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TP53 mutation status and consensus molecular subtypes of colorectal cancer in patients from Rwanda

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Abstract

Background Mutations in the *TP53* tumor suppressor gene are well-established drivers of colorectal cancer (CRC) development. However, data on the prevalence of *TP53* variants and their association with consensus molecular subtype (CMS) classification in patients with CRC from Rwanda are currently lacking. This study addressed this knowledge gap by investigating *TP53* mutation status concerning CMS classification in a CRC cohort from Rwanda.

Methods Formalin-fixed paraffin-embedded (FFPE) tissue blocks were obtained from 51 patients with CRC at the University Teaching Hospital of Kigali, Rwanda. Exons 4 to 11 and their flanking intron-exon boundaries in the TP53 gene were sequenced using Sanger sequencing to identify potential variants. The recently established immunohistochemistry-based classifier was employed to determine the CMS of each tumor.

Results Sequencing analysis of cancerous tissue DNA revealed *TP53* pathogenic variants in 23 of 51 (45.1%) patients from Rwanda. These variants were predominantly missense types (18/23, 78.3%). The most frequent were c.455dup (p.P153Afs*28), c.524G > A (p.R175H), and c.733G > A (p.G245S), each identified in three tumors. Trinucleotide sequence context analysis of the 23 mutations (20 of which were single-base substitutions) revealed a predominance of the [C > N] pattern among single-base substitutions (SBSs) (18/20; 90.0%), with C[C > T]G being the most frequent mutation (5/18, 27.8%). Furthermore, pyrimidine bases (C and T) were preferentially found at the 5′ flanking position of the mutated cytosine (13/18; 72.2%). Analysis of CMS subtypes revealed the following distribution: CMS1 (microsatellite instability-immune) (6/51, 11.8%), CMS2 (canonical) (28/51, 54.9%), CMS3 (metabolic) (9/51, 17.6%), and CMS4 (mesenchymal) (8/51, 15.7%). Interestingly, the majority of *TP53* variants were in the CMS2 subgroup (14/23; 60.1%).

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Conclusion Our findings indicate a high frequency of *TP53* variants in CRC patients from Rwanda. Importantly, these variants are enriched in the CMS2 subtype. This study, representing the second investigation into molecular alterations in patients with CRC from Rwanda and the first to explore *TP53* mutations and CMS classification, provides valuable insights into the molecular landscape of CRC in this understudied population.

Keywords *TP53*, Mutation spectrum, Mutation pattern, Consensus molecular subtypes, Mismatch repair, Colorectal cancer, Rwanda, Africa

Introduction

Colorectal cancer (CRC) represents a significant global health burden, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related mortality [1]. In 2022 alone, an estimated 1.9 million new CRC cases and 903,859 deaths were reported worldwide [1]. The substantial impact of CRC extends to Africa, where it ranks among the top 10 cancer groups across all 54 African countries. In 2022, Africa reported an estimated 70,428 (5.9% of all cancers) new CRC cases and 46,087 (6.0% of all cancers) deaths. The risk of dying from CRC before the age of 75 years was estimated to be 0.59% [2]. Rwanda, with a population exceeding 13.6 million in 2022, faces a substantial cancer burden. GLOBOCAN 2022 estimates suggest 7,122 new cancer cases and 4,887 cancer-related deaths [3]. CRC ranks among the top ten malignancies, contributing 353 new cases and 251 deaths annually [3]. Although significant efforts have been made to improve CRC diagnosis and treatment in Rwanda [4, 5], molecular research on CRC lags considerably behind than in European countries [6] and the USA [7]. We recently reported the first molecular study of CRC in Rwandan patients, investigating mutational profiles of the cancer-related genes *APC* and *KRAS* [8]. This study highlights the need for further comprehensive molecular analyses to elucidate the unique characteristics of CRC in patients from Rwanda.

