Contribution of bi-allelic germline MUTYH mutations to early-onset and familial colorectal cancer and to low number of adenomatous polyps: case-series and literature review

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Abstract In the absence of a polyposis phenotype, colorectal cancer (CRC) patients referred for genetic testing because of early-onset disease and/or a positive family history, typically undergo testing for molecular signs of Lynch syndrome in their tumors. In the absence of these signs, DNA testing for germline mutations associated with other known tumor syndromes is usually not performed. However, a few studies in large series of CRC patients suggest that in a small percentage of CRC cases, bi-allelic MUTYH germline mutations can be found in the absence of the MUTYH-associated polyposis phenotype. This has not been studied in the Dutch population. Therefore, we analyzed the MUTYH gene for mutations in 89 patients with microsatellite-low or stable CRC cancer diagnosed before the age of 40 years or otherwise meeting the Bethesda criteria, all of them without a polyposis phenotype. In addition, we studied a series of 693 non-CRC patients with 1–13 adenomatous colorectal polyps for the MUTYH hotspot mutations Y179C, G396D and P405L. No bi-allelic MUTYH mutations were observed. Our data suggest that the contribution of bi-allelic MUTYH mutations to the development of CRC in Dutch non-polyposis patients that meet clinical genetic referral criteria, and to the development of low number of colorectal adenomas in non-CRC patients, is likely to be low.

Keywords Colorectal cancer · Adenomatous polyps · MUTYH · Young age · Familial · Bethesda criteria

Introduction

Colorectal cancer (CRC) is one of the most frequent solid tumors worldwide. In the Netherlands, it is the second most common type of cancer for women and third most common type for men, with more than 12,000 new cases reported in 2009 [1]. Although most CRC cases are sporadic, approximately 15–25 % of all CRC patients have a positive family history [2, 3], indicating genetic predisposition to CRC. The two best-characterized types of hereditary CRC are Lynch syndrome and familial adenomatous polyposis (FAP). These syndromes are autosomal dominant inherited disorders that account for approximately 3 % and 0.1–1 % of CRC diagnoses [4]. In addition to FAP, other polyposis syndromes have been recognized, including MUTYH associated Polyposis (MAP) accounting for 0.5–1 % of CRC diagnosis [5, 6] and Peutz–Jeghers syndrome, juvenile polyposis and other rare syndromes, each contributing to a small part of familial colorectal cancer. Unfortunately, for most of the remaining familial CRC cases, which usually do not present with a polyposis phenotype, underlying genetic factors are still unclear [7].

Typically, after referral for clinical genetic studies of colorectal cancer and in the absence of a polyposis...
phenotype, referred to in this paper as a ‘non-polyposis’, patients and families meeting particular clinical criteria are studied for signs indicative of Lynch syndrome. In the past, the Amsterdam criteria were used for selection, however, nowadays, the revised Bethesda criteria [8] are more commonly used. Characteristic features of Lynch syndrome include an increased risk for developing CRC, on average at a younger age, a predisposition for extracolonic malignancies including endometrial, ovarian and gastric carcinoma, and a positive family history [9]. After tumor testing for Lynch syndrome-associated features, microsatellite instability and/or loss of staining for mismatch repair (MMR) gene coded proteins, patients suspected of having Lynch syndrome are subsequently tested for germline MMR gene mutations. Patients and families with tumors that are not indicative of Lynch syndrome, and without a polyposis phenotype, are subsequently not routinely offered DNA testing for tumor syndrome genes and usually counseled on the basis of their family history with respect to cancer risks and appropriate surveillance programs. Additional genes for hereditary CRC may be identified in the future and testing of those genes may become part of the diagnostic strategy. However, it is possible that known tumor syndrome genes may present with phenotypes, including non-polyposis CRC, that are not traditionally associated with germline defects in those genes. Although these genes are not routinely tested in early-onset and/or familial CRC, such testing might be warranted. MUTYH is one of the genes to be considered testing in this setting.

