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

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Analysis of *MUTYH* genotypes and colorectal phenotypes in patients with *MUTYH*-associated polyposis

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Background & aims:
Biallelic mutations in the base excision DNA repair gene *MUTYH* lead to *MUTYH*-associated polyposis (MAP) and predisposition to colorectal cancer (CRC). Functional studies have demonstrated significant differences in base recognition and glycosylase activity between various *MUTYH* mutations, notably for the 2 mutations most frequently reported in MAP patients: Y179C and G396D (previously annotated as Y165C and G382D). Our goal was to establish correlations between genotypes and colorectal phenotype of patients with MAP.

Methods:
In this multicenter study, we analyzed genotype and phenotype data from 257 MAP patients. Data included age at presentation of MAP, polyp count, and the occurrence, location, and age at presentation of CRC.

Results:
Patients with a homozygous G396D mutation or compound heterozygous G396D/Y179C mutations presented later with MAP and had a significantly lower hazard of developing CRC than patients with a homozygous Y179C mutation ($P < .001$). The mean ages of CRC diagnosis in patients were 58 years (homozygous G396D) and 52 years (compound heterozygous G396D/Y179C) versus 46 years (homozygous Y179C; $P = .001$, linear regression).

Conclusions:
Our study identified the phenotypic effects of Y179C as relatively severe and of G396D as relatively mild. These clinical data are in accord with findings from in vitro functional assays. Genotypic stratification may become useful in the development of guidelines for counseling, surveillance, and management of families with MAP.

MUTYH-associated polyposis (MAP) is an autosomal recessive inherited disorder that is characterized by the development of colorectal adenomatous polyps (generally between 10 and 500) and subsequently colorectal carcinoma (CRC), in the majority of cases.[1–5] In addition, MAP is associated with extracolonic manifestations, including duodenal adenomas and carcinomas.[5,6]

Mutations causing MAP are distributed across the *MUTYH* locus, and various population-specific mutations have been reported.[7] The majority of changes found in the *MUTYH* gene are missense mutations, of which Y179C and G396D (previously annotated as Y165C and G382D; see Patients and Methods section) represent approximately 73% of *MUTYH* mutations found in western populations.[7]

The MUTYH protein has glycosylase activity and is involved in the base excision repair of DNA damage, including that caused by oxidation of guanine to 8-oxo-7,8-dihydro-2=-deoxyguanosine (8-oxoG).[1] A mispairing can occur between 8-oxoG and adenine, leading to a G:C>T:A trans-version. The MUTYH protein recognizes an 8-oxoG:A mismatch and subsequently excises the adenine base using a base-flipping mechanism. The Y179C mutation is thought to affect mismatch specificity and flipping of the adenine into a base specificity pocket, while the G396D mutation appears to interfere with 8-oxoG recognition. Characterization of mutant *Escherichia coli* proteins demonstrated that the Y82C change, corresponding to human Y179C, had a more deleterious effect on the rate of adenine removal than G253D, corresponding to human G396D.[1] Parker *et al* [8] showed that different MAP-associated *MUTYH* mutations had significantly different functional effects on base excision repair in human cell lines. Recently, Ali *et al* [9] showed that glycosylase activity and DNA binding activity in vitro is severely defective in Y179C, but partially retained in G396D.

Despite these in vivo and in vitro studies, little is known about clinical genotype-phenotype correlations in MAP. The aim of this study was to evaluate clinical characteristics of MAP, including age at presentation, polyp count, and the occurrence, location, and age at diagnosis of CRC in patients with different *MUTYH* genotypes. Differences in these aspects of the disorder may have implications for counseling, screening, and management of patients and their families. Patients were first grouped into those with either 2 nontruncating mutations, 1 nontruncating and 1 truncating, or 2 truncating mutations. As truncating mutations generally have a more deleterious effect on the protein activity than non-truncating changes, we wanted to study whether these mutations might indeed result in a more severe phenotype. We then compared the phenotypes of patients with different biallelic combinations of the 2 most common mutations, Y179C and G396D.

