Clinical and molecular aspects of MUTYH- and APC-associated polyposis

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Chapter 3

MUTYH heterozygotes

3.1

Increased colorectal cancer incidence in obligate carriers of heterozygous mutations in *MUTYH*

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Background & aims:
MUTYH-associated polyposis (MAP) is an autosomal recessive disorder caused by mutations in the *MUTYH* gene. Patients with MAP are at extremely high risk of colorectal cancer, but the risks of colorectal and other cancers in heterozygous carriers of a single *MUTYH* mutation are uncertain. We performed a retrospective study of cancer incidence and causes of death among obligate *MUTYH* heterozygote individuals.

Methods:
MAP index cases were identified from polyposis registers in Germany, The Netherlands, and the United Kingdom. Cancer incidence, cancer mortality, and all-cause mortality data were collected from 347 parents of unrelated MAP index cases and the spouses of 3 index cases who were also found to be heterozygous for single *MUTYH* mutations. These data were compared with appropriate national sex-, age-, and period-specific population data to obtain standardized mortality ratios (SMR) and standardized incidence ratios (SIR).

Results:
There was a 2-fold increase in the incidence of colorectal cancer among parents of MAP cases, compared with the general population (SIR, 2.12; 95% confidence interval [CI]: 1.30– 3.28). Their colorectal cancer mortality was not increased significantly (SMR, 1.02; 95% CI: 0.41–2.10) nor was overall cancer risk (SIR, 0.92; 95% CI: 0.70–1.18), cancer mortality (SMR, 1.12; 95% CI: 0.83–1.48), or overall mortality (SMR, 0.94; 95% CI: 0.80–1.08).

Conclusions:
The risk of colorectal cancer in heterozygous carriers of single *MUTYH* mutations who are relatives of patients with MAP is comparable with that of first-degree relatives of patients with sporadic colorectal cancer. Screening measures should be based on this modest increase in risk.

Inherited factors contribute to an estimated 35% of colorectal cancers (CRCs),[1] but characterized genetic syndromes account for less than 5% of cases. Genetic variants of low penetrance are likely to account for most familial risk.[2,3] *MUTYH*-associated polyposis (MAP) is an autosomal recessive colorectal adenoma and carcinoma predisposition syndrome caused by biallelic germ-line mutations in the *MUTYH* gene. If untreated, MAP confers an extremely high risk of CRC.[4–8] MUTYH is a component of the highly conserved base excision repair system that plays a major role in protecting against oxidative DNA damage and its mutagenic consequences. It removes adenines mis-incorporated opposite 8-oxo-7,8-dihydro-2'-deoxyguanosine.[9] Its association with colorectal tumorigenesis may, in part, reflect high levels of reactive oxygen species in the colorectum that are generated by commensal bacteria and dietary carcinogens.[10–12]

The clinical significance of carrying a single *MUTYH* mutation has been unclear. The 2 most common MAP-associated *MUTYH* mutations (Y179C and G396D, previously known as Y165C and G382D) are present as heterozygous changes in approximately 1%–2% of individuals in North American and northern European populations,[8,13–16] but, in clinical practice, heterozygote individuals are usually identified during genetic testing in the families of MAP index cases.

A number of studies have reported an overrepresentation of *MUTYH* heterozygote individuals among CRC cases,[8,13,14,17–21] and independent statistical significance was reached in 3 studies.[8,14,21] Some case-control studies investigating *MUTYH* heterozygote CRC risk may not reach statistical significance because of the very large sample size required to assess rare alleles. The present study set out to investigate CRC incidence, all-cancer incidence and mortality, and all-cause mortality in the largest series of *MUTYH* heterozygote individuals to date by retrospective study of 347 parents and 3 heterozygous spouses of patients in whom MAP had been confirmed by molecular genetic analysis and comparison of their data to appropriate national age-, sex-, and period-specific figures for the general population.