CRC development is driven by the progressive accumulation of genetic and epigenetic alterations within cells [9]. These aberrations activate oncogenes and inactivate tumor suppressor genes, such as *TP53* [10–12]. Located on chromosome 17, *TP53* encodes a 393-amino acid protein with critical cellular functions [13, 14], including its well-established role as a master regulator of the cellular stress response [15]. TP53 exerts its tumor suppressive functions through the regulation of over 300 genes, highlighting its centrality to cellular stress responses [16]. This critical role likely explains the high prevalence of *TP53* mutations (over 50%) observed in various cancers [17]. Unlike other tumor suppressor genes, which are often inactivated by truncating mutations [18], *TP53* dysfunction frequently arises from missense mutations in its DNA binding domain [10]. The most frequent *TP53* base change in all cancers is C>T, with 20% of these mutations occurring at CpG sites and most are associated with cytosine to uracil deamination [19, 20]. These mutations are thought to be catalyzed by members of the cytidine deaminase family including the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) [19] and activation-induced cytidine deaminase (AICDA) [21]. These enzymes show a strong preference for deaminating cytidine residues depending on the nitrogenous base that comes before the mutated base [22]. For example, APOBEC enzymes favor C residues flanked by pyrimidine bases (C or T) [21, 23, 24], while AICDA is involved in alterations that occur in cytidine preceded by purine (G or A) [21]. Interestingly, CRC studies have shown that patients with missense mutations leading to TP53 protein accumulation in the nucleus exhibit a poorer prognosis [18]. Building on our previous work investigating mutational profiles of *APC* and *KRAS* in CRC of patients from Rwanda (mutation rates of approximately 50% and 40%, respectively) [8], this study addresses the critical gap in knowledge regarding *TP53* mutations within this population.

CRC classification traditionally relied on a system categorizing tumors into colorectal cancer subtypes such as CCS1 (chromosomal instability), CCS2 (microsatellite instability), and CCS3 (microsatellite stable) [25]. However, in 2015, a major shift occurred when an international consortium, committed to large-scale data sharing, established a novel classification system based on gene expression profiles [26]. This collaborative effort led to the identification of four distinct CRC groups, now recognized as consensus molecular subtypes (CMS). The novel CMS classification system categorizes CRCs into four distinct subtypes: CMS1 (microsatellite instability [MSI]-immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) [26]. Numerous studies and clinical trials have established the prognostic value of CMS classification [27–29]. However, broader implementation in clinical practice is hindered by the higher costs and time requirements associated with gene expression profiling [30]. To overcome the limitations of gene expression profiling, an immunohistochemistry-based approach combined with MSI/mismatch repair deficiency (MSI/MMR-D) assessment has been developed [31]. This method utilizes five CMS markers (CDX2, HTR2B, FRMD6, ZEB1 and CK AE1/AE3) for classification. This approach offers improved efficiency and affordability, allowing for the confident identification of CMS1 (MSI) and CMS4 (mesenchymal) tumors.

However, it cannot currently distinguish between CMS2 and CMS3, which are both epithelial subtypes. Despite this limitation, such an approach represents a significant advancement in CRC management by facilitating the identification of CMS1 (good prognosis) and CMS4 (poor prognosis) [32, 33].

The CMS2 subgroup is characterized by canonical Wnt signaling pathway activation [26], of which β-catenin is a major component [34]. The Wnt signaling pathway regulates several biological processes, including cell proliferation and self-renewal of some tissues in the body [34]. Its activation is considered an important step in cancer progression and is characterized by higher β-catenin expression in the nucleus of tumor cells [35]. Therefore, Li et al., considered it a potential tie-breaker between the CMS2 and CMS3 groups [36]. Building upon the work of Li et al., who incorporated β-catenin staining to distinguish CMS2 (β-catenin positive) from CMS3 (β-catenin negative) within the immunohistochemistry framework [36], we employed this modified technique to classify CRC in patients from Rwanda. This approach holds promise for improved clinical stratification of CRC patients in resource-limited settings like Rwanda, potentially guiding more optimal treatment strategies. The present study aimed to characterize the mutational profile of *TP53* in patients with CRC from Rwanda, including the association between *TP53* mutations and CMS subtypes classified using the recently developed immunohistochemistry-based CMS classifier.

