Asia–Pacific Journal of Clinical Oncology

Edited By: Stephen ACKLAND, Australia and Mengzhao WANG, China

Impact factor: 2.601

2020 Journal Citation Reports (Clarivate Analytics): 200/243 (Oncology)

Online ISSN: 1743-7563

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First Published: 30 January 2022

Received: 6 June 2021 | Revised: 27 July 2021 | Accepted: 4 December 2021

DOI: 10.1111/ajco.13749

ORIGINAL ARTICLE

WILEY

Molecular characteristics of young-onset colorectal cancer in Vietnamese patients

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Funding information
This work was supported by the Department of Science and Technology of Ho Chi Minh City, grant number 16/2019/HD-QPTKHCN.
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Funding information
Department of Science and Technology of Ho Chi Minh City, Grant/Award Number: 16/2019/HD-QPTKHCN

Abstract

Background: Colorectal cancer (CRC) is one of the most common cancer globally. Understanding the genetic characteristics of CRC is essential for appropriate treatment and genetic counseling.

Methods: The genetic profile of CRC tumor tissues was identified using next-generation sequencing of 17 target genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *SMAD4*, *BMPR1A*, *MUTYH*, *STK11*, *PTEN*, *TP53*, *ATM*, *CDH1*, *CHEK2*, *POLE*, and *POLD1*) in a cohort of 101 Vietnamese patients diagnosed with young-onset CRC. Corresponding germline genetic alterations of determined somatic mutations were subsequently confirmed from patients' blood samples.

Results: Somatic mutations were determined in 96 out of 101 CRC patients. Two-thirds of the tumors harbored more than two mutations, and the most prevalent mutated genes were *TP53* and *APC*. Among confirmed germline mutations, 10 pathogenic mutations and 11 variants of unknown significance were identified.

Conclusions: Given the burden of CRC and the gradually reducing cost of genetic testing, multigene panel screening can benefit young-onset CRC patients as well as their relatives.

KEYWORDS
germline mutation, next-generation sequencing, somatic mutation, young-onset colorectal cancer

1 | INTRODUCTION

Colorectal cancer (CRC) is a heterogeneous disease with complex molecular mechanisms and one of the most prevalent cancers in Vietnam in both genders.[1] Although this disease is usually diagnosed in patients who are more than 60 years old,[2] the incidence of young-onset CRC in patients who are 50 years old or less is increasing worldwide and becoming a global burden;[3,4] this trend may explain why the recommended age for initiating screening in an average-risk adult has been reduced from 50 to 45 by the American Cancer Society.[5] In most cases, the disease occurs sporadically, and hereditary cases in which mutations of causative genes are detected typically account for less than 10% of CRC patients.[6] However, studies have shown that when reducing the age for genetic testing to less than 50, the frequency of germline mutations almost doubles.[7,8] Young-onset CRC has attracted numerous studies due to the burden of the disease and the impact of treatment on young individuals, as well as the potential hereditary risk faced by patients' relatives.[8,9]

The development of sequencing techniques allows a better understanding of the molecular mechanisms of CRC. Genetic testing for germline mutations is part of the recommendation for patients with a high risk of hereditary CRC;[10] however, universal genetic testing has been shown to detect further causative mutations.[11] Once germline mutations have been found, both the screening and management of

patients and their relatives change significantly. Thus, subjects who are at high risk of CRC genetically would benefit from an early screening strategy.[12] High penetrance genes that are associated with an inherited risk of CRC are mismatched repair genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* for Lynch syndrome, *APC* for familial adenomatous polyposis, *SMAD4*, *BMPR1A* for Juvenile polyposis, *MUTYH* for *MUTYH*-associated polyposis, and *STK11*, *PTEN*, *TP53*.[13] These genes are the primary components of the traditional genetic testing panel for inherited CRC. Additionally, the current strategy for genetic testing for CRC also includes moderate penetrance genes, such as *ATM*, *CDH1*, *CHEK2*, *POLE*, and *POLD1*.[14]