Although bi-allelic MUTYH mutations are typically associated with the adenomatous polyposis syndrome known as MUTYH-associated polyposis (MAP) [10], CRC in the absence of a polyposis phenotype has been observed in a few patients with germline bi-allelic MUTYH mutations in large (population based) CRC series [11–17]. For this reason we have searched for the presence of bi-allelic MUTYH mutations in two independent cohorts of Dutch CRC patients that had been referred for genetic testing and counseling. In addition, we have studied the frequency of such mutations in a large Dutch cohort of non-CRC patients with low number of adenomatous polyps because this frequency in our population was unknown and therefore the potential clinical use of MUTYH analysis in this type of patients difficult to assess.

Materials and methods

Groningen CRC study population

Patients diagnosed with colorectal cancer before the age of 40 years, referred after January 1st 2005 to the department of Genetics of the University Medical Center Groningen for genetic study, were included in the study, irrespective of their family history. Only one patient per family was included. Patients with more than 20 polyps, and those with tumor microsatellite instability and/or loss of immunohistochemical staining for MMR proteins (methods published previously [18–20] were excluded. In total, 47 CRC patients were selected (16 men and 31 women; see Table 1 for other characteristics). DNA was isolated from peripheral blood lymphocytes using standard techniques. DNA testing was approved by the institute’s medical ethical review board.

Leiden CRC study population

From the clinical diagnostic and research registries at the department of Clinical and Human Genetics of the Leiden University Medical Center, we selected CRC patients meeting Amsterdam and/or Bethesda criteria with MSI-low or stable CRC, normal IHC and less than 20 polyps. Presence of MMR gene mutations, a polyposis phenotype, lack of details on personal medical and/or family medical history were exclusion criteria. Only one patient per family was included. In total, 42 CRC patients were selected for DNA analysis (20 men and 22 women; see Table 1 for other characteristics). DNA was isolated from peripheral blood lymphocytes using standard techniques. DNA testing was approved by the institute’s medical ethical review board.

Wageningen colorectal polyp study population

DNA was obtained from 668 healthy controls and 693 individuals previously gathered in an endoscopy-based case control study, which focused on gene-environment interactions and colorectal adenoma risk. In this study, participants were recruited among those undergoing endoscopy of the large bowel in ten outpatient clinics in the Netherlands between June 1997 and June 2002. The colorectal adenoma cases include both men and women, from 18 years of age up to age 75 at diagnosis, with no family history of CRC and with no history of CRC, partial colorectal resection or inflammatory bowel disease. Colonoscopy was performed for follow-up after previously detected colorectal adenomas or gastrointestinal complaints. Cases were selected for the presence of at least one histologically confirmed colorectal adenomatous polyp (see Table 1). The age at which polyps was detected in this population is shown in Fig. 1. DNA was isolated from peripheral blood lymphocytes using standard techniques [21]. The Medical Ethics Committee of Radboud
Groningen and Leiden MUTYH analysis

Mutation scanning of the coding region of the MUTYH gene was performed by denaturing gradient gel electrophoresis (DGGE) combined with direct sequencing of PCR fragments showing aberrant gel patterns in DGGE analysis, as published previously [20, 22]. Denaturing gradient gel electrophoresis has been widely used and has been shown to be a sensitive mutation detection method [23].

Wageningen population MUTYH analysis

At the National Institute for Public Health and the Environment (RIVM) MUTYH analysis of the hotspots Y179C, G396D and P405L in the controls and polyp patients was performed using the Pyrosequencing™ technique (http://www.pyrosequencing.com/) [24] as reported previously [25]. In Caucasian populations, a bi-allelic status for the hot spot mutations p.Y179C and/or p.G396D is reported in up to 70 % of MAP patients. Furthermore, 90 % of the western MAP population carries at least one of these mutations [26]. P405L is the third hotspot mutation in the Dutch population [22].

