

# **Paper VII**



# **MUTYH Mutations Do Not Cause HNPCC or Late Onset Familial Colorectal Cancer**

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**Key words:** familial adenomatous polyposis coli, hereditary non-polyposis colorectal cancer, late onset familial colorectal cancer, *MUTYH* mutations

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## **Abstract**

Recently, carriers of biallelic mutations in the base excision repair gene *MUTYH*, have been demonstrated to have a predisposition for multiple adenomas and colorectal cancer. Still, many questions remain unanswered concerning *MUTYH*. We have addressed the following: Do biallelic *MUTYH* mutation carriers invariably demonstrate FAP, and may *MUTYH* be a gene causing HNPCC, HNPCC-like or dominantly inherited late onset colorectal cancer? We examined affecteds from our total series of HNPCC, HNPCC-like and dominantly inherited late onset colorectal cancer kindreds not demonstrated to have any MMR mutations. Bloodsamples from 96 patients were subjected to sequencing of exon 7 and exon 13 in the *MUTYH* gene. Two heterozygotes and one homozygote for the European founder mutations were found. The homozygous carrier did not meet criteria for FAP/AFAP. We conclude that *MUTYH*, when mutated, causes a rare recessively inherited disorder including colorectal- and duodenal cancers. It is not verified that heterozygous carriers of *MUTYH* mutations have an increased risk of cancer, and they do not explain the occurrence of familial colorectal cancer in the population.

## **Introduction**

Recently, carriers of biallelic mutations in the base excision repair gene, *MUTYH* at 1p34.1, have been demonstrated to have a predisposition for multiple adenomas and colorectal cancer (CRC) causing autosomal recessive inherited disease [1]. The gene encodes a glycolase which is involved in repair of DNA damage caused by reactive oxygen species generated during aerobic metabolism [2, 3]. In variant forms this ability is reduced [4]. Oxidative damage to DNA can lead to degenerative diseases, aging and cancer [2].

Sequence variants in *MUTYH* are found in different ethnic groups [1]. Two common *MUTYH* mutations,

Y165C and G382D, account for approximately 85% of the mutations occurring in individuals of Caucasian ancestry [1]. Other *MUTYH* mutations known to be associated with colorectal cancer (CRC) are E466X, Y90X and 1395delGGA [1, 5]. Combined data from previous studies suggest that about 1% of colorectal cancer cases can be attributed to biallelic *MUTYH* mutations [3, 6-8]. Families classified as Familial Adenomatous Polyposis (FAP) or Attenuated FAP (AFAP), but without a detected mutation in the Adenomatous Polyposis Coli (APC) gene, may be caused by biallelic *MUTYH* mutations [9].

Phenotypic expression of the *MUTYH* mutation in the heterozygous form is not clear. Kairupan et al. [9]

demonstrated that the *MUTYH* mutations Y165C, G382D and 1395delGGA were not present in the APC mutation-positive FAP population they investigated, suggesting that it does not act as a modifier gene in the development of FAP. The mismatch repair genes also have roles in the removal of oxidative damage [10]. It is demonstrated that the MSH2/MSH6 complex is physically associated with *MUTYH* at an MSH6 binding site and is required to stimulate *MUTYH* activity [10, 11]. Disruption of the MSH2/MSH6 complex causes HNPCC, and it has been reported that *MUTYH* mutations are associated with colorectal cancer risk and can be associated with the clinical features of HNPCC [9, 12]. Kambara et al. [8] suggest that *MUTYH* variation does not initiate progression of cancer, but acts as a promoter in a subset of CRCs. Croitoru et al. [13] demonstrated that heterozygous mutations in Y165C and G382D may confer a potentially important low-penetrant risk of CRC.

A number of families fit the clinical criteria for FAP, AFAP, HNPCC or appear to be inherited, but mutations in known genes have not yet been found. About 30% of FAP cases are apparently "sporadic" (new mutations) giving a one in two risk to their offspring, but low risk to their siblings. If these cases are caused by *MUTYH* mutations and recessive inheritance, the risk to siblings is one in four and the risk to offspring is low in small sibships. The findings have clinical implications for the families as to evaluating the risk of developing cancer and mode of surveillance.

Many questions remain unanswered concerning *MUTYH*. We have addressed the following: 1) Do biallelic *MUTYH* mutation carriers invariably demonstrate FAP? 2) May *MUTYH* be a gene causing HNPCC, HNPCC-like or dominantly inherited late onset colorectal cancer (LOCRC)?

Previously [14, 15] we have identified a number of families with aggregation of CRCs and/or colorectal polyps. We have used the tools available to identify known CRC syndromes in these families, but in a majority no definite cause has been demonstrated. We here report the results of screening these families for *MUTYH* mutations.

## Patients and methods

The patients included in the study had been referred to the Section of Genetic Counselling at the Norwegian Radium Hospital. They were included and classified according to their family history of colorectal and other cancers applying wide clinical criteria [16]. The family history was expanded and information on the site and classification of cancers and polyps and age at diagnosis

were obtained and verified in the medical files and/or in the National Cancer Registry whenever possible.

We examined affecteds from our total series of HNPCC, HNPCC-like and LOCRC kindreds demonstrated not to have any MMR mutations. Blood samples from 96 patients diagnosed with either cancer and/or tubular adenomas were examined. Thirty-one belonged to HNPCC families, i.e. they met the Amsterdam criteria II [17]; 43 were HNPCC-like, i.e. they almost met the Amsterdam criteria II and/or had cases with multiple primaries; and 22 belonged to families with LOCRC, i.e. presence in the family of four or more HNPCC-related cancers all diagnosed over 50 years of age [18]. Sixty-three index persons had at least one cancer and 33 index persons had one or more adenomas. Median age of first tumour was 49 years (23-78) for colorectal cancers (n=53), 49 years (29-76) for tubular adenomas (n=33), 54 years (44-62) for endometrial cancers (n=4) and 57 years (39-69) for other cancers (n=6).

