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Digenic inheritance of MSH6 and MUTYH variants in familial colorectal cancer

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Abstract
We describe a family severely affected by colorectal cancer (CRC) where whole-exome sequencing identified the coinheritance of the germline variants encoding MSH6 p.Thr1100Met and MUTYH p.Tyr179Cys in, at least, three CRC patients diagnosed before 60 years of age. Digenic inheritance of monoallelic MSH6 variants of uncertain significance and MUTYH variants has been suggested to predispose to Lynch syndrome-associated cancers; however, cosegregation with disease in the familial setting has not yet been established. The identification of individuals carrying multiple potential cancer risk variants is expected to rise with the increased application of whole-genome sequencing and large multigene panel testing in clinical genetic counseling of familial cancer patients. Here we demonstrate the coinheritance of monoallelic variants in MSH6 and MUTYH consistent with cosegregation with CRC, further supporting a role for digenic inheritance in cancer predisposition.

KEYWORDS
digenic inheritance, familial colorectal cancer, Lynch syndrome, MSH6, MUTYH, whole-exome sequencing

1 | INTRODUCTION

Approximately 25% of colorectal cancers (CRCs) are diagnosed in patients with a family history of CRC. However, the majority of familial CRC cannot be explained by clear-cut genetic defects, which hampers appropriate genetic counselling. The most frequent form of hereditary CRC is Lynch syndrome (OMIM#120435), which predisposes to cancers that develop in a context of DNA mismatch repair (MMR) deficiency, including CRC and endometrial cancer. It is caused by heterozygous, pathogenic variants affecting the DNA MMR genes, MLH1, MSH2, MSH6, or PMS2. MUTYH-associated polyposis (MAP: OMIM#608456) is a recessively inherited CRC syndrome caused by biallelic variants in the base-excision repair gene MUTYH. The potential of monoallelic, pathogenic MUTYH variants to predispose to CRC remains debatable. Some MUTYH variants confer greater functional defects in vitro and are associated with more severe clinical phenotypes, such as the variant encoding p.Tyr179Cys compared to p.Gly396Asp.

Digenic inheritance of monoallelic MSH6 and MUTYH variants has been suggested to predispose to Lynch syndrome-associated cancers;...
however, cosegregation of both variants within CRC families has not yet been demonstrated. Here, we demonstrate, for the first time, the coinheritance of monoallelic variants in MSH6 and MUTYH consistent with the cosegregation with CRC, further supporting a role for digenic inheritance in cancer predisposition.

2 MATERIAls AND METHODS

2.1 Patients

Clinicopathological data of family members was obtained during consultations at the department of Clinical Genetics of the Amsterdam University Medical Centre, Vrije Universiteit Amsterdam. DNA was extracted from peripheral blood and formalin-fixed paraffin-embedded tissues using standard techniques. All patients provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center, The Netherlands (protocol P01.019).

2.2 Whole-exome sequencing

Whole-exome sequencing was outsourced to BGI (BGI-Shenzhen, Shenzhen, China); exome libraries were constructed with the BGI capture kit, followed by sequencing on the Complete Genomics’ Sequencing Platform (Complete Genomics Inc., San Jose, California). Filtering and variant prioritization was performed as previously described. All variants were selected based on a maximum population frequency <0.01 (in 1000 Genomes phase 3, ExAC 1.0, ESP6500SI-V2 or GoNL release 5).

2.3 Variant screening

The MSH6 (p.Thr1100Met) and MUTYH (p.Tyr179Cys) variants were validated and investigated in additional family members by using Sanger sequencing of PCR products obtained under standard PCR conditions. The following M13-tailed primer sets were used: 5'-TGT AAA ACG ACG GCC AGT AAA ACC CCC AAA CGA TGA A-3' and 5'-CAG GAA ACA GCT ATG ACC TGC TCC TCT TCA CAG-3' for MSH6, and 5'-GAC GTT GTA AAA CGG CCA GTC CCT AGG GTA GGG GAA ATA GG-3' and 5'-CAG GAA ACA GCT ATG ACC ATG AGT TCC TAC CCT GCC ATC-3' for MUTYH (M13-tails are underlined).