PATIENTS AND METHODS

Patients and Samples

The study was approved by national and/or local ethics review boards at each center (the Multi-centre Research Ethics Committee for Wales, ref. 06/MRE09/19, University of Bonn Ethics Review Board No. 063/04, and Leiden University Medical Centre Ethics Review Board No. P01.019). Unrelated MAP index cases with 2 confirmed mutations in *MUTYH* were identified from polyposis registers in Germany, The Netherlands,

and the United Kingdom. Index cases who had been identified originally because of investigations for a family history of CRC or polyposis were not eligible. Of 174 index cases recruited to the study, 158 had been identified because of symptomatic presentation of polyposis or CRC, and 16 did not have symptoms and were identified through CRC screening programs for the general population. Seventy-one index cases were from Germany, 55 from The Netherlands, and 48 from the United Kingdom. All-cause mortality and cancer incidence and mortality data were collected on the obligate *MUTYH* heterozygote parents of the MAP index cases and on 3 spouses of MAP patients who were also identified as heterozygote individuals because their offspring were affected by MAP. Causes of deaths were confirmed with death certificates, and cancer diagnoses were verified by regional cancer registries or hospital records. If a parent had been diagnosed with CRC, the relevant pathology reports were obtained, and tissue blocks that had been stored from the time of their CRC surgery were requested for molecular genetic analysis.

MUTYH Mutational Analysis in Archived Tissues

It was possible that some parents who had been affected by CRC might themselves have had MAP (ie, have 2 rather than 1 inherited *MUTYH* mutation). Therefore, for these parents, DNA that was already stored or that was extracted from cores of their paraffin-embedded, noncancerous tissues was analyzed for *MUTYH* mutations. Exons 1–16 of *MUTYH* were polymerase chain reaction amplified as 21 fragments (primer sequences available on request) and screened for mutations using automated sequencing. Mutations were described using the most up to date annotation for *MUTYH* (NM_001128425), which meant that nucleotide and amino acid numbering after nucleotide position 157 (amino acid 53) differed by 42 nucleotides (14 amino acids) from some previous reports.

Table 1. Time Periods for Which Appropriate National Mortality Rates Were Available

Rate	Germany	The Netherlands	United Kingdom
CRC mortality	1970–2005	1970–2006	1940–2005
All-cancer mortality	1970–2005	1970–2006	1940–2005
All-cause mortality	1970–2005	1970–2006	1901–2005

NOTE. Data were obtained from the Office for National Statistics for the United Kingdom, from the National Comprehensive Cancer Centers and Eindhoven Comprehensive Cancer Center for The Netherlands, and from the Epidemiologisches Krebsregister Saarland for Germany.

Statistical Analysis

Time "at risk" for mortality analyses was from the date of birth unless the date of birth was before the relevant national population rates were available, in which case it was from the date at which rates were available, until the date of death, loss to follow-up, or the last date for which the relevant population rates were available, whichever came first (Table 1).

Time "at risk" for cancer incidence analyses covered the periods 1971–2003 for the United Kingdom, 1960–2006 for The Netherlands, and 1970–2005 for Germany. Follow-up was counted from the date of birth or from the start of these periods, whichever was first, until the date of cancer diagnosis, death, or the end of these periods, whichever came first. The expected numbers of cancer cases and deaths in the cohort were calculated using sex-, age-, period-, and nation-specific rates and were compared with the observed number of cases or deaths to obtain standardized mortality ratio (SMR) and standardized incidence ratio (SIRs). Exact 95% confidence intervals (CI) were estimated by assuming that the observed number of cases is a Poisson count and that the exact number of cases is known without error in STATA version 9 (Stata Corporation, College Station, TX).

Seventy-seven of 350 parents were excluded from analysis of CRC SIRs, 74 from all-cancer SIRs, 74 from CRC SMRs and 73 from all-cancer SMRs, and 70 from all-cause SMRs either because their data were incomplete (eg, unknown date of birth or death), their CRC diagnosis or death occurred before population rate data were available, it was unknown whether they had or died from CRC, or because they lived and died in a different country to the index case with MAP.

RESULTS

MAP Families

One hundred seventy-four apparently unrelated MAP families were identified in which the index cases had 2 confirmed *MUTYH* mutations (Supplementary Table 1). In 3 families, MAP was already known to have recurred in the subsequent generation because the spouse of the affected index case was also a carrier. For these 3 families, data were collected on both the parents and the spouse of the index case. Data were therefore collected initially on 351 apparently heterozygous individuals.