Materials and methods

Patients and tissue samples

Between 2020 and 2022, we prospectively recruited 148 Rwandan patients undergoing colonoscopy at the Department of Internal Medicine, University Teaching Hospital of Kigali (CHUK), Rwanda. Of these, 129 (87.1%) provided informed consent for the study and colonoscopy biopsies were procured from each patient. Following histopathological examination, CRC was confirmed in 58 patients (45.0%). Subsequent analyses, including TP53 gene sequencing, MMR assessment, and CMS classification, were successfully performed on tissue samples from 51 cases.

Histopathological diagnosis

Tissue biopsies were initially evaluated microscopically at CHUK (Rwanda). Subsequently, tissue slides were reviewed by pathologists at Hamamatsu University School of Medicine (HUSM), Japan. Histopathological characteristics of the carcinoma samples were determined based on the extent of glandular differentiation/formation using a two-tiered grading system: low-grade (formally well- to moderately differentiated) and high-grade (formally poorly differentiated), based on the World Health Organization (WHO 5th edition) tumor classification [37].

TP53 gene sequencing, mutation detection, and interpretation

For mutational analysis, genomic DNA was isolated from FFPE tissue sections using the QIAamp DNA FFPE Advanced UNG Kits (Qiagen, Hilden, Germany) at the Department of Tumor Pathology, HUSM, Japan. Exons 4–11 of the TP53 gene were then amplified by PCR using HotStarTaq DNA polymerase (Qiagen) and specific primers listed in Supplementary Table S1. The amplified PCR products were purified and subjected to Sanger sequencing, as previously described [8, 22, 38].

Sanger sequencing data were aligned to the *TP53* reference genomic sequence (i.e., NC_000017.11) to identify mutations. Identified variants were then annotated and interpreted following the Human Genome Variation Society (HGVS) guidelines and the recommendations outlined by the joint consensus of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology [39, 40]. Variant filtering was performed to prioritize those with a minor allele frequency (MAF) of less than 1% in the 1000 Genomes and gnomAD databases [41, 42]. The ACMG guidelines for classification were used to evaluate the pathogenicity of these candidate variants [43]. Further reports on their clinical significance from public archives, such as ClinVar [44], were also evaluated. For a detailed description of our mutation detection and interpretation pipeline, please refer to our previous work [22].

Single-nucleotide variant data from *TP53* mutations in CRC of patients from Rwanda, were categorized into six classic substitution patterns based on the mutated pyrimidine (C>A, C>G, C>T, T>A, T>C, and T>G) [45]. Each of the six patterns can have 16 permutations considering the flanking bases (one each on the left and right sides from the four DNA bases), resulting in a total of 96 possible substitution patterns [20, 45]. To understand the context of the trinucleotide sequence at the mutation site, we considered the immediate flanking nucleotides around the mutated nitrogenous base.

Immunohistochemistry-based CMS classification

Immunohistochemistry was performed to evaluate the expression of MMR proteins and CMS markers in 51 CRC tissue samples. FFPE blocks were sectioned at 4 µm thickness. On one hand, following deparaffinization, MMR protein expression (MLH1, MSH2, MSH6, and PMS2) was assessed using the Ventana BenchMark Ultra Autostainer (Ventana, Tucson, AZ, USA) with the Ventana MMR RxDx Panel, which includes primary antibodies specific for each protein: MLH1 (clone M1), MSH2 (clone G219-1129), MSH6 (clone SP93), and PMS2 (clone

A16-4). A 3,3′-diaminobenzidine (DAB) chromogen (Dako, Carpinteria, CA, USA) was used for visualization, and hematoxylin was employed for nuclear counterstaining. Immunohistochemical evaluation classified tumors as MMR-deficient (MMR-D) if moderate to strong expression was lost in one or more MMR proteins. Conversely, tumors with retained moderate to strong staining for all four MMR proteins were classified as MMR-proficient (MMR-P) [46].