PATIENTS AND METHODS

Patients

This multicenter study was a collaboration among 3 research groups from the universities of Bonn, Cardiff, and Leiden. Informed consent was obtained according to protocols approved by the appropriate national and/or local ethics review boards. The study cohort consisted of 257 patients with MAP, including 185 apparently unrelated index patients (72%), 66 siblings (26%), and 6 other relatives with biallelic *MUTYH* mutations (1 nephew, 4 children, and 1 parent). Information was collected on genotype, age at presentation of MAP, number of polyps, therapy and surgery, and the occurrence and age at diagnosis of CRC. CRC staging was performed according to the modified Astler–Coller (MAC) guidelines. Carcinoma in situ included intraepithelial and intramucosal carcinoma, and patients with a carcinoma in situ were included in the CRC group. The number of polyps was divided into the following groups: <10, 10 –100, >100, and other ("multiple," "polyposis," and unknown).

Our series included 16 siblings (6%) who were not tested for *MUTYH* mutations because they were either already deceased (n = 7) or their DNA was not available for analysis. These siblings were assumed to be biallelic carriers based on their phenotype—that is, they had CRC before 35 years of age, or 10 or more adenomas. Because of the severe phenotype of the siblings (leading to a number of them dying young), excluding them from our analyses could have resulted in a selection bias towards surviving siblings.

Genotyping

To describe mutations, we used the most up-todate annotation for *MUTYH* (http://chromium.liacs.nl/ LOVD2/home.php?select_db=*MUTYH*, http://www.ncbi. nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=190358496). The coding DNA reference sequence is extended at the 5= end of exon 3 with bases from expressed sequence tags, and exon 3 is alternatively spliced in several ways. Therefore, nucleotide and amino acid numbering after nucleotide position 157 (amino acid 53) differs by 42 nucleotides (14 amino acids) from some previous reports; for example, Y165C is Y179C, and G382D is G396D. Not all mutations reported here are formally proven pathogenic, since functional testing has not been done for all variants.

Genotyping was performed as described previously.[1,2,4,5] We classified genotypes into 6 groups: (1) homozygotes for a nontruncating mutation (no truncating mutations; n = 185), (2) compound heterozygotes for a nontruncating and a truncating mutation (1 truncating mutation; n = 50), (3) homozygotes for a truncating mutation (2 truncating mutations; n = 22), (4) G396D homozygotes (G396D/G396D; n = 20), (5) G396D/Y179C compound heterozygotes (G396D/Y179C; n = 64), and (6) Y179C homozygotes

(Y179C/Y179C; n = 60). Separate analyses for biallelic combinations of mutations other than the G396D and Y179C were not done because the corresponding numbers of MAP patients were too small.

Statistical Analysis

All tests were performed within the subgroups 1–3 and 4 – 6 with SPSS 14.0 (SPSS, Chicago, IL). A P value of <.05 was considered to be statistically significant. Differences in age at presentation of MAP and age at diagnosis of CRC between genotype groups were evaluated with 1-way analysis of variance or a trend analysis (linear regression), when a trend was expected. Fisher's exact test was performed to compare the location of CRC in the colon and the MAC stages of the tumors. To compare the number of polyps between the different genotypes, a trend analysis (linear-by-linear association) was performed.

We performed a survival analysis to calculate the CRC hazard for the different genotypes. Patients were assumed to be informative until they had a (complete or partial) colectomy, contact was lost, or they died. After these occurrences patients were censored. The development of CRC was considered an end point. For graphic visualization, a Kaplan–Meier analysis was done. We used the Wald test criterion of the Cox proportional hazard regression model to evaluate whether the CRC hazard differed significantly among the different mutation groups.

RESULTS

Thirty-six different mutations were found in 185 index patients (Table 1). The Y179C mutation represented 44% of all mutations (164/370), and the G396D represented 24% of all mutations (90/370). Truncating mutations (64/370) represented 17% of all changes. At presentation, 175 of 257 patients (68%, including 141 index cases and 34 relatives) were already symptomatic, and 60 patients (23%, 31 index cases and 29 relatives) were asymptomatic and referred for clinical screening because of the family history. These asymptomatic cases were equally distributed among the different genotypes. In 22 cases, the reason for referral for screening that led to the diagnosis was not known (9%, 22/257). The mean age at presentation of MAP in symptomatic patients was 45 years (n = 172; SD :10.1 years; range 12– 68 years).