Tumours from eighty-five affecteds had been subjected to immunohistochemistry (IHC) for the presence of MLH1, MSH2 and MSH6 gene products using standard procedures [14, 15, 19, 20]. Blood samples from all patients demonstrating lack of gene product in tumour by IHC (n= 66) had been subjected to sequencing of the corresponding gene and searching for large rearrangements in *MLH1* and *MSH2* [14, 15, 21].

All blood samples were subjected to sequencing of exon 7 (Y165C) and exon 13 (G382D) in the *MUTYH* gene with PCR reactions as previously described [6]. DNA sequencing was performed using a DYEnamic ET dye terminator cycle sequencing kit on a MegaBACE™1000 (Amersham Biosciences, Uppsala, Sweden).

The medical files verifying diagnoses were obtained with written permission from living persons, or with permission from descendants of the dead ones. Written informed consent was underlying all requests for tumour specimens. If the patient was dead, the living relative(s) at risk consented. For mutation analyses, informed consent was obtained in writing following genetic counselling. Because all activity reported was approved health care, all information was kept in our medical files.

## Results

Two heterozygotes for the European founder mutations were found:

- one patient was heterozygous for G382D. She had an ovarian cancer at the age of 69 and one colon adenoma. Her daughter had three tubular adenomas 48 years old, three tubular adenomas 49 years old

and one at the age of 56, but a blood sample was not available. The family had been classified as HNPCC-like. Immunohistochemical staining with antibodies against MLH1, MSH2 and MSH6 proteins of the patient's ovarian cancer showed presence of all three proteins and mutation analyses for MMR genes were not undertaken;

- the other patient was heterozygous for Y165C. He had two tubular adenomas at the age of 49 and a hypophyseal adenoma at 50. His two siblings had not had cancers or adenomas. His mother had CRC at 40 years, and endometrial cancer at 50. She developed duodenal and jejunal polyps demonstrated to include two invasive cancers, and was demonstrated to be homozygous for Y165C. Her sister had contracted CRC at 42. The family had been classified as close to the Amsterdam criteria II [17].

## Discussion

In our series, selected to have dominantly inherited colorectal cancer, or suspected to have, we found no excess of *MUTYH* mutations. We concluded that the clustering of colorectal cancers in the families was not caused by *MUTYH* mutations.

The prevalence of *MUTYH* mutations in the series examined was  $2/96=2\%$ , which is not more than expected by chance alone. Both mutation carriers were selected because of presence of adenomas, which consequently could not be included in the analysis of the mutation carriers. The major clinical findings were, however, that one of the patients had ovarian cancer and the other a hypophyseal adenoma. In addition, the latter had a homozygous mother with HNPCC-like phenotype including endometrial carcinoma, who later in life presented with AFAP-like disease. None of the findings indicated that *MUTYH* might cause dominantly inherited HNPCC or LOCRC, and none of the families included relatives with FAP.

Other studies have shown 1.35-1.67% Y165C and G382D mutations in a control population [9, 12, 13], 1.6% in HNPCC patients [12] and 2-2.34% in CRC patients [7, 13]. The numbers are probably underestimated since the mutation screening methods do not detect all disease-causing sequence changes. The carrier frequency in British and North American populations has been estimated to be 2% [4, 22].

In the first reports on homozygous for *MUTYH* mutations having colorectal cancer, patients were selected on the basis of having multiple polyps (AFAP). The two heterozygous mutation carriers and also the homozygous mutation carrier in our study only had a few adenomas. Others have also shown that

heterozygous and homozygous mutation carriers are not consistent with multiple adenomatous polyps [6, 7, 12, 13, 23]. It seems like homozygous for *MUTYH* mutations may present AFAP, but they do not invariably do so.

The homozygous (Y165C/Y165C) mutation carrier did not meet the diagnostic criteria for FAP, but developed duodenal and jejunal polyps. This is keeping with other reports: [12, 13]. Nielsen et al. [24] revealed several cases of gastro-duodenal polyps in homozygous mutation carriers in *MUTYH*-associated polyposis coli and Kairupan et al. [9] reported one patient with a duodenal polyp.

Farrington et al. [23] demonstrated that heterozygous mutations predispose to CRC later in life, but the effect was small and of borderline significance. Croitoru et al. [13] reported that heterozygous *MUTYH* mutations (Y165C and G382D) may confer a potentially important low-penetrance risk of CRC. Such reports should be interpreted with caution, because when an *MUTYH* mutation is found in a colorectal cancer kindred, half of the affected relatives are expected to have the same mutation. One might have expected more reports if the positive finding were true. It is possible that negative findings have not been reported. None of our LOCRC families demonstrated *MUTYH* mutations.

In conclusion, our findings were in keeping with our interpretation of the literature: homozygous carriers of *MUTYH* mutations are at increased risk of colorectal cancer. They may present FAP or AFAP, but they do not invariably do so. Heterozygous carriers of *MUTYH* mutations may or may not have slightly increased risk of colorectal cancer, but they do not explain the occurrence of familial colorectal cancer in the population. From a practical clinical genetic point of view, *MUTYH* mutations cause a rare autosomally recessively inherited disorder characterized by colorectal and duodenal cancers.

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