MUTYH Mutation Status of Parents With CRC

Twenty-two parents (and none of the heterozygous spouses) had been diagnosed with CRC. Analysis of blood DNA banked on 1 of the 22 confirmed heterozygosity for

the *MUTYH* mutation Y179C. Archived noncancerous tissues in paraffin blocks were available and of adequate quality for molecular genetic analysis for 7 of the 21 other parents who had been affected by CRC. This confirmed a single *MUTYH* mutation in 6 (Y179C in 4 and G396D in 2) parents and homozygosity for P157L in 1 parent. The pathology reports were obtained for all parents with CRC and confirmed that the 1 homozygous parent had a phenotype consistent with MAP because she also had multiple colorectal adenomas when she presented symptomatically at 74 years of age. None of the others reported multiple colorectal adenomas. The homozygous parent was excluded from the study.

CRC Incidence and Mortality

The 21 parents with CRC had been diagnosed at a mean age of 70 years (standard deviation [SD], 7 years; range, 58 –82 years). To assess whether or not more monoallelic *MUTYH* mutation carriers were affected by CRC than would have been expected in the relevant general populations, SIRs were calculated for each country separately and for all countries together. One parent with colorectal cancer had to be excluded from the analyses because of unknown date of diagnosis. Significantly more parents were diagnosed with CRC than expected (SIR, 2.12; $P < .01$, X^2 test, Table 2). We also calculated SIRs for males and females separately. Whereas the SIR in females was increased significantly (SIR, 2.72; 95% CI: 1.45–4.65), the increase in incidence in males was not significant (SIR, 1.50; 95% CI: 0.61–3.10), but numbers of cases when the sexes were considered separately were small. We only knew or could infer a limited number of genotypes of parents with colorectal cancer and, from these data, could not identify any differences in allele-specific risks of CRC: 5 of 65 Y179C carriers, 2 of 32 G396D carriers, and 13 of 176 carriers of other or uncertain mutations had CRC (Y179C vs G382D, P = .579; Y179C vs other/uncertain, P = .563; G382D vs other/ uncertain, P = .586). In 15 of 20 parents included in the CRC SIR and SMR analyses, CRCs had been diagnosed before the diagnosis of polyposis in their offspring. In 1 of the 5 parents who had CRC diagnosed after polyposis was recognized in their offspring, the diagnosis of CRC was made by screening. Eight parents (4 male and 4 female) had died from CRC, and SMRs for CRC were not different to those in the general population (Table 3).

All-Cause Mortality and Cancer Incidence

Two hundred forty (134 male and 106 female) of the 350 obligate heterozygote individuals had already died. Ages at death were known in 227, and these deaths occurred at mean ages of 74 years (SD, 15 years; range, 26–106 years) for females and 68 years (SD, 13 years; range, 29–94) for males. The mean ages at last contact for those who were

not known to have died were 72 years (range, 47–92 years) for females and 73 years (range, 54–98 years) for males. There were no differences in all-cause mortality (SMRs, Table 4), cancer incidence (SIRs, Table 5), or cancer mortality (cancer SMRs, Table 6) compared with appropriate general populations. The types and numbers of cancers observed are shown in Table 7.

Table 2. Standardized CRC Incidence Ratios

				95% Confidence intervals	
Country	Observed	Expected	CRC SIR	Lower	Upper
United Kingdom	4	1.77	2.27	0.62	5.79
The Netherlands	9	3.49	2.58[a]	1.18	4.90
Germany	7	4.18	1.68	0.67	3.45
All countries	20	9.43	2.12[b]	1.30	3.28

NOTE. Exact SIRs, and confidence intervals were calculated. Observed refers to the number of *MUTYH* heterozygote individuals who were diagnosed with CRC (SIR).
[a]$P < .05$.
[b]$P < .01$.

Table 3. Standardized CRC Mortality Ratios

				95% Confidence intervals	
Country	Observed	Expected	CRC SMR	Lower	Upper
United Kingdom	1	1.46	0.69	0.02	3.82
The Netherlands	4	3.22	1.24	0.34	3.18
Germany	2	2.20	0.91	0.11	3.28
All countries	7	6.88	1.02	0.41	2.10

NOTE. Exact SMRs and confidence intervals were calculated. Observed refers to the number of *MUTYH* heterozygotes who died from CRC (SMR).

Table 4. Standardized Mortality Ratios for All-Cause Mortality

				95% Confidence intervals	
Country	Observed	Expected	All-cause SMR	Lower	Upper
United Kingdom	48	61.67	0.78	0.57	1.03
The Netherlands	57	63.13	0.90	0.68	1.17
Germany	75	67.37	1.11	0.88	1.40
All countries	180	192.17	0.94	0.80	1.08

NOTE. Exact SMRs and confidence intervals were calculated. Observed refers to the number of deceased *MUTYH* heterozygote individuals.