The mean age at last contact was 54 years (n = 253; SD : 12.0 years; range, 14 –84 years). Of 254 patients for whom data were available, 148 (58%) developed CRC. In 3 patients, information was insufficient to exclude CRC. The mean age at diagnosis

of CRC was 48 years (SD :9.6 years; range, 21–70 years). In total, 49 of 148 patients (33%) had synchronous or metachronous carcinomas (between 2 and 5 tumors). The proportion of cases with synchronous or metachronous carcinomas in G396D homozygotes and Y179C homozygotes was similar— 4 of 9 (44%) and 18 of 42 (43%), respectively. Colectomy had been performed on 200 patients (78%, 200/257) because of CRC or multiplicity of adenomas.

MAP: Age at Presentation and Polyp Count

G396D homozygotes presented at a mean age of 51 years and G396D/Y179C compound heterozygotes at a mean age of 50 years, whereas Y179C homozygotes presented at a younger mean age of 43 years (Table 2). The mean age at presentation was inversely correlated with the number of Y179C alleles carried (Table 2; P = .001, linear regression). The number of alleles carrying truncating mutations had no significant effect on age at presentation (Table 2).

Twenty percent of G396D homozygotes had fewer than 10 polyps, and 15% had more than 100 polyps in the colon (Figure 1A). In contrast, only 2% of Y179C homozygotes had fewer than 10 polyps, and 29% had more than 100 polyps.

Table 1. Frequency of Mutations Found in Our Study

Mutation	n	Mutation	n	Mutation	n	Mutation	n
c.55C>T, p.R19X	1	c.536A>G, p.Y179C	164	c.734G>A, p.R245H	7	c.1101del, p.R368fs	1
c.289C>T, p.R97X	1	c.545G>A, p.R182H	1	c.739C>T, p.R247X	2	c.1145delC, p.A385fs	19
c.312C>A, p.Y104X	4	c.628C>T, p.Q210X	1	c.749G>A, p.G250D	1	c.1171C>T, p.Q391X	3
c.325C>T, p.R109W	1	c.643G>A, p.V215M	1	c.820C>T, p.R274W	1	c.1187G>A, p.G396D	90
c.389-1G>A	1	c.647A>G, p.G216E	1	c.824-829dupCAGGAG, p.G276_D277insAG	1	c.1214C>T, p.P405L	20
c.391T>A, p.W131R	1	c.690G>A, p.Q230Q	2	c.884C>T, p.P295L	5	c.1227_1228dupGG, p.E410fs	2
c.463-1G>C	1	c.691-1G>A	1	c.925C>T, p.R309C	1	c.1437_1439delGGA, p.E480del	7
c.470C>T, p.P157L	1	c.713A>G, p.N238S	1	c.933+3A>C	11	c.1438G>T, p.E480X	10
c.504+19_504+31del13	1	c.722G>A, p.R241Q	1	c.1012C>T, p.Q338X	2	c.1518+2T>C	2

Table 2. Mean and Median Age at Presentation of MAP in Symptomatic Patients by Genotype Group

Genotype	n	Mean (median) age at presentation (y)	Range	95% CI
1. No truncating mutation	125	46[a] (45)	12–68	43.9–47.5
2. One truncating mutation	35	42[a] (41)	21–60	38.9–45.3
3. Two truncating mutations	12	49[a] (50)	32–65	41.7–55.4
4. G396D/G396D	12	51[b] (54)	36–62	44.9–56.6
5. G396D/Y179C	38	50[b] (51)	12–68	46.1–53.6
6. Y179C/Y179C	42	43[b] (44)	24–65	40.1–45.4

CI, confidence interval; MAP, MUTYH-associated polyposis.
[a]*P* = .587 (linear regression).
[b]*P* = .001 (linear regression).