Besides germline mutations, somatic mutations in CRC provide useful information regarding disease pathophysiology, prognosis, and novel treatments target. Molecular characteristics of oncogenes, such as *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and their associated clinicopathological features have been extensively studied in Vietnam;[15] however, information on tumor suppressor and mismatched repair genes is still not available. Furthermore, the molecular characteristics of CRC in young-onset, as well as other CRC patients, is well-established in some populations,[7,8,16] but these data have not been described for Vietnamese patients. Hence, this study aims to evaluate the genetic profile of CRC-associated genes in Vietnamese patients diagnosed with young-onset CRC by using an advanced, next-generation sequencing (NGS) platform.

2 | MATERIALS ANS METHODS

2.1 | Subjects

The protocol for this study was approved by the Ethical Committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (approval number 291/ĐHYD-HDDD). CRC patients who were aged 50 or less at onset were prospectively recruited at the University Medical Center, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. The CRC diagnosis was based on pathology specimens and confirmed as adenocarcinoma by two experienced pathologists. A total of 101 patients who had undergone colorectal tumor resection agreed to take part in this study. Formalin-fixed paraffin-embedded (FFPE) tissues from the surgery, four milliliters of peripheral blood, and clinical information on all the subjects were collected.

2.2 | DNA extraction

DNA samples were extracted from paired tumor tissues and peripheral blood from all the participants. DNA from tumor tissue was extracted from pathologically diagnosed samples using GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA samples were quantified for purity and concentration using a QFX Fluorometer (DeNovix, Delaware, United States). The minimum concentration of DNA was 2.5 ng/μL. Genomic DNA was extracted from blood cells using an Illustra Blood GenomicPrep Mini Spin Kit (GE healthcare, Illinois, United States) according to the manufacturer's protocol.

2.3 | Primers designed for multiplex-PCR

Primers used for multiplex-PCR of the coding regions of 17 target genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *SMAD4*, *BMPR1A*, *MUTYH*, *STK11*, *PTEN*, *TP53*, *ATM*, *CDH1*, *CHEK2*, *POLE*, and *POLD1*) were designed based on AmpliSeq Gene principles using Design Studio software (https://login.illumina.com/platform-services-manager). The designed primers were considered to be qualified when the maximum length of the amplified regions was 140 bp and acceptable coverage was greater or equal to 95%. These primers were synthesized by Illumina (California, United States) and divided into three master mixes (pool 1, pool 2, and pool 3).

2.4 | Library preparation and NGS

DNA extracted from FFPE tissues was amplified using a multiplex-PCR reaction with three pools of primers. DNA products (amplicons) were treated with FuPa reagent to remove excessive primers and adapters were subsequently added to distinguish between samples. The DNA library was purified by AMPure XP beads and amplified. The final concentration of the library was quantified using a QFX Fluorometer before dilution to the final concentration of 2 nM.

The sequencing reactions were performed using a MiniSeq system (Illumina, California, United States) and a MiniSeq High Output kit (Illumina, California, United States). The number of samples in each run was calculated to assure 600 to 1000x coverage for the FFPE samples.

2.4.1 | Data analysis

NGS data were analyzed using BaseSpace Sequence Hub software (Illumina, California, United States) with human genome 19 as a reference. Identified variants were classified based on the ClinVar database for germline mutations and the Catalogue of Somatic Mutations in Cancer (COSMIC) database for somatic mutations.[17,18]

2.4.2 | Direct sequencing

Genetic variants identified in FFPE tissues by NGS were subsequently confirmed by direct sequencing using appropriate primers in both FFPE tissues and corresponding blood samples. The protocol for direct sequencing was developed in previous studies.[19–21]

Germline variants were designated according to guidelines from the Human Genome Variation Society. The impact of detected novel missense mutations was assessed by sorting intolerant from tolerant (SIFT) and polymorphism phenotyping-2 (PolyPhen-2).[22,23]

