutY homologue gene, MutYH. MutYH is often also termed MYH. MAP tumours display a mutational signature of somatic guanine-to-thymine transversion mutations in the adenomatous polyposis coli and K-ras genes, reflecting the normal role of MutYH in the base excision repair of adenines misincorporated opposite 7,8-dihydro-8-oxoguanine, a prevalent and stable product of oxidative damage to DNA. However, the full genetic pathway of MAP tumorigenesis has not been elucidated.

FAP (familial adenomatous polyposis) and MAP (MutYH-associated polyposis)
Most CRCs (colorectal cancers) are thought to develop from pre-existing adenomas, and CRC risk is increased in patients who have multiple adenomatous polyps in the colorectum. FAP is a well-characterized autosomal dominant disorder in which hundreds or thousands of colorectal adenomas develop, usually during late childhood or early adult life [1]. This leads to inevitable CRC unless prophylactic surgery is performed to remove the large bowel. Patients with FAP may also develop extra-colonic manifestations of the disorder, including skin and bone cysts, duodenal adenomas and cancer, desmoid tumours and asymptomatic retinal abnormalities. FAP is caused by inherited mutations in the APC (adenomatous polyposis coli) gene. An attenuated form of the disease, AFAP (attenuated familial adenomatous polyposis), also occurs, associated with smaller numbers of adenomas and later clinical presentation. The risk of CRC is very high, but typically occurs later in life than in FAP. AFAP is less well characterized than FAP. It is caused by mutations in the 3′ and 5′ ends of the APC gene and in the alternatively spliced region of exon 9 [1]. Recently, we have shown that colorectal phenotypes indistinguishable from FAP and AFAP can also be associated with biallelic inherited mutations of the BER (base excision repair) gene, MutYH (human MutY homologue), in the absence of demonstrable inherited mutations of APC [2–4]. The MutYH gene is also commonly called MYH, although this terminology is strictly incorrect, as it is the gene symbol for the myosin heavy-chain encoding gene. MutYH–associated polyposis is increasingly being referred to as MAP to distinguish it from FAP. The established role of MutYH is in BER of adenine residues that have been misincorporated opposite guanine or 8-oxoG (7,8-dihydro-8-oxoguanine) [5]. The pattern of somatic mutation observed in adenomas and CRCs from MAP patients, together with functional data on mutant MAP–associated alleles, supports a causal relationship between MutYH–associated deficiency in BER and colorectal tumorigenesis in MAP [2,6].

MutYH and BER
Reactive oxygen species generated during aerobic metabolism represent a potent source of DNA damage. Notably, the oxidation product 8-oxoG is stable and readily mispairs with adenine (instead of cytosine). Unless repaired, this leads to G:C to T:A transversion at the next round of DNA replication. MutYH is a DNA glycosylase that plays a key role in BER of 8-oxoG:A and G:A mismatches by removing the mismatched adenine. Other key components include OGG1 (8-oxoG DNA N-glycosylase 1), an orthologue of MuTM, that removes 8-oxoG from duplex DNA, and MTH1, a MutT orthologue that hydrolyses 8-oxoG to prevent its incorporation into nascent DNA (Figure 1). Until the recent recognition of MAP, no inherited deficiencies of BER had been associated with disease.

Identification and characterization of MAP
MAP was first identified during the investigation of ‘family N’, in which three of seven siblings were affected by AFAP–like phenotypes in the absence of an identifiable inherited truncating mutation in APC [2]. Characterization of somatic mutations of APC in colorectal tumours from the affected siblings showed that the majority (15 out of 18) were G:C to T:A transversions, suggesting the possibility of an inherited deficiency in the repair of 8-oxoG-related mutations. Al-Tassan et al. [2] also noted that the somatic APC mutations occurred almost exclusively at GAA sequences to generate TAA stop codons that predicted protein truncation. This specificity was observed despite the presence of an equal number of non-GAA sequences in APC that were potentially mutable to stop codons by G:C to T:A transversions.

Key words: adenomatous polyposis coli (APC), colorectal cancer, familial adenomatous polyposis (FAP), MutYH (MYH), MutYH–associated polyposis (MAP).
Abbreviations used: APC, adenomatous polyposis coli; AFAP, attenuated familial adenomatous polyposis; BER, base excision repair; CRC, colorectal cancer; FA, 2-fluoro-2-deoxyadenosine; FAP, familial adenomatous polyposis; MAP, MutYH–associated polyposis; MMR, mismatch repair; MSH, microsatellite instability; MuTM, human MutY homologue; N′-glycosylase 1, 8-oxoG DNA N′-glycosylase 1.
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The enzymes participate in the prevention and repair of 8-oxoG-associated DNA damage. MutT is an 8-oxo-dGTPase, preventing incorporation into nascent DNA. MutM is a DNA glycosylase that excises the oxidized base from 8-oxoG·C pairs. MutY is a DNA glycosylase that excises adenine residues that have been misincorporated opposite 8-oxoG.