The number of Y179C alleles was inversely correlated with the proportion of cases with fewer than 10 polyps (P = .006, linear-by-linear association). Patients with 2 truncating mutations more often had a polyp count above 100 (48%) than patients with no (26%) or 1 truncating (15%) mutation (Figure 1B), but no significant trend was seen. We did see a significant trend (P = .04, linear-by-linear association) in patients with fewer than 10 polyps; 9% of patients with no truncating mutations had fewer than 10 polyps versus none of the patients with 2 truncating mutations.

CRC: Location, Stage, Hazard, and Age at Diagnosis

The location of the CRC was known in 138 of 148 patients. In 61 patients (44%), the carcinomas were situated in the descending colon (ie, distal of the transverse colon), and in 77 patients (56%) in the ascending or proximal part of the colon. There were no significant differences in CRC location between genotype groups. Eighty-five of 148 patients (57%) developed a nonmetastatic CRC (MAC stages carcinoma in situ, A, or B), and 51 of 148 (34%) developed a metastatic CRC (MAC stages C or D). The stage of the tumor was unknown in 12 of 148 patients (8%). No significant differences in MAC stages were seen between the different genotypes (Table 3).

A Cox regression was undertaken to compare CRC hazard in the different genotype groups. A significant difference in CRC hazard was found between the missense genotypes Y179C and G396D (P < .001) (Figure 2A). The mean age at diagnosis of CRC was 58 years for G396D homozygotes, 52 years for G396D/Y179C compound heterozygotes, and 46 years for Y179C homozygotes (Table 4). The mean CRC age was inversely correlated with the number of Y179C alleles carried (P < .001, linear regression). The CRC hazard in the groups with no, 1, or 2 truncating mutations was not significantly different (P = .421) (Figure 2B). The mean age at diagnosis of CRC was not correlated with the number of truncating alleles carried (P = .810, linear regression).

G396D combined with mutations other than G396D and Y179C

We identified G396D as a mutation associated with a less severe phenotype than Y179C; therefore, we next analyzed phenotypes in patients who were G396D compound heterozygotes carrying second mutations other than Y179C. In the 21 MAP patients with these genotypes, we found a significantly greater CRC hazard for patients who were compound heterozygotes for G396D/truncating or other missense mutations than for patients with G396D/Y179C or G396D/G396D (Figure 2C). The mean age at diagnosis of CRC in this group was 48 years, compared with 52 years for G396D/Y179C, and 58 years for G396D/G396D (Table 4). The mean age at presentation of MAP was 45 years in symptomatic patients with a G396D/truncating or other missense

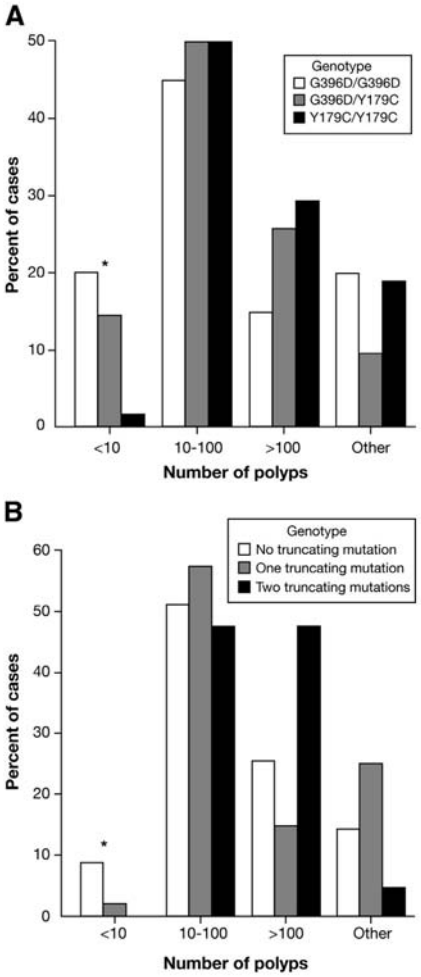

Figure 1. Number of polyps by genotype groups. *Other* means "multiple," "polyposis," or unknown. (*A*) Genotype groups: G396D/G396D, G396D/Y179C, and Y179C/Y179C. *<10 polyps: $P = .006$, linear-by-linear association. (*B*) Genotype groups: no truncating mutation, one truncating mutation, and two truncating mutations. *<10 polyps: $P = .043$, linear-by-linear association.

mutation (n = 15, excluding 6 patients who were asymptomatic and referred for clinical screening because of their family history), which was not significantly different from G396D/G396D and G396D/Y179C patients (Table 2).