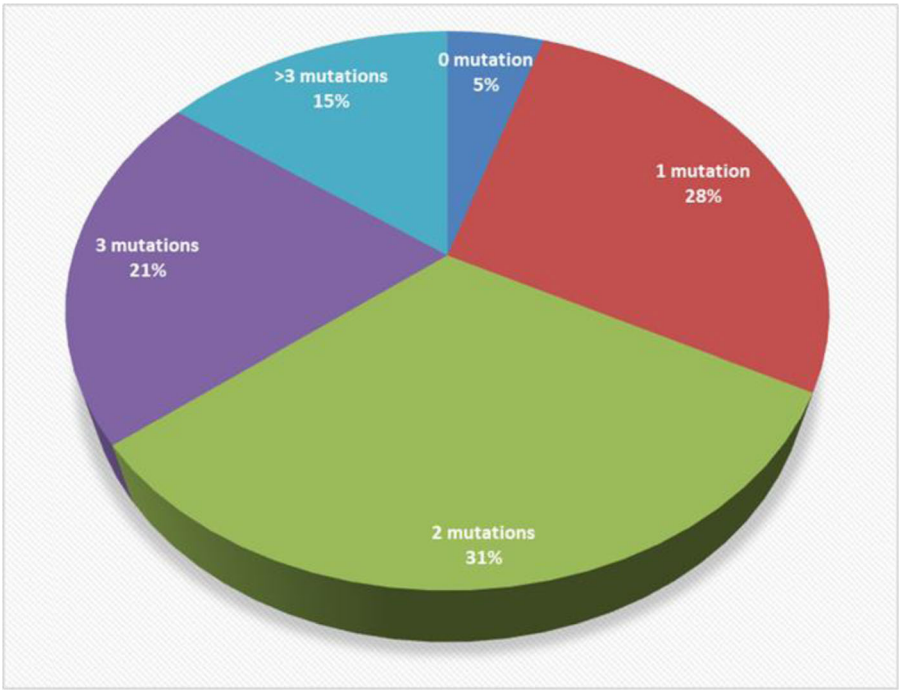

FIGURE 1 The distribution of number of somatic mutations in colorectal cancer patients [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Clinical characteristics of CRC patients

The clinical characteristics of 101 participants are summarized in Table 1. All patients were self-identified as Kinh Vietnamese. The mean age at CRC diagnosis in this study was 38.6, and males accounted for 48.5% of the sample. Most of the patients did not have a history of polyps or a family history of CRC. The dominant symptoms were abdominal pain and hematochezia. After surgery, most patients were found to have invasive stage III to IV tumors, according to the classification of the Union for International Cancer Control.[24]

3.2 | Molecular characteristics of CRC patients

The details of somatic mutations of all the tested genes are summarized in Supplementary Table 1. The most common genetic mutations were detected in *TP53*, *APC*, and *SMAD4* with a prevalence of 65.3%, 52.5%, and 17.8%, respectively, while there were no detectable mutations in *STK11* or *EPCAM*. The distribution of somatic mutations is shown in Figure 1. More than two-thirds of the patients had at least two somatic mutations. Most of the somatic mutations of *APC* are nonsense while the majority of *TP53* mutations were missense. Mutations of Lynch-associated genes (*MLH1*, *MSH2*, *PMS2*, *MSH6*, and *EPCAM*) were found in 29 out of the 101 CRC cases (Supplementary Table 1).

The results of direct sequencing show that germline pathogenic mutations were found in 10 patients; variants of uncertain significance (VUS) were also detected in another 11 patients. The germline mutations and their associated syndromes are described in Table 2. Most of the pathogenic germline mutations were associated with Lynch syndrome and familial adenomatous polyposis. Two patients with *APC* mutations had history of colorectal polyps and family history of coloretal cancer while these characteristics were not found in the rest of germline mutation-carriers. One mutation of *CDH1*, traditionally associated with hereditary diffuse gastric cancer, was found. The detected VUS and their predicted impact by SIFT and PolyPhen-2 are described in Table 3. Most of the VUS were predicted to be deleterious except for the variant c.1273G > A (p.V425I) on *CDH1*. The clinicopathologic characteristics stratified by mutation status of 101 CRC patients are reported in Table 1.