On the other hand, an automatic immunohistochemistry platform, the HISTOSTAINER (Nichirei Bioscience, Tokyo, Japan), was employed for staining CMS protein markers. The Histofine Simple Stain MAX PO (Nichirei Bioscience) was utilized as previously described [27, 31, 36]. A panel of five antibodies targeting CDX2 (1:100, 082323 A, Biocare medical, Concord, CA, USA), HRT2B (1:100, HPA012867, Sigma-Aldrich, St. Louis, MO, USA), FRMD (1:50, 21039-1-AP, Proteintech, Chicago, IL, USA), CK AE1/AE3 (1:100, AE1/AE3-601-L-CE, Novocastra, Newcastle, UK), and ZEB1 (1:500, HPA027524, Sigma-Aldrich) was used for CMS classification. Building upon established protocols [36], we incorporated an additional anti-β-catenin antibody (1:100, B-CAT-L-CE, Novocastra) to discriminate between CMS2 and CMS3 subtypes. The immunohistochemical staining utilized DAB (Dako) as a chromogen and hematoxylin for nuclear counterstaining. Staining intensity was scored semi-quantitatively (0=absent, 1=weak, 2=moderate, 3=strong). β-catenin evaluation focused on the presence or absence of unequivocal cells exhibiting nuclear staining, as previously described [35]. A Leica DMD 108 microscope (Leica Biosystems, Wetzlar, Germany), was used for both visualization of the staining signal and capture of representative photomicrographs.

CMS classification followed a tiered approach. Cases with MMR-D were directly assigned to the CMS1 subtype. Next, an online platform (https://crcclassifier.shinyapps.io/appTesting/) developed by Trinh et al. [31]. was utilized to calculate the prediction probability (p) of a case belonging to either mesenchymal-like or epithelial-like subtype. As in the previous approach by Trinh et al. [31], cases with a p-value exceeding 0.6 (60%) were classified as mesenchymal-like, corresponding to CMS4. The remaining epithelial cases (potentially CMS2 or CMS3, displaying nuclear staining for CDX2 and low ZEB1 expression) were differentiated based on β-catenin expression: positive staining indicated CMS2, whereas negative staining indicated CMS3. Details regarding this classification scheme were previously described by Li X. et al. [36].

Publicly available *TP53* mutation data from China and the US

Somatic *TP53* mutation data from China were collected from the International Cancer Genome Consortium (ICGC) data portal [47]. The data from the US belong to Memorial Sloan Kettering (MSK) Cancer Center (NY, USA) [48] and were retrieved from the cBioPortal platform (https://www.cbioportal.org/datasets) where they are deposited as "Rectal Cancer (MSK, Nature Medicine 2019)." From the ICGC and cBioPortal platforms, mutation data were downloaded as files containing tab-separated values. They were converted into Excel files for summary and organization and later transferred into SPSS 29.0 version software for statistical analysis.

Statistical analysis

Statistical analyses were performed using the Statistical Product and Service Solutions version 29.0 software (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, depending on sample size considerations. Student's t-test was employed for comparisons of normally distributed continuous variables, such as age. A p-value of less than 0.05 was considered statistically significant.

Results

Clinicopathological characteristics of Rwandan patients with CRC analyzed for *TP53* mutations

The clinicopathological characteristics of 51 patients with CRC are listed in Table 1. The mean age ± standard deviation was 61.0 ± 12.5 years and ranged from 31 to 89 years. The male: female ratio was approximately 1:2. The tumors were located in the colon (8 cases) or rectum (43 cases), and histological grading showed a distribution between low-grade (18 cases, 35.3%) and high-grade (33 cases, 64.7%) cases. One patient (2.0%) had a family history of cancer, 33 patients (64.7%) had no family history of cancer, and 17 (33.3%) had no information regarding their family history of cancer.