Results

Details of the results are shown in Table 1. Mutations are reported referring to the MUTYH Genomic sequence: NG_008189.1 (http://www.ncbi.nlm.nih.gov/gene/4595) [27]. Mutations Y179C, G396D and P405L have previously been published as Y165C, G382D and P391L, respectively. In the Groningen, Leiden and Wageningen series, no bi-allelic MUTYH mutations were identified. Mono-allelic mutations were observed in 0/47, 2/42

Table 1: Study population characteristics and MUTYH analysis results

<table>
<thead>
<tr>
<th>Population</th>
<th>Selection criteria</th>
<th>CRC characteristics</th>
<th>Polyps</th>
<th>Bethesda II</th>
<th>Amsterdam II</th>
<th>MUTYH</th>
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<tbody>
<tr>
<td>Groningen: Dutch, white Caucasian</td>
<td>CRC &lt; 40 yrs, MSS tumor and normal tumor MMR protein staining</td>
<td>Mean age: 33.9 yrs (Range: 22–39 yrs)</td>
<td>6 patients with adenomatous polyps (range: 1–8 polyps)</td>
<td>47/47 (100 %)</td>
<td>8/47 (17.0 %)</td>
<td>Full gene analyzed Mut/mut: 0/47 Mut/wt: 0/47 Wt/Wt: 47/47 (100 %)</td>
</tr>
<tr>
<td>Leiden: Dutch, white Caucasian</td>
<td>CRC Bethesda criteria positive &lt; 20 polyps</td>
<td>Mean age 52.2 yrs (Range: 29–71)</td>
<td>11 patients with adenomatous polyps (range 1–4 polyps)</td>
<td>42/42 (100 %)</td>
<td>30/42 (71.4 %)</td>
<td>Full gene analyzed Mut/mut: 0/42 Mut/wt: 2/42 (4.8 %; 1 Y179C and 1 G396D) Wt/wt: 40/42 (95.2 %)</td>
</tr>
<tr>
<td>Wageningen: Dutch, white Caucasian</td>
<td>One or more adenomatous polyps Colonoscopy performed because of clinical complaints or follow-up after previous polyp</td>
<td>Not applicable</td>
<td>100 % had between 1 and 13 adenomatous polyps: 1–2 polyps in 69.7 %; 3–4 in 16.2 %; 5–6 in 8.2 %; 7–8 in 3.8 % and 8–13 polyps in 2.1 % of cases. Ages at diagnosis 35–75 years (see Fig. 1)</td>
<td>0/693</td>
<td>0/693</td>
<td>3 hotspot mutations analyzed: Y179C; G396D and P405L Mut/mut 0/693 Mut/wt: 15/693 (2.1 %; 4 Y179C, 11 G396D)</td>
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</table>

CR colorectal, CRC colorectal cancer, IHC immunohistochemical staining for the Lynch syndrome-associated MMR gene-coded proteins, MMR DNA mismatch repair genes, MSI microsatellite instability, MSS microsatellite stable, MSI-L microsatellite instability-low, Mut MUTYH gene germline mutation, Wt wild type MUTYH allele, Yrs age in years
* not significantly different from the heterozygote frequency of 2.2 % in 668 Dutch controls (p > 0.1)
(4.8 %) and 15/693 (2.1 %) cases in the Groningen, Leiden and Wageningen series, respectively. These frequencies were not significantly different from the 15/668 (2.2 %) frequency observed in the controls ($p = 0.1$ and 0.85, respectively, Fisher exact). This heterozygote frequency corresponds to published population frequencies of 1–2 % [11–13].