DISCUSSION

This multicenter genotype-phenotype study presents the largest series of MAP patients reported so far. We found that Y179C homozygotes presented earlier and had a significantly greater CRC hazard than G396D homozygotes and G396D/Y179C compound heterozygotes. The more severe phenotype in Y179C homozygotes is consistent with a recent clinical study[10] and with functional studies.[1,8,9]

Several factors have been reported to contribute to the differing functional effects of these mutations. First, cells with biallelic Y179C mutations appear to contain lower levels of the MUTYH protein than cells with biallelic G396D mutations, in which levels are comparable to those of MUTYH wild-type cell lines.[8] Second, Y179C shows a lesser ability to recognize an 8-oxoG:A mismatch than G396D8 and has severely defective glycosylase and DNA binding activity.[9] Remarkably, in vivo, Y179C and G396D exhibit the same low levels of cleavage of a mispaired adenine from an 8-oxoG. This noticeable difference between in vitro activity and in vivo activity of the G396D mutation (high vs low, respectively) may be explained, at least in part, by its influence on the phosphorylation status of MUTYH.[8,11,12] We found that compound heterozygosity for G396D and a second mutation, other than G396D or Y179C (n = 21), was associated with a more severe phenotype (ie, higher CRC hazard and earlier presentation) than G396D homozygosity or G396D/Y179C compound heterozygosity. Parker *et al*[8] showed that 1145delC/G396D and Y179C/G396D mutant cell lines have MUTYH protein levels that are about 50% compared with wild-type cells. They suggest that residual MUTYH levels were due to expression of the G396D mutant alleles, and that the latter were probably also responsible for the binding and cleavage activities observed in these cells. Apparently, the combination of Y179C and G396D mutations may lead to a better glycosylase function (binding to and cleaving of 8-oxoG:8 mispairs) than G396D combined with some other mutations. Although G396D is a relatively mild mutation, not every combination of G396D with another *MUTYH* mutation may give a relatively mild phenotype.

In contrast to the differences in phenotypic expression we observed between the most common *MUTYH* missense mutations, we found no significant difference between truncating and nontruncating mutations except for polyp count (ie, the number of truncating alleles was inversely correlated with the proportion of cases with <10 polyps). Truncating mutations generally have a more deleterious effect than nontruncating mutations on the protein activity; as a result, a more severe phenotype might be anticipated.

Table 3. MAC Stage of CRC by Genotype Group

Genotype	MAC stage, n (%)		
	CIS, A, B	C, D	Unknown
1. No truncating mutation, n = 109[a]	65 (60%)	36 (33%)	8 (7%)
2. One truncating mutation, n = 28[a]	15 (54%)	9 (32%)	4 (14%)
3. Two truncating mutations, n = 11[a]	5 (45%)	6 (55%)	0 (0%)
4. G396D/G396D, n = 9[b]	6 (67%)	2 (22%)	1 (11%)
5. G396D/Y179C, n = 32[b]	20 (62%)	12 (38%)	0 (0%)
6. Y179C/Y179C, n = 42[b]	28 (67%)	12 (28%)	2 (5%)

CIS, carcinoma in situ; CRC, colorectal cancer; MAC, modified Astler–Coller.
[a]P = .440 (Fisher's exact test).
[b]P = .437 (Fisher's exact test).