TP53 mutational status among patients with CRC from Rwanda

Analysis of the TP53 gene using Sanger sequencing and the evaluation of the detected variants based on ACMG guidelines for classification revealed 23 (45.1%) cases with pathogenic variants out of a total of 51 CRC patients in Rwanda (Table 2 and Supplementary Table S2; representative electropherograms in Supplementary Figure S1). Among these mutations, single base substitutions were the most frequent (20/23, 87.0%), with the remaining classified as frameshift mutations (n=3, 13.0%). Analysis of the distribution of *TP53* mutations using the cBioPortal mutation mapper [49] revealed an enrichment within exons 4 to 8 (Fig. 1A). Remarkably, all identified

Table 1 Clinicopathological characteristics of Rwandan patients with colorectal cancer ($n=51$)

Characteristics	Number of cases	Percentage or years old
Age (years old)		
Mean ± Standard deviation		61.2 ± 12.5
Range		31–89
Age group		
< 50	9	17.6%
≥ 50	42	82.4%
Sex		
Female	33	64.7%
Male	18	35.3%
Anatomic site		
Cecum	1	2.0%
Ascending colon	3	5.9%
Transverse colon	1	2.0%
Descending colon	2	3.9%
Sigmoid colon	1	2.0%
Rectum	43	84.3%
Family history of cancer		
Yes	1	2.0%
No	33	64.7%
Not known	17	33.3%
Grade		
Low-grade	18	35.3%
High-grade	33	64.7%

mutations, except for p.E68K, resided within the DNA binding domain of *TP53* (residues 100–288). Mutations in exon 5 (13/23, 56.5%) and exon 7 (7/23, 30.4%) were the most prevalent. Interestingly, three specific mutations–c.455dup (p.P153Afs*28), c.524G>A (p.R175H), and c.733G>A (p.G245S)–each occurred three times, representing the most frequent mutations identified (Table 2 and Supplementary Table S2). The mutational landscape revealed a predominance of missense mutations (18/23, 78.3%), as illustrated in Fig. 1A-B and Supplementary Table S3. These mutations typically lead to the production of a full-length protein with altered functionality. Frameshift mutations, on the other hand, were less frequent (3/23, 13.0%) and can introduce premature stop codons, resulting in a truncated or non-functional TP53 protein [50, 51]. For instance, the c.455dup mutation (p.P153Afs*28) introduces a frameshift and likely leads to a truncated protein of 179 amino acids compared to the wild-type TP53 protein with 393 amino acids [52]. Moreover, investigation of the association between these pathogenic mutations and clinicopathological factors (age, sex, anatomic site, tumor grade) revealed no statistically significant associations (Table 3). These findings suggest that *TP53* mutations are frequent events in CRC of patients from Rwanda and exhibit distinct characteristics.

We compared the distribution of *TP53* variants in Rwandan patients with publicly available data from China (number of *TP53* variants: 156) [47] and data from the US (number of *TP53* variants: 286) [48] (Supplementary Table S3). For all regions, we observed a higher number of missense variants compared with other types of mutations (i.e., frameshift variants, nonsense variants, and splice-site variants) (Rwanda: 18/23, 78.3%, China: 120/156, 76.9%, and the US: 183/286, 64.0%). The results suggest that missense variants are the most frequent *TP53* mutations in Rwandan patients as well as in China and the US.

Trinucleotide sequence context analysis of CRC in patients from Rwanda

Analysis of *TP53* single-nucleotide variant data ($n=20$) from Rwandan CRC cases revealed a predominance of C to other nucleotide substitutions (C>N; $n=18$, 90.0%) as shown in Supplementary Table S4. Of the C>N mutations, C>T transitions with a [CCG] trinucleotide (C[C>T]G) were the most frequent (5/18, 27.8%). Overall, the [CCG] trinucleotide occurred in 8 (40.0%) of 20 cases of single base substitutions, whereas [CTG] and [GCG] occurred in 3 (15.0%) cases each (Supplementary Table S5). Furthermore, analysis of the 5′ flanking bases preceding mutated C residues demonstrated a preference for pyrimidines (C or T). Specifically, 10/18 (55.6%) mutations occurred within a CC>N context, while 3/18 (16.7%) occurred within a TC>N context, totaling 13/18 (72.2%) pyrimidine-preceded mutations. This finding aligns with the observed enrichment of C>N substitutions, particularly C[C>T]G transitions, within *TP53* mutations in CRC of patients from Rwanda.