4 | DISCUSSION

Traditionally, direct sequencing of large genes for cancer research in Vietnam required considerable resources and sometimes a specific strategy, such as mRNA sequencing.[25] Advances in NGS have simplified the analysis of large and complex genes[16,26] and provided powerful tools for CRC research and diagnostic applications.[27]

The use of tumor sequencing revealed that almost all the colorectal cancer tissues harbor at least one somatic mutation in the genes tested. More than half of the patients carried *APC* somatic mutations and most of them were nonsense. Compared to the Cancer Atlas Genome,[16] we

TABLE 1 Clinicopathologic characteristics of young-onset CRC patients

Patients' characteristics	All patients (N = 101)	Germline mutation-carrier (N = 10)	VUS-carrier (N = 11)	No mutation or VUS (N = 80)
Age, (Mean ± SD)	38.6 ± 7.2	40.5 ± 8.4	38.0 ± 6.4	38.5 ± 7.2
Gender, N (%)				
Male	49 (48.5)	7 (70.0)	7 (63.6)	35 (43.8)
Female	52 (51.5)	3 (30.0)	4 (36.4)	45 (56.2)
Demographic, (Mean ± SD)				
Height (cm)	162.6 ± 8.0	163.8 ± 10.2	161.7 ± 8.6	162.5 ± 7.8
Weight (kg)	57.8 ± 10.7	57.9 ± 14.2	60.1 ± 15.5	57.6 ± 9.6
BMI (kg/m^2)	21.8 ± 3.1	21.4 ± 3.9	22.6 ± 3.9	21.8 ± 3.0
Family history of cancer, N (%)	4 (3.9)	1 (10.0)	0	3 (3.8)
History of colorectal polyp, N (%)	2 (1.9)	2 (20.0)	0	0
Comorbidities, N (%)				
Hypertension	3 (2.9)	0	1 (9.1)	2 (2.5)
Type 2 diabetes	3 (2.9)	1 (10.0)	0	1 (1.3)
Symptoms, N (%)				
Hematochezia	44 (43.6)	4 (40.0)	5 (45.5)	35 (43.8)
Abdominal pain	57 (56.4)	8 (80.0)	5 (45.5)	44 (55.0)
Constipation	23 (22.8)	0	3 (27.3)	20 (25.0)
Diarrhea	23 (22.8)	4 (40.0)	2 (18.2)	17 (31.3)
Change in bowel habit	26 (25.7)	4 (40.0)	2 (18.2)	20 (25.0)
Tiredness	9 (8.9)	2 (20.0)	0	7 (8.8)
Weight loss	24 (23.8)	5 (50.0)	1 (9.1)	18 (22.5)
Anal pain	6 (5.9)	0	0	6 (7.5)
Time from symptom to diagnosis (day), (Median - min, max)	60 (2-720)	30 (0-360)	75 (5-360)	60 (2-72)
Tumor site, (N%)				
Right colon	24 (23.8)	2 (20.0)	3 (27.3)	19 (23.8)
Transverse colon	7 (6.9)	2 (20.0)	1 (9.1)	4 (5.0)
Left colon	36 (35.6)	2 (20.0)	5 (45.5)	29 (36.3)
Rectum	34 (33.7)	4 (40.0)	2 (18.2)	28 (35.0)
Invasion, N (%)	84 (82.3)	9 (90.0)	10 (90.9)	65 (81.3)
Macro pathology, N (%)				
Protruded	50 (49.5)	4 (40.0)	5 (45.5)	41 (51.3)
Ulcerative	35 (34.7)	2 (20.0)	5 (45.5)	28 (35.0)
Protruded and ulcerative	12 (11.9)	3 (30.0)	1 (9.1)	8 (20.0)
Infiltrative	4 (3.9)	1 (10.0)	0	3 (3.7)
UICC stage, N (%)				
I	1 (1.0)	0	0	1 (1.2)
II	7 (6.9)	1 (10.0)	0	6 (7.5)
III	73 (72.3)	9 (90.0)	10 (90.9)	54 (67.5)
IV	20 (19.8)	0	1 (9.1)	19 (23.8)

UICC: Union for International Cancer Control.