Screening of OGG1, MTH1 and MutYH (MYH) identified germline biallelic MutYH missense mutations, Y165C and G382D, in each of the three affected siblings. In contrast, family members who were heterozygous for either mutation showed no abnormality on colonoscopy. In vitro assay of the glycosylase activities of the corresponding Escherichia coli mutations MutY Y82C and G253D revealed minimal residual activity in the case of the Y82C mutant and a significant, but more modest, reduction in the activity of G253D. By linking the pattern of somatic APC mutations to the presence of functionally significant biallelic mutations of MutYH, Al-Tassan et al. [2] provided the crucial first evidence that MAP represented a novel recessive colorectal adenoma and carcinoma predisposition syndrome. The functional consequences of the Y165C and G382D mutations were later studied in human MutYH proteins that were shown to be severely compromised [6].

Mutation analysis of MutYH has now been undertaken in several series of patients with FAP-like and AFAP-like phenotypes in whom no inherited APC mutation could be identified [3,4,7–11]. Biallelic MutYH mutations have been identified in approx. 50% of cases reported by Sampson et al. [4] and by Sieber et al. [7]. Duodenal adenomas have been reported in some patients [4,7], and clinical studies of further series of patients are required to establish whether other extra-colonic manifestations also occur at significant frequencies. More than 20 mutations that are likely to be pathogenic have been identified to date. Non-truncating mutations appear to cluster in known functional domains of MutYH, while truncating mutations have been identified throughout the coding region (Figure 2). While Y165C and G382D appear to be much the most frequent mutations in Northern Europeans, other recurrent mutations have been identified in Italian (1395delGGA) and Portuguese (1186–1187insGG) patients, and the truncating mutation E466X has been identified in at least four apparently unrelated Gujarati families (Figure 3) [4,8,10].

Functional analysis of MutYH mutations
Few MutYH mutations have so far been subject to functional analysis. Chmeil et al. [6] investigated the Y165C and G382D mutants and showed that they have a reduced capacity to complement MutY deficiency in E. coli. They also assessed substrate affinity using the substrate analogue FA (2′-fluoro-2′deoxyadenosine). Both mutations severely diminished the ability to recognize FA and to discriminate between guanine and 8-oxoG. The glycosylase activity of both mutant and wild-type proteins was also noted to be less efficient in the sequence context that had previously been noted to be preferentially mutated in APC in MAP tumours [2,3]. Two further missense mutations, R227W and V232F, that lie close to, or within, the putative MSH6-binding domain of MutYH,
Figure 2 | Likely pathogenic mutations affecting MutYH that have been reported in the literature

Only mutations that have been identified in MAP cases with biallelic mutations are included. Approximate positions of putative functional domains are indicated as shaded areas in relation to a representation of the MutYH coding region. APE1, apurinic endonuclease 1; HhH, helix-hairpin-helix; PCNA, proliferating-cell nuclear antigen; RPA, replication protein A.

Figure 3 | Relative frequency of MutYH mutations in MAP patients as currently reported in the literature

Only mutations from cases with biallelic mutations are included.

have also been investigated [13]. Neither mutation affected MSH6 binding, but both mutant proteins were compromised in A:8-oxoG binding, in their glycosylase activities and in complementation of MutY deficiency in E. coli.

MutYH and MMR (mismatch repair)
The DNA MMR system serves to increase the fidelity of DNA replication and genetic recombination and the proteins involved also have roles in transcription-coupled repair, meiosis, cell cycle arrest and apoptosis. Three of the human homologues of E. coli MutS, called MSH2, MSH3 and MSH6, form the heterodimers hMutSα (MSH2–MSH6) and hMutSβ (MSH2–MSH3). These recognize base–base mismatches and short insertion-deletion loops (hMutSα) and long insertion-deletion loops (hMutSβ) [14,15]. MutYH has been shown to interact directly with hMutSα by binding to the MSH6 subunit, and this stimulates the DNA binding and glycosylase activity of MutYH with 8-oxoG:A mismatches. Thus MutYH-mediated BER may co-operate with MMR to prevent 8-oxoG-mediated mutagenesis [16].