TP53 mutations and CMS classification in patients with CRC from Rwanda

We investigated the relationship between *TP53* mutational status and CMS classification in patients with CRC from Rwanda. CMS, a recently developed molecular classification of CRC [26, 31, 36], was originally based on tumor transcriptomic data. However, a recently described immunohistochemistry-based method, paralleling the transcriptomic approach, has been established [36]. We employed this immunohistochemistry strategy in CRC series from Rwanda. Our approach involved immunohistochemical analysis of four MMR proteins and six CMS protein markers, followed by an evaluation of their expression status (Figs. 2 and 3). Based on this analysis, we were able to classify the 51 CRC cases from Rwanda into the four established CMS subtypes: CMS1 (microsatellite instability-immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) (Fig. 4). The results from this study shows that CMS groups are distributed as follows: CMS1 ($n=6$, 11.8%), CMS2 ($n=28$,

Table 2 List of *TP53* pathogenic and likely pathogenic variants that were identified in patients with colorectal cancer from Rwanda ($n=23$)

Sample ID	Locus	Coding DNA	Protein description	MAF	ClinVar	ACMG classification
CRC03	chr17:7674945	c.586 C>T	p.R196*	0.0000040	Pathogenic	Pathogenic
CRC04	chr17:7674220	c.743G>C	p.R248P	NA	Pathogenic	Pathogenic
CRC06	chr17:7674221	c.742 C>T	p.R248W	0.0000040	Pathogenic	Pathogenic
CRC07	chr17:7675217	c.395 A>G	p.K132R	0.000	Pathogenic	Pathogenic
CRC08	chr17:7674230	c.733G>A	p.G245S	0.0000035	Pathogenic	Pathogenic
CRC11	chr17:7676167	c.202G>A	p.E68K	NA	No entry	Likely pathogenic
CRC13	chr17:7675086	c.526T>A	p.C176S	NA	CIP	Likely pathogenic
CRC18	chr17:7675161	c.455dup	p.P153Afs*28	NA	Pathogenic	Pathogenic
CRC23	chr17:7675086	c.526T>G	p.C176G	NA	US	Likely pathogenic
CRC24	chr17:7674230	c.733G>A	p.G245S	0.0000035	Pathogenic	Pathogenic
CRC27	chr17:7676047	c.322G>T	p.G108C	0.0000080	US	Likely pathogenic
CRC30	chr17:7675097	c.515T>A	p.V172D	NA	CIP	Likely pathogenic
CRC37	chr17:7675088	c.524G>A	p.R175H	0.0000040	Pathogenic	Pathogenic
CRC39	chr17:7674230	c.733G>A	p.G245S	0.0000035	Pathogenic	Pathogenic
CRC42	chr17:7675088	c.524G>A	p.R175H	0.0000040	Pathogenic	Pathogenic
CRC44	chr17:7675161	c.455dup	p.P153Afs*28	NA	Pathogenic	Pathogenic
CRC45	chr17:7673806	c.814G>A	p.V272M	0.0000040	Pathogenic	Pathogenic
CRC46	chr17:7674220	c.743G>C	p.R248P	NA	Pathogenic	Pathogenic
CRC48	chr17:7675086	c.526T>G	p.C176G	NA	US	Likely pathogenic
CRC52	chr17:7674229	c.734G>A	p.G245D	0.0000040	Pathogenic	Pathogenic
CRC55	chr17:7675088	c.524G>A	p.R175H	0.0000040	Pathogenic	Pathogenic
CRC56	chr17:7673764	c.856G>T	p.E286*	NA	Pathogenic	Pathogenic
CRC58	chr17:7675161	c.455dup	p.P153Afs*28	NA	Pathogenic	Pathogenic

NA: Not available. CIP: Conflicting interpretations of pathogenicity. US: Uncertain significance

54.9%), CMS3 ($n=9$, 17.6%), and CMS4 ($n=8$, 15.7%) (Table 4 and Supplementary Table S6). And these results are consistent with the ones from previous reports [36, 53–56] whereby the distribution of CMS subtypes was: CMS1 (13.6–23.1%), CMS2 (26.3-55.8%), CMS3 (13.0–24.0%), and CMS4 (6.5–36.2%) (Table 5). Remarkably, in the present study, pathogenic *TP53* mutations were most frequently observed in CMS2 tumors (14 out of 23 cases with pathogenic mutations, 60.9%) (Table 4). These findings suggest a high prevalence of pathogenic *TP53* mutations specifically within CMS2 group of Rwandan patients with CRC.