TABLE 2 Germline mutations and their associated syndromes

Patient	Mutation	Associated syndrome
YCRC-3	*PMS2* c.341_348del (p.L114Pfs*22)	Lynch syndrome
YCRC-4	*APC* c.1905insG (p.G637Wfs*14)	Familial adenomatous polyposis (FAP)
YCRC-59	*PMS2* c.1738A > T (p.K580*)	Lynch syndrome
YCRC-62	*CDH1* c.377del (p.P126Rfs*89)	Hereditary diffuse gastric cancer
YCRC-87	*MSH2* c.1165C > T (p.R389*)	Lynch syndrome
YCRC-91	*MSH2* c.2038C > T (p.R680*)	Lynch syndrome
YCRC-92	*APC* c.3927_3931delAAAGA (p.E1309Dfs*4)	Familial adenomatous polyposis (FAP)
YCRC-100	*MSH6* c.394_395delCA (p.Q132fs)	Lynch syndrome
YCRC-101	*MLH1* c.1975C > T (p.R659*)	Lynch syndrome
YCRC-110	*MSH6* c.1572_1573delCA (p.Y524*)	Lynch syndrome

TABLE 3 VUS and their calculated impact by SIFT and Polyphen-2

Patient	Variant	SIFT score	PolyPhen-2 score
YCRC-2	*MUTYH* c.934-2A > G	n/a	n/a
YCRC-24	*APC* c.6691A > T (p.I2231F)	0.02 Affect protein function	0.997 Probably damaging
YCRC-50	*MSH2* c.2203A > G (p.I735V)	0.00 Affect protein function	0.991 Probably damaging
YCRC-52	*MLH1* c.2173C > T (p.R725C)	0.00 Affect protein function	1.000 Probably damaging
YCRC-60	*ATM* c.4375G > A (p.G1459R)	0.02 Affect protein function	0.999 Probably damaging
YCRC-64	*MSH2* c.73G > T (p.G25C)	0.09 Tolerated	0.941 Possibly damaging
YCRC-75	*APC* c.6691A > T (p.I2231F)	0.02 Affect protein function	0.997 Probably damaging
YCRC-76	*MLH1* c.1487C > G (p.P496R) *PMS2* c.737C > G (p.P246R)	0.00 Affect protein function 0.02 Affect protein function	0.189 Benign 0.962 Probably damaging
YCRC-88	*MLH1* c.649C > T (p.R217C)	0.05 Affect protein function	1.000 Probably damaging
YCRC-96	*MLH1* c.649C > T (p.R217C)	0.05 Affect protein function	1.000 Probably damaging
YCRC-97	*CDH1* c.1273G > A (p.V425I)	0.42 Tolerated	0.013 Benign

SIFT: sorting intolerant from tolerant.
PolyPhen-2: polymorphism phenotyping v2.

found that the frequency of *APC* mutations was significantly lower in this study. The lack of *APC* mutations in this population is similar to what has been observed in African Americans diagnosed with CRC.[28] *APC* is known as a "gatekeeper" tumor suppressor gene and the inactivated mutations of *APC* are considered as the initial step in the multistep tumorigenesis of CRC.[29] *APC*-mutation-negative tumors are considered to have distinct molecular characteristics, and the mutation status of *APC* has been reported as a prognostic marker for CRC.[28,30] Unlike *APC*, it has been suggested that *TP53* plays an important role in the later stage of CRC tumorigenesis, and *TP53* mutations are associated with a poor prognosis in CRC.[31,32] The results of *TP53* mutations here were similar to those found in previous studies in which the prevalence of the mutation was 60–80% and the majority of mutations were missense.[16,33]