Table 5. Standardized All-Cancer Incidence Ratios

Country	Observed	Expected	Cancer SIR	95% Confidence intervals Lower	Upper
United Kingdom	14	12.02	1.16	0.64	1.95
The Netherlands	23	28.27	0.81	0.52	1.22
Germany	23	25.01	0.92	0.58	1.38
All countries	60	65.31	0.92	0.70	1.18

NOTE. Exact SIRs, and confidence intervals were calculated. Observed refers to the number of parents who were diagnosed with any cancer (SIR). Eighty-four parents (40 male and 44 female) had been diagnosed with at least 1 cancer, and 62 (32 male and 30 female) had died from cancer.

DISCUSSION

Several previous studies have attempted to assess CRC risk in *MUTYH* heterozygote individuals using case-control or kin-cohort approaches,[13,15–25] but all were based on much smaller numbers than the present study, which identified a statistically significant 2-fold increase in CRC incidence. Three of the previous studies also identified a statistically significant increase in CRC risk among *MUTYH* heterozygote individuals. Farrington *et al*[8] identified a significant excess of G396D heterozygote individuals among CRC cases over the age of 55 years; Peterlongo *et al*[14] found a significant excess of *MUTYH* heterozygote individuals among CRC cases from hereditary nonpolyposis CRC-like families that were negative for mismatch repair gene mutations; and, recently, Cleary *et al.*[21] identified an increased age-and sex-adjusted odds ratio for CRC of 1.48 in heterozygote individuals. The most recent and largest meta-analysis of case-control studies reported only a nonsignificant increase in CRC risk in *MUTYH* heterozygote individuals (odds ratio, 1.11; 95% CI: 0.90–1.37; for Y179C heterozygote individuals alone, the odds ratio was 1.24 [95% CI: 0.83–1.84]), but it included studies that undertook very limited genetic testing that would misclassify a significant proportion of heterozygote individuals,[22] and it did not include the study of Cleary *et al.*[21]

The parents in the current study were assumed to be *MUTYH* heterozygote individuals because, in every case, 2 mutations had been characterized in their offspring with MAP. However, we could not confirm directly the genetic status of most parents because 240 of 350 were already dead and without stored DNA samples. Nonpaternity could have led to an underestimate of CRC in fathers, although the apparent excess of affected mothers in the current study was not statistically significant.

We did attempt to clarify the genetic status of those parents who had been affected

Table 6. Standardized All-Cancer Mortality Ratios

Country	Observed	Expected	Cancer SMR	95% Confidence intervals Lower	Upper
United Kingdom	12	12.65	0.95	0.49	1.66
The Netherlands	18	15.73	1.14	0.68	1.81
Germany	20	16.18	1.24	0.75	1.91
All countries	50	44.56	1.12	0.83	1.48

NOTE. Exact SMRs, and confidence intervals were calculated. Observed refers to the number of parents who died from cancer (SMR). Eighty-four parents (40 male and 44 female) had been diagnosed with at least 1 cancer, and 62 (32 male and 30 female) had died from cancer.

Table 7. Cancers Identified in 84 Parents of MAP Index Cases

Cancer	No. of cases	Mean age at diagnosis, *y* (range)
Colorectal[a,b]	21	70 (58–82)
Lung[b]	16	70 (57–85)
Breast[a]	6	60 (45–90)
Gastric[c]	6	67 (54–78)
Prostate[d]	6	72 (59–80)
Leukemia	5	64 (52–93)
Lymphoma[b]	3	61 (55–67)
Bladder[d]	3	66 (59–77)
Uterine[c]	2	47
Laryngeal[e]	2	57
Osteosarcoma	2	78 (72–83)
Ovarian	1	72
Cervical	1	<70
Testicular	1	Unknown
Duodenal	1	55
Pancreatic	1	67
Liver	1	59
Plasmocytoma	1	60
Fibrosarcoma	1	68
Glioblastoma	1	65
Astrocytoma[e]	1	64
Thyroid	1	Unknown
Cancer, primary unknown	9	60 (33–79)