Finally, we determined whether the above MMR status and CMS group data were associated with the trinucleotide sequence context of the *TP53* single-nucleotide variant data (Supplementary Table S5). The results indicated that there was no significant association between MMR status, CMS group, age, or sex and trinucleotide sequence context. In addition, there was no association between MMR status and *TP53* mutation in our cohort of CRC patients from Rwanda (Table 3).

Discussion

In the present study, Sanger sequencing was used to analyze *TP53* mutations in DNA samples isolated from CRC tissue specimens from 51 Rwandan patients. Twenty-three pathogenic mutations were identified from our analysis. These mutations were predominantly missense variants (78.3%) followed by truncating mutations (21.7%). With respect to the trinucleotide sequence context, our results indicated that C[C>T]G transitions were the most frequent among the single base substitution mutations (5/18, 27.8%). The analysis of CMS molecular subtypes by immunohistochemistry revealed the following distribution: CMS1 (11.8%), CMS2 (54.9%), CMS3 (17.6%), and CMS4 (15.7%). Interestingly, the prevalence of pathogenic *TP53* mutations was the highest in CMS2 (60.1%). These findings suggest that *TP53* mutations are frequent events in CRC patients from Rwanda and exhibit distinct characteristics, such as a frequent prevalence of pathogenic mutations within the CMS2 subtype. This study represents the second molecular analysis of CRC patients from Rwanda and the first to examine *TP53* mutations and CMS classification. Our data provide valuable insight that will contribute to a more comprehensive understanding of the molecular pathways underlying CRC in Rwanda.

The rate of *TP53* mutation was markedly higher with approximately 45.1% of the mutated cases harboring pathogenic/likely pathogenic variants. Evaluation of these variants revealed that 78.3% were missense variants, whereas 21.7% were truncating variants. Missense variants result in the production of a protein lacking the tumor suppressor activity of wild-type TP53 [57]. Despite