Discussion

Our findings of zero bi-allelic MUTYH germline mutations suggest that the contribution of these bi-allelic mutations to the development of low number of adenomatous polyps in non-CRC patients, or to the development of early-onset and familial colorectal cancer in Dutch patients, is likely to be small. In our health care insurance setting, a cut-off of 10 % chance of finding a germline mutation is traditionally used to decide for or against testing for a particular gene. Although the size of our clinical genetics study population was limited, the chance of observing zero bi-allelic mutations in a sample of 89 individuals from a population with an 10 % proportion of such bi-allelic mutations is extremely small (8.5*10\(^{-5}\) or smaller). Still, because of the autosomal recessive nature of bi-allelic MUTYH mutations, we might have observed a higher frequency of mutations in CRC cases selected for negative family history or those with affected siblings only. On the other hand, although the issue is still under debate, mono-allelic MUTYH mutations may cause a small increase in CRC risk [28] and parents of patients with MUTYH bi-allelic mutations more frequently have CRC than can be expected in the general population [29]. Therefore, MUTYH mutations could also be expected in families with CRC in multiple generations. The published studies on bi-allelic MUTYH mutations observed in non-polyposis colorectal cancer patients are summarized in Table 2. These studies had different designs, making comparisons difficult. Bi-allelic MUTYH mutations were identified in MSI low or stable CRC patients, ranging in age between 31 and 48 years with zero (6 cases) or a small number of polyps (2 cases, 3 and 12 adenomas respectively) [16, 17]. However, polyp counts were unavailable for 2 of the patients in the Riegert-Johnson series [16]. The twelve patients with bi-allelic mutations in the Croituro series [13] had not been preselected using MSI and/or IHC findings. Seven of these patients had less than 10 polyps, their ages at CRC diagnosis ranged between 35 and 66 years. In total, in four of the studies, no MSI and/or IHC had been performed, which makes it difficult to extrapolate their findings to patients that are referred for clinical genetic testing who are typically first analyzed for these tumor characteristics. Six out of 7 studies analyzed MUTYH for hotspot mutations only. Therefore, the frequency of MUTYH mutations might have been somewhat underestimated and the same is true for our non-CRC polyp series which because of its large size has been analyzed for 3 hotspot mutations only.

Another important finding in the reported studies is that when bi-allelic mutations in the absence of a multiple polyp phenotype are present, this is not limited to those CRC cases with early-onset disease. Given the commonly known natural history of the MAP syndrome phenotype, which, like FAP, is associated with increasing number of polyps with increasing age, this is a somewhat unexpected finding. Likely environmental and other genetic factors might explain this difference of polyp count in CRC patients with bi-allelic MUTYH mutations. These study findings therefore suggest that age might not be an appropriate selection criterion for deciding when to look for MUTYH bi-allelic mutations in CRC patients without or with only few polyps. In our study, we might have observed a higher frequency of bi-allelic MUTYH if later-onset colorectal cancer cases would have been included. However, we deliberately selected only younger age-at-onset cases or those otherwise meeting the Bethesda criteria, reflecting the patients typically referred for genetic analysis.

As previously reported, certain tumor features, molecular and histological might better help direct the physician, i.e. pathologist, towards a MAP etiology of CRC. These features include a proximal location, mucinous histotype, increased presence of tumor infiltrating lymphocytes and a specific somatic KRAS mutation (the c.34G $\rightarrow$ T in codon 12), since these were found to be relative common in MAP related CRCs [30, 31].

Taken together, the literature and present findings suggest that bi-allelic MUTYH mutations in non-polyposis CRC patients and in non-CRC patients with low number of adenomatous polyps are relatively rare. Given the present costs of DNA testing, including that of testing mutation hotspots only, and the fact that only a limited number of gene tests per patient are covered by Dutch health care insurance, we suggest that germline MUTYH testing should not yet be part of the routine genetic analysis of patients with non-polyposis colorectal cancer or of a low number of adenomatous polyps in our country. Other countries may face similar financial constraints. In the meantime tumor analysis, especially KRAS hot spot analysis, could be implemented as a pre-screening test that helps select patients with CRC who are eligible for MUTYH mutation screening [31]. A more widespread use of MUTYH analysis should, however, be considered when genetic testing becomes more affordable, for example as part of a targeted analysis gene panel in next generation sequencing.
### Table 2 Literature overview