NOTE. Seven parents had more than 1 type of cancer as shown in footnotes below.
[a]Two were diagnosed with CRC and breast cancer.
[b]Two had CRC and lung cancer, 1 of whom also had lymphoma.
[c]One had gastric and uterine cancers.
[d]One had prostate and bladder cancers.
[e]One had laryngeal cancer and an astrocytoma.

by CRC because it was anticipated that any who by chance carried biallelic *MUTYH* mutations were likely to be found in this group and that their inclusion would introduce bias toward overestimating heterozygote risk. Indeed, 3 families from the current cohort were already known to have pseudodominant transmission of MAP because of the occurrence of biallelic mutations in 2 generations.[26,27] Pseudodominance was

confirmed in a fourth family during this study. Nine parents had metastatic cancer with an unknown primary site, and 1 had hepatic cancer that could not be confirmed as a definite primary tumor. Some of these parents might have had CRC, and, if so, this would have led to a small underestimate of heterozygote CRC risk.

To avoid ascertainment bias in favor of families in which a parent had a history of CRC, families were not eligible for the study when the index case with MAP had been identified as a consequence of a parent (or any other relative) having adenomas or CRC. Sixteen of the index cases who were included in the study had been identified as a result of population screening programs for CRC in the absence of symptoms or a family history, but all the others had all presented symptomatically and been found to have polyposis with or without CRC.

However, we could not eliminate all potential sources of bias. To allow for changing cancer incidence over time, parents were only included in analyses if information including dates of birth and diagnosis could be confirmed. These data were more likely to be known to polyposis registers when a parent had been affected by CRC, leading to a bias in favor of inclusion of parents with CRC. Whereas only 5% (1/21) of the parents with CRC were excluded because these details were unknown, 23% (76/329) of those unaffected by CRC were excluded for the same reasons (P = .034, Fisher exact test). However, an analysis including all parents still showed a significantly increased CRC risk (SIR, 1.87; 95% CI: 1.16–2.87; P < .05, χ^2 test). Less obvious sources of bias may also be present. For example, studies in which large population-based cohorts of patients with CRC have been tested for *MUTYH* mutations suggest that, up to 30% of individuals with biallelic *MUTYH* mutations (homozygotes and compound heterozygotes) may develop CRC in the absence of polyposis.[8,13,22,24] Families in the present study were identified from polyposis registers, and our cohort is therefore biased toward index cases with multiple adenomas. This may have selected for parents who carried additional (currently unidentified) genetic variants that predisposed them to CRC and that were transmitted to their offspring, leading to the development of multiple colorectal tumors in the context of biallelic *MUTYH* mutations.

We have previously shown that the phenotypic effects of homozygosity for Y179C are more severe, in terms of both CRC hazard and earlier age at presentation, than those of homozygosity for G396D or compound heterozygosity for G396D/Y179C.28 Balaguer *et al* have suggested that different CRC risks might also be associated with heterozygosity for different *MUTYH* mutations, but their observations did not reach statistical significance.[22] We could not genotype most parents, limiting our opportunity to assess allele-specific CRC risks to the parents of homozygous MAP index cases (where both parents must have carried the same mutant allele), and these were insufficient

in number for such an analysis to reach statistical significance. The mechanisms underlying CRC in *MUTYH* heterozygote individuals are unknown but could involve somatic mutation of the wild-type *MUTYH* allele, analogous to the situation in Lynch syndrome (hereditary nonpolyposis CRC). Consistent with this possibility, 2 studies[13,23] have identified more frequent chromosome 1p loss of heterozygosity (corresponding to the chromosomal location of *MUTYH*) in CRCs from carriers of germ-line *MUTYH* variants than in CRCs from noncarriers, although the numbers studied were too small for statistical significance.

Population screening for CRC has been implemented in many countries including Germany and the United Kingdom, but the age from which screening is recommended varies. The distribution of ages at which CRCs were diagnosed in obligate *MUTYH* heterozygote parents in the present study (mean, 70 years; range, 58 –82 years) was very similar to that in the general population, and heterozygote individuals would be expected to benefit from population-screening measures. It is generally accepted that screening should be enhanced when there is a strong family history of CRC or multiple adenomas, and a number of guidelines have been produced to aid decision making in clinical practice.[29,30] However, it has been unclear whether such measures are indicated for the heterozygous relatives of patients with MAP and practice has been inconsistent. The 2-fold increase in CRC risk identified in the present study is comparable with the relative risk of 2.24 seen in individuals from the general population who have at least 1 first-degree relative affected by CRC.[31] The present study indicates that screening measures for CRC in the heterozygous relatives of MAP patients need be no more intensive than for this group.