2.4 Tumor analysis

MMR deficiency in tumor samples was assessed by microsatellite instability analysis and immunohistochemical detection of the four MMR proteins (MLH1, MSH2, MSH6, and PMS2). KRAS codon 12/13 mutations were screened with Sanger sequencing.

2.5 Functional MMR assay

In vitro MMR activity assay was performed as previously described.

3 RESULTS

We performed germline whole-exome sequencing on three CRC patients diagnosed before 60 years of age (III-1, III-7, III-8, Figure 1A)
<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>RefSeq accession number</th>
<th>mRNA change</th>
<th>Protein change</th>
<th>Population frequency&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ClinVar classification&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Franklin classification&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Cancer gene census</th>
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<tbody>
<tr>
<td>1</td>
<td>EBNA1BP2</td>
<td>NM_001159936</td>
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<td>—</td>
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<td>—</td>
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<tr>
<td>1</td>
<td>CAPN9</td>
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<td>—</td>
<td>VUS</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
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<td>c.3229C &gt; T</td>
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<td>Uncertain</td>
<td>VUS</td>
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<tr>
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<td>—</td>
<td>VUS</td>
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<tr>
<td>7</td>
<td>KIAA1324L</td>
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<td>c.2369 T &gt; C</td>
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<td>—</td>
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<td>TRIP6</td>
<td>NM_003302</td>
<td>c.822G &gt; C</td>
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<td>7</td>
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<td>8</td>
<td>PDP1</td>
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<td>Likely benign</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: Chr, chromosome; VUS, variant of uncertain significance.
<sup>a</sup>Population frequency (gnomAD 2.1.1).
<sup>b</sup>ClinVar clinical significance (ClinVar database version August 5, 2019).
<sup>c</sup>Franklin by Genoox (accessed on May 20, 2020).
and who belonged to a CRC family comprising of seven cancer patients divided over two generations. Twenty-two rare variants were shared by the three patients (Tables 1 and S1), including variants in the MSH6 (NM_000179.2: c.3299C > T, p.Thr1100Met) and MUTYH (NM_001128425.1: c.536A > G, p.Tyr179Cys) genes, while the other 20 genes could not be clearly linked to cancer predisposition. The identified MSH6 variant was classified as a variant of uncertain significance (VUS) in the Leiden Open Variant Database and the InSiGHT DNA Variant Database.14,15 The MUTYH variant is the most common pathogenic variant found in the Netherlands.2

Fourteen relatives, all unaffected by cancer or polyposis, were genotyped for these MSH6 and MUTYH variants, identifying one additional carrier of both variants, five MSH6-only carriers and four MUTYH-only carriers. In all probability, the mothers of the sequenced patients, II-1 and II-2, who were affected by ovarian cancer bellow age 74 and CRC at 38 years old respectively, were obligate carriers of both variants; however, DNA was unavailable for testing and, formally, inheritance through the fathers to the sequenced individuals (III-1, III-7, III-8) cannot be excluded. MMR deficiency was not detected in the colorectal carcinoma of patient III-1, which also lacked the KRAS mutation typical for MAP tumors (c.34C > T; Table S2). Functional analysis of the MSH6 p.Thr1100Met variant showed retained MMR function in vitro (Figure 1B).