<table>
<thead>
<tr>
<th>Authors</th>
<th>Population</th>
<th>Country/Ethnicity</th>
<th>Polyps</th>
<th>MSI/IHC in tumors</th>
<th>Type of MUTYH analysis</th>
<th>MUTYH mut/ wt and wt/wt</th>
<th>MUTYH mut/ wt (%)</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Ashton et al. [11]. | Either Amsterdam or Bethesda positive patients n = 442
Group 1: No mutation of hMLH1 or hMSH2 N = 233 (162/233 had developed CRC)
Group 2: confirmed mutation of hMLH1 or hMSH2 N = 209 | Caucasian, from the state of New South Wales | Few adenomas identified, not further specified | Not reported | Y179C and G396D, the whole gene was screened when a mono- or bi-allelic mutation was found | Group 1: mut/ wt: 5/233 (2.1 %) Wt/wt: 228/233 (97.9 %) | Group 1: 0/233 (0 %) | Group 2: mut/ wt: 2/209 (0.96 %) Wt/wt: 207/209 (99.0 %) | Controls: Mut/wt: 4/296 (1.35 %) Wt/wt: 292/296 (98.6 %) |
| Casper et al. [12]. | CRC only: n = 23
Adenoma only: n = 75
CRC and adenoma N = 33
Total group: N = 131 ages 18–89 yrs
Median 68 yrs (89 M/42F) | Caucasian | <15 adenomatous polyps: 100/108 (92.6 %)
≥15 adenomatous polyps: 8/108 (7.4 %) | Not reported | Hotspot mutations Y179C and G396D | mut/wt: 3/131 (2.3 %) Wt/wt: 127/131 (96.9 %) | 1/131 (0.77 %) | 2 of 3 patients with mut/wt had polypos (>15 adenomatous polyps)
The mut/mut patient developed CRC at age of 44 and in the latter 14 yrs 107 adenomatous polyps were removed (MAP phenotype)
One additional patient was an uncertain bi-allelic mutation carrier: in addition to 1 pathogenic mutation he/she carried an unclassified Q338H variant. He had 27 adenomas at age 67 yrs. |
| | Crohn’s disease N = 72
Ulcerative colitis N = 20
Other non-CRC colorectal disease N = 13
Total group: N = 112 (51 M/61F) | Caucasian | Not applicable | Not applicable | Hotspot mutations Y179C and G396D | mut/wt: 1/112 (0.9 %), possibly 2/112 (1.8 %), see comments Wt/wt: 110/112 (98 %) | 0/112 (0 %), possibly 1/112 (0.8 %), see comments | One Crohn’s patient was an uncertain bi-allelic mutation carrier: in addition to 1 pathogenic mutation he/she carried an unclassified Q338H variant. | Controls: no colorectal disease, n = 116 (54 M/62F) |
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<th>MUTYH mut/wt (%)</th>
<th>Comments</th>
</tr>
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</table>
| Croitoru et al. [13]. | CRC: 20–74 yrs  
N = 1238 | Canadian, Ontario population                                      | FAP patients were excluded from the study but criteria for FAP diagnosis (>100 adenomas and/or APC mutations?) was not reported  
Range of polyp counts in study group as a whole not reported | Not reported | Hotspot mutations Y179C and G396D. The whole gene was screened in hotspot mutation carriers to look for a second mutation | mut/wt: 29/1238 (2.3 %)  
w/t: 1197/1238 (96.6 %) | 12/1238 (0.97 %) | Ages at CRC diagnosis in bi-allelic mutation carriers ranged between 35 and 67 years; Adenoma counts ranged between 0 and at least 48 (one patient was reported to have 48 polyps, another “polyposis”) |
| Giraldes et al. [14]. | CRC ≤ 50 yrs (mean 44.1 yrs)  
N = 140  
Family history of CRC in 36/140 (26.3%) of patients  
8/140 (5.8%) of the cases fulfilled Amsterdam II criteria | Spanish, not further specified | Patients with a history of polyps were excluded  