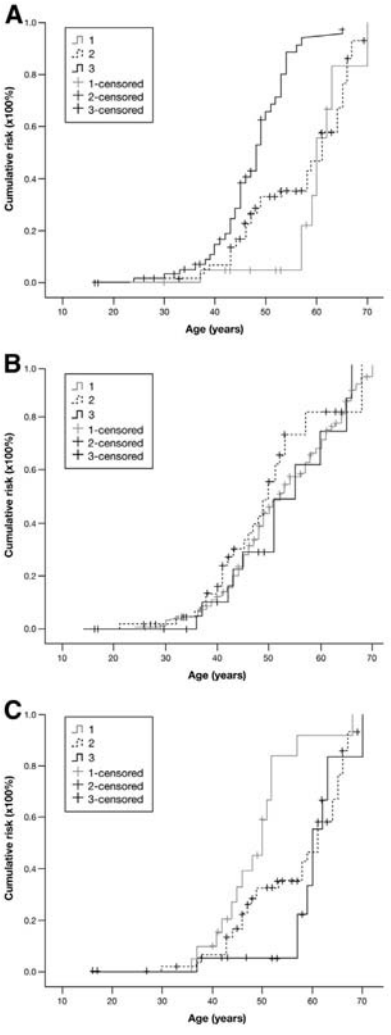

Figure 2. CRC hazard by genotype groups. (*A*) *1* = G396D/G396D; *2* = G396D/Y179C; *3* = Y179C/Y179C. The CRC hazard, using Cox regression, was significantly different between group, 1 and 3 ($P <$ .001) and between group, 2 and 3 ($P <$ .001). (*B*) *1* = no truncating mutation; *2* = one truncating mutation; *3* = two truncating mutations. The CRC hazard, using Cox regression, was not significantly different between groups. (*C*) *1* = G396D/missense or truncating; *2* = G396D/Y179C; *3* = G396D/G396D. The CRC hazard, using Cox regression, was significantly different between group, 1 and 2 (P = .008) and between group, 1 and 3 (P = .006).

Table 4. Mean and Median Age at Diagnosis of CRC by Genotype Group

Genotype	n	Mean (median) age at diagnosis of CRC (*y*)	Range	95% CI
1. No truncating mutation	109	48[a] (48)	24–70	46.5–50.1
2. One truncating mutation	28	46[a] (47)	21–68	42.1–49.1
3. Two truncating mutations	11	50[a] (51)	36–66	43.0–57.2
4. G396D/G396D	9	58[b] (60)	37–70	51.5–65.2
5. G396D/Y179C	32	52[b] (49)	30–67	47.8–55.3
6. Y179C/Y179C	42	46[b] (47)	24–65	43.8–48.5

CI, confidence interval; CRC, colorectal cancer.
[a]$P = .810$ (linear regression).
[b]$P < .001$ (linear regression).

It is possible that complete loss of MUTYH function reduces cell survival and leads to less tumor formation than if some functional MUTYH protein remains.

A potential shortcoming of our study was the inclusion of affected siblings and other relatives. This could lead to bias because phenotypes in siblings may be more similar than phenotypes of unrelated index cases who share the same *MUTYH* genotype, but not alleles at other loci that modify the MAP phenotype. We therefore repeated our analyses for index cases only (ie, excluding all relatives). This did not alter the significance (or nonsignificance) of our findings, except for polyp count (data available upon request). The proportion of cases with fewer than 10 polyps was no longer significantly correlated with the number of truncating alleles carried (data not shown). The 60 patients who were asymptomatic at diagnosis of MAP and referred for clinical screening because of the family history were equally distributed among the genotypes and are unlikely to have introduced significant bias into our study. Although the natural history of an inherited disorder is ideally assessed by the prospective follow-up of a birth cohort, such data were not available for our analysis. Our analysis probably gives an overestimation of the cumulative CRC risk because the majority of the cases we studied presented clinically with MAP-associated symptoms. Two groups of patients could be underrepresented in our study: patients with small numbers of polyps who may not come to medical attention, and patients with CRC but without polyps who are rarely tested for *MUTYH* mutations. However, the first group is most likely to be small, as Gismondi *et al*[3] previously did not find any *MUTYH* mutations in 141 patients with 1–9 polyps. With respect to the second group, biallelic *MUTYH* mutations were found in 0.5%– 1.0% of CRC patients, and no polyps were present in 25%– 42% of these patients.[10,13,14]

Colonoscopic surveillance of MAP patients has been recommended to commence at 18 –20 years of age.[15] Based on the range of age at diagnosis of CRC (Table 4), it may be appropriate to initiate surveillance later for G396D homozygotes and G396D/Y179C compound heterozygotes. Ideally, long-term prospective studies of MAP should be undertaken to extend the evidence base for refinement of clinical guidelines.

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