4 | DISCUSSION

Digenic inheritance of monoallelic MSH6 and MUTYH variants has been suggested to predispose to Lynch syndrome-associated cancers. The involvement of both MSH6 and MUTYH in oxidative DNA damage repair and their physical interaction enhancing MUTYH’s repair activity, substantiates the association of variants in these genes.16 From earlier studies, the inheritance of monoallelic MUTYH variants seemed primarily relevant in patients carrying MSH6 VUSs, which are less strongly associated with MMR deficiency than pathogenic MSH6 variants (Table S2).5–9 Furthermore, a digenic inheritance model was proposed once before for CRC predisposition in a carrier of variants in the oxidative DNA damage repair genes MUTYH and OGG1.17 Although the functional evidence of combined defects in oxidative DNA damage repair genes is still lacking, the coinheritance of MSH6 and MUTYH variants in at least three, but likely five cancer cases within one family warrants further mechanistic and clinical studies. The absence of cancer and numerous polyps in nondigenic carriers further substantiates this association. Tumor analysis of the tumor of one of the digenic carriers and the in vitro MMR activity assay indicated retention of MMR function of MSH6 p.Thr1100Met protein. In addition, the genetic marker for MAP-tumors (KRAS c.34G > T) was absent in this tumor, which points toward retained MUTYH repair activity. The combined inheritance of both genetic variants could still result in impaired repair of oxidative DNA damage. More extensive somatic mutation analysis to assess this was, however, not possible, because of low quality of the DNA sample and the unavailability of additional tumor material.

Next to MSH6 and MUTYH, CUX1 has been described as a cancer-driving gene.18 CUX1 is implicated in inflammatory bowel disease and various cancer types, although primarily due to loss-of-function somatic mutations.18,19 This gene codes for several isoforms, including the ubiquitously expressed p200 CUX1, which, among other functions, has been shown to stimulate the repair of oxidized DNA bases by OGG1.20 The identified CUX1 (NM_001202543: c.1438A > G, p.Ser480Gly) variant, however, was classified as likely benign by the Franklin variant classification tool.21 Additional gene reportedly linked to tumorogenesis include RYR3,22 EBNA1BP2,23 TRIP6,24 and CAPN9.25 The RYR3 (NM_001036: c.7812C > G, p.Asn2604Lys) and EBNA1BP2 (NM_001159936: c.1034A > T, p.Asn345Ile) variants were classified as likely benign and benign, respectively, while the TRIP6 (NM_003302: c.822G > C, p.Glu274Asp) and the CAPN9 (NM_006615: c.55G > T, p.Ala19Ser) variants were classified as VUS.21 TRIP6 promotes cell migration and invasion through Wnt/β-catenin signaling and was shown to be upregulated in colorectal tumors.24 Therefore, TRIP6 variants that increase protein stability or expression could potentially stimulate colorectal tumorigenesis. In addition, lost-of-function variants in CAPN9 might promote tumor formation, as Calpain-9 induces cell cycle arrest and apoptosis, and low expression predicts a poorer prognosis in gastric cancer patients.25 The contribution of the genetic variants, other than MSH6 and MUTYH, to cancer risk cannot be completely excluded. However, none of these variants have been functionally investigated and especially the variants predicted as benign or likely benign are less likely to contribute to an increased cancer risk. Besides, none of these genes have, to date, been associated with a genetic predisposition to any types of cancer.

In conclusion, with the increased application of whole-genome sequencing or large multigene panel testing in clinical genetic counseling, the number of identified individuals carrying multiple potential risk variants is expected to rise. Here, we demonstrate the coinheritance of MSH6 and MUTYH variants consistent with the cosegregation with cancer, further supporting a role for digenic inheritance in CRC predisposition. Our results reiterate that digenic inheritance should be considered as cause of genetic diseases.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.
AUTHOR CONTRIBUTIONS

Tom van Wezel, Noel F. C. C. de Miranda, and Hans Morreau conceived and designed the study. Dina Ruano performed next-generation sequencing analyses. Noel F. C. C. de Miranda and Stephanie A. Schubert performed analysis and interpretation of whole-exome sequencing data. Mark Drost and Yvonne Tiersma performed functional analysis. Maartje Nielsen and Liselotte P. van Hest performed patient counseling and clinical data acquisition. Hans Morreau performed the pathology review of the samples. Tom van Wezel, Noel F. C. C. de Miranda, Mark Drost, and Niels de Wind supervised the work. Stephanie A. Schubert, Noel F. C. C. de Miranda, and Tom van Wezel wrote the manuscript. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy restrictions.

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REFERENCES


SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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