Range of polyp counts in study group as a whole not reported | Not applicable | Similar to the CRC group | wt/wt: 1232/1255 (97.9 %) | 0/1255 (0 %) | Cohort includes proven Lynch syndrome patients  
All patients with bi-allelic MUTYH mutations had MSI-S tumors. Ages at diagnosis were 43, 45, 46 and 47 yrs respectively. |
| Piroshky et al. [15]. | CRC: total  
N = 75 (30 M/45F)  
Mean age at CRC diagnosis of total group = 50.4 yrs  
MAP group (n = 15): Clinical diagnosis of MAP syndrome defined as between 10–99 CR adenomas + pedigree suggestive of AR inheritance  
FAP group (n = 15)  
FAP clinically defined as > 100 CR adenomas  
CRC patients meeting Amsterdam II criteria (n = 15)  
CRC patients meeting Bethesda but not Amsterdam II criteria (n = 15)  
Sporadic CRC (n = 15)  
age > 60 yrs  
Polyps present in 47/75 (65 %) patients:  
<10 polyps: 21/47 (47.7 %)  
10–99 polyps: 7/47 (15.9 %)  
100–500 polyps: 3/47 (6.8 %)  
>500 polyps: 2/47 (4.5 %)  
Undetermined number of polyps: 11/47 (23.4 %) | Not reported | Hotspot mutations Y179C and G396D | Mut/wt: 3/75 (4.0 %)  
Wt/wt: 70/75 (93.3 %) | 2/75 (2.7 %) | 1 bi-allelic mutation was found in the MAP group and one in the FAP group |
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<tbody>
<tr>
<td>Riegert-Johnson et al.</td>
<td>CRC ≤ 50 yrs N = 229</td>
<td>USA, not further</td>
<td>Information on polyp count was available for 17% of patients (mean = 2 polyps, no range given)</td>
<td>MSS or MSI-L No IHC data reported</td>
<td>Hotspot mutations Y179C and G396D</td>
<td>mut/wt: 6/229 (2.6%) wt/wt: 219/229 (95.6%)</td>
<td>4/229 (1.7%)</td>
<td>Ages at diagnosis of the 4 patients with bi-allelic MUTYH were 31, 39, 46 and 48 yrs respectively. Polyp count was available for the second and third of these patients: 12 and 3 adenomas, respectively.</td>
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<tr>
<td>Wang et al. [17]</td>
<td>Group 1: Screening colonoscopy N = 400</td>
<td>USA, not further specified</td>
<td>Group 1: 87/400 with adenomatous polyps (range 1–3 polyps)</td>
<td>MSS: 382/444 CRC MSI-H: 62/444 CRC Of these 60/62 had loss of staining for at least one MMR gene</td>
<td>Hotspot mutations Y179C and G396D</td>
<td>Group 1: mut/wt: 5/400 (1.3%) wt/wt: 395/400 (98.7%)</td>
<td>Group 1: 0/400 (0%)</td>
<td>Both patients with bi-allelic mutations had a MSI-S CRC, diagnosed at age 39 and 46 yrs, respectively.</td>
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<td>Group 2: CRC patients n = 444</td>
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<td>Group 2: no information on polyps reported</td>
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<td></td>
<td>CRC ≥ 51 yrs n = 328</td>
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<tr>
<td></td>
<td>CRC ≤ 50 yrs n = 116</td>
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CR colorectal, CRC colorectal cancer, FAP familial adenomatous polyposis, IHC immunohistochemical staining for the Lynch syndrome-associated MMR gene-coded proteins, MSI microsatellite instability, MSI-L microsatellite instability low, MSS microsatellite stable, Mut MUTYH gene germline mutation, Yrs age in years, Wt wild type MUTYH allele
References