It has been shown that CRC patients with mismatched repair gene mutations present unique clinicopathological characteristics.[34,35] Mutations of these genes leading to a defective DNA mismatched repair were found in almost one-third of the population studied while this phenomenon was reported from 10 to 20% in the general CRC population.[36–38] Other studies in young-onset CRC also reported that the frequency of defective DNA mismatched repair was age of onset-dependent and ranged from 19.7% to 41.0%.[39,40] These differences might be explained by the fact that CRC tumors in the young-onset population are highly associated with Lynch Syndrome but not the epigenetic inactivation of *MLH1*.[41,42] These mismatch repair results provide useful data, particularly for novel treatment targets.[43,44] However, understanding the roles of these mutations (e.g., driver or passenger mutations) requires further research and remains challenging.[45] The diverse results of somatic mutations once again highlight the heterogeneous nature of CRC pathophysiology.

The identification of CRC-associated germline mutations is useful for the screening, treatment, and follow-up of patients and also their relatives. The prevalence of germline mutations in this population was 9.9%, which means that one in ten patients with young-onset CRC would benefit from multigene testing. Besides the clinical benefit of identifying germline mutations, cost-effectiveness should be considered so as to maximize the application of multigene testing. The prevalence of germline mutations in this study was lower compared to previous studies.[7,8] This underestimation can be explained by the VUS and the multigene panel of choice. First, due to limited data on the genotype-phenotype correlation in Vietnamese CRC patients, there is insufficient data to classify the detected VUS, which were mostly predicted as deleterious mutations. Second, the lack of certain genes, such as *PALB2*, *CDKN2A*, *GREM*, *AXIN2*, *NTHL1*, and *MSH3*, in the multigene panel could reduce the ability to detect mutations.[10] Notably, one patient in this cohort presented with a *CDH1* mutation, which is traditionally associated with familial gastric cancer. The mutation c.377del of *CDH1* leading to a truncated E-cadherin protein was considered as a causative factor for CRC tumorigenesis through its interaction with APC protein.[46,47]

Limitations of this study are the lack of comparison between clinical/genetic profile of young-onset and the general CRC population, systemic microsatellite evaluation, copy number analysis, and methylation analysis of tumor tissues. This information would provide greater knowledge for the understanding of CRC molecular and pathologic mechanisms. Further studies are required to describe in more detail the landscape of CRC in Vietnamese patients.

5 | CONCLUSION

To our best knowledge, this is the first study in Vietnam providing information on the comprehensive mutation spectrum of both somatic and germline mutations in young-onset CRC patients. These data provide useful information for understanding the molecular characteristics of CRC and appropriate treatment targets and support genetic counseling. The results of germline mutations also suggest a beneficial role of multigene testing in patients with young-onset CRC given the decreasing price of NGS in Vietnam.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request: please contact hoanganhvu@ump.edu.vn.

CODE AVAILABILITY

Not applicable

AUTHORS' CONTRIBUTIONS

H.V. and M.D. designed the study. T.N., T.T., B.N., and K.L. recruited the patients. K.L., L.L., C.H., H.N., H.V., T.D., and M.D performed genetic experiments, analyzed the data. H.V. and M.D. wrote the manuscript. All authors read and approved the manuscript.

ETHICS APPROVAL

The studies involving human participants were reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam.

CONSENT TO PARTICIPATE

The patients provided their written informed consent to participate in this study.

CONSENT FOR PUBLICATION

All the patients and their family members fully understood and agreed by signing in the informed consent that their disease information can be published anonymously.

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SUPPORTING INFORMATION

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How to cite this article: Do MD, Nguyen TH, Le KT, et al. Molecular characteristics of young-onset colorectal cancer in Vietnamese patients. *Asia-Pac J Clin Oncol*. 2022;1-8. https://doi.org/10.1111/ajco.13749