Heterozygosity and CRC risk
It is reasonable to suppose that, in heterozygotes, somatic inactivation of the wild-type MutYH allele occurs in a proportion of colonic crypt stem cells. If such ‘second hits’ are associated with the acquisition of a mutator phenotype at the cellular level (a supposition for which there is no experimental evidence at present), then predisposition to CRC might result. Consistent with this possibility, two studies [12,17] have identified more frequent chromosome 1p loss of heterozygosity (corresponding to the chromosomal location of MutYH) in CRCs from carriers of germline MutYH variants than in CRCs from non-carriers, although the numbers studied are very small. However, studies of the relatives of patients with MAP have not provided robust evidence of an excess of CRC among heterozygous carriers [4,12]. Some studies have noted more heterozygotes among cases with multiple colorectal adenomas than among controls, but in
No somatic involvement of MutYH in CRC?

Most genes that result in a very high risk of CRC when mutated in the germline have also been shown to be subject to somatic inactivation in sporadic CRC. APC is mutated in the germline in FAP and is subject to biallelic somatic mutation in the majority of colorectal adenomas and carcinomas. The MMR genes are mutated in the germline in hereditary non-polyposis CRC and MLH1 is inactivated (usually via promoter methylation) in some 10–15% of sporadic CRCs. Germline mutations of SMAD4 are associated with juvenile polyposis (hamartomatous colorectal polyps and later CRC risk), while somatic inactivation is associated with the later stages of adenoma progression. Only one study has so far addressed the question of whether somatic inactivation of MutYH plays a significant role in sporadic colorectal tumorigenesis. Halford et al. [21] found no somatic mutations of MutYH in any of 75 unselected CRCs or 35 CRC cell lines. MutYH mRNA and protein was expressed in all the cell lines, indicating that epigenetic silencing was also unlikely to occur at a significant frequency.

Pathways to CRC in MAP

Established genetic pathways of CRC development include those associated with chromosomal instability and MSI (microsatellite instability). The steps to CRC in MAP have not been extensively studied at the present time. It has been shown that MSI is not a feature of MAP tumours [2,22]. In addition to the initiating G-to-T transversion mutations of APC, a proportion of MAP adenomas show a specific, and apparently exclusive, activating missense mutation, G12C of K-ras [22,23], that also results from G to T transversion. Lipton et al. [22] reported the most comprehensive study of MAP tumours to date, and found that they appeared to be near diploid on flow cytometry and showed no evidence of mutations affecting BRAF (v-raf murine sarcoma viral oncogene homologue B1), SMAD4 or TGFβ (transforming growth factor β).

MutYH and clinical management

Current data indicate that the colorectal phenotype in MAP is extremely variable. Most early studies have been undertaken in ‘polyposis registers’. Although these have established a clear association between biallelic mutations of MutYH and adenomatous colorectal polyposis, such studies are inevitably subject to ascertainment bias. It is clear that some cases have very few (if any) adenomas [4,12]. At present, it would be wise to adopt a cautious approach, including annual or biennial colonoscopic surveillance, for individuals with biallelic mutations, probably commencing in teenage years and certainly by their early twenties. Although duodenal adenomas have been reported in MAP patients, the case for surveillance is unclear (as the therapeutic options for duodenal adenoma present their own limitations and risks), and prospective studies of upper gastrointestinal endoscopy are required. Surgical options for colorectal disease need to be tailored to the individual patient, since tumour burden can vary from a count of one to many hundreds. A significant proportion of patients will, however, require definitive surgery to remove the large bowel, as for FAP. It is not yet clear whether there is an increased risk of cancers outside the gastrointestinal tract, and surveillance protocols are likely to require modification as the phenotype and natural history of MAP is elucidated.

Since there is little evidence at the present time that there is a clinically significant increased risk of CRC in MutYH heterozygotes, screening measures beyond those recommended in the general population cannot be justified. However, this is a position that may need to be revised as more extensive data are collected.

Genetic testing for MutYH (MYH) is indicated in those with phenotypic features of MAP, primarily to guide surveillance needs in relatives. MAP needs to be distinguished from FAP and AFAP, as it is the siblings, rather than offspring, of MAP cases who are most likely to require further investigation. Gene testing can be used to identify which siblings of MAP cases are at risk themselves and also to clarify the genetic status of spouses of those with biallelic mutations, so that (usually) their offspring can be reassured. In due course, there may also be a place for wider MutYH gene testing of incident CRC cases [24], since current data indicate that polypos number may be very low or even zero in cases with CRC and biallelic mutations. The siblings of such apparently sporadic CRC cases are anticipated to be at high risk of CRC themselves and could be offered surveillance if indicated following cascade genetic testing. One major current caveat is the uncertainty surrounding heterozygote risk. This needs to be clarified as a matter of urgency so that individuals who are found to carry a single mutant allele can be advised unambiguously about their risks and management.

Note added in proof (received 13 June 2005)

Since submission of this manuscript, Farrington et al. [25] have reported a 1.68-fold excess CRC risk for heterozygous carriers of G382D aged >55 years (CI 1.07–2.95).

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References


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