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Supplementary Table 1. Genotypes of 174 MAP Index Cases

MUTYH mutation 1	*MUTYH* mutation 2	No. MAP index cases
c.536 A>G, p.Y179C	c.1187 G>A, p.G396D	40
c.536 A>G, p.Y179C	c.536 A>G, p.Y179C	40
c.1187 G>A, p.G396D	c.1187 G>A, p.G396D	13
c.536 A>G, p.Y179C	c.933+3 A>C	9
c.536 A>G, p.Y179C	c.1145delC, p.A385fs	7
c.536 A>G, p.Y179C	c.1214 C>T, p.P405L	6
c.1438 G>T, p.E480X	c.1438 G>T, p.E480X	5
c.1187 G>A, p.G396D	c.1214 C>T, p.P405L	4
c.1187 G>A, p.G396D	c.734 G>A, p.R245H	2
c.536 A>G, p.Y179C	c.690 G>A, p.Q230Q	2
c.312 C>A, p.Y104X	c.312 C>A, p.Y104X	2
c.1214 C>T, p.P405L	c.1214 C>T, p.P405L	2
c.1187 G>A, p.G396D	c.1145delC, p.A385fs	2
c.1437_1439delGGA, p.E480del	c.1437_1439delGGA, p.E480del	2
c.536 A>G, p.Y179C	c.734 G>A, p.R245H	2
c.734 G>A, p.R245H	c.1145delC, p.A385fs	2
c.1187 G>A, p.G396D	c.1101delC, p.R368fs	1
c.1187 G>A, p.G396D	c.933+3 A>C	1
c.1187 G>A, p.G396D	c.647 A>G, p.G216E	1
c.1187 G>A, p.G396D	c.713 A>G, p.N238S	1
c.1187 G>A, p.G396D	c.1012 C>T, p.Q338X	1
c.536 A>G, p.Y179C	c.1518+2 C>T	1
c.536 A>G, p.Y179C	c.391 T>A, p.W131R	1
c.1214 C>T, p.P405L	c.739 C>T, p.R247X	1
c.536 A>G, p.Y179C	c.691-1 G>A	1
c.1187 G>A, p.G396D	c.325 C>T, p.R109W	1
c.1171 C>T, p.Q391X	c.1171 C>T, p.Q391X	1
c.1228_1229insGG	c.1228_1229insGG	1
c.536 A>G, p.Y179C	c.739 C>T, p.R247X	1
c.1214 C>T, p.P405L	c.1145delC, p.A385fs	1
c.1214 C>T, p.P405L	c.933+3 A>C	1
c.536 A>G, p.Y179C	c.925 C>T, p.R309C	1
c.749 G>A, p.G340D	c.1145delC, p.A385fs	1
c.536 A>G, p.Y179C	c.1437_1439delGGA, p.E480del	1
c.504+19_504+31del13	c.734 G>A, p.R245H	1
c.722 G>A, p.R241Q	c.1187 G>A, p.G396D	1
c.1145delC, p.A385fs	c.1437_1439delGGA, p.E480del	1
c.536 A>G, p.Y179C	c.824_829dupCAGGAG, p.G276_D277insAG	1
c.470 C>T, p.P157L	c.1187 G>A, p.G396D	1
c.289 C>T, p.R97X	c.1214 C>T, p.P405L	1
c.884 C>T, P295L	c.1437_1439delGGA, p.E480del	1
c.820 C>T, R274W	c.1518+2 T>C	1
c.536 A>G, p.Y179C	c.1012 C>T, p.Q338X	1
c.536 A>G, p.Y179C	c.1171 C>T, p.Q391X	1
c.628 C>T, p.Q210X	c.1145delC, p.A385fs	1
c.463-2 G>C	c.1145delC, p.A385fs	1
c.643 G>A, p.V215M	c.884 C>T, P295L	1
c.884 C>T, P295L	c.884 C>T, P295L	1
c.55 C>T, p.R19X	c.1145delC, p.A385fs	1
c.545 G>A, p.R182H	c.884 C>T, P295L	1

NOTE. All cases had confirmed biallelic mutations (MUTYH mutations 1 and 2) that are shown as both the nucleotide change and the predicted protein change.