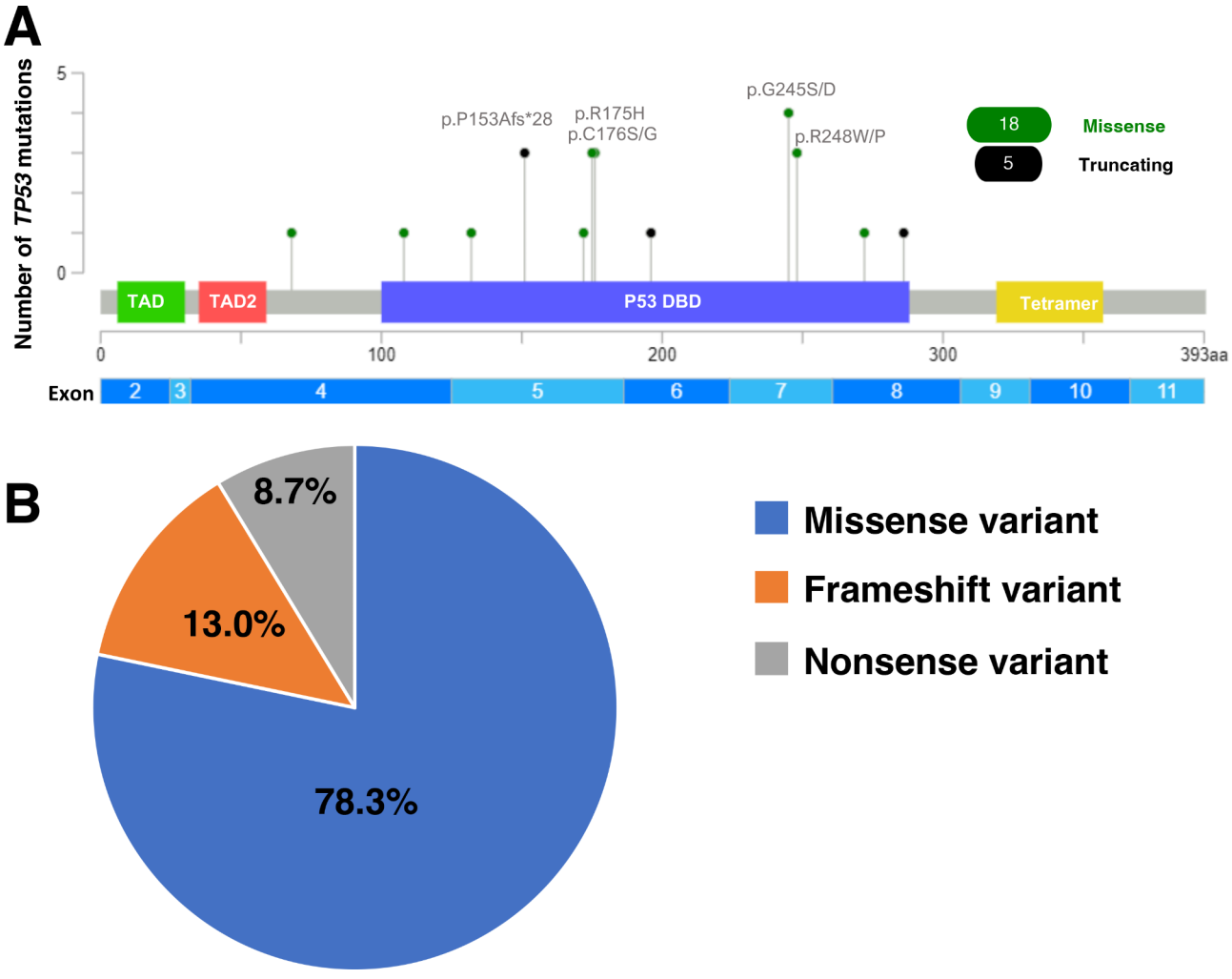

Fig. 1 Location and type of *TP53* mutations in colorectal cancer (CRC) of patients from Rwanda. **A**: Types and locations of exonic *TP53* mutations ($n = 23$) on the protein structure using the cBioPortal mutation mapper. Lollipop plots represent mutation frequencies, with the height proportional to frequency. The most frequent codon with mutations is 245 (3× p.G245S, 1× p.G245D). Colors denote mutation type: green - missense, black - truncating, pink - others. TAD: TP53 transactivation motif (residues 6–30), TAD2: TP53 transactivation motif 2 (residues 35–59), DBD: DNA binding domain (residues 100–288), Tetramerization motif (residues 319–357). **B**: Distribution of *TP53* mutation types ($n = 23$) detected in patients with CRC from Rwanda. The pie chart shows the proportion of missense (blue), frameshift (orange), and nonsense (gray) variants. Missense variants are the most frequent type of mutations in Rwanda at 78.3%

losing normal function, mutated TP53 can acquire new oncogenic functions to promote cell transformation, tumor progression, metastasis, and resistance to chemotherapy [57]. Some missense mutations gain such a function by binding to different transcription factors, which promotes tumor growth [58]. In contrast, truncating mutations result in the absence of protein or premature protein termination, which disrupts TP53 function altogether [59]. Therefore, both missense and truncating variants inactivate the TP53 suppressor gene through either a gain or loss of function, respectively [56]. The inactivation of TP53 gene is considered an important event in tumorigenesis [16]. In CRC, *TP53* missense mutations may confer resistance to tumor cells against systemic therapy [60], and therefore, favor neoplastic proliferation [57]. Conversely, tumor-associated antigens and/or tumor-specific antigens that arise as a result of some *TP53* mutations with missense variants sensitize cells to targeted therapies, such as immunotherapy [58]. Because of this increased sensitivity [61–63], the results of this study may contribute to the selection of treatment for 45.1% of the patients with *TP53* mutations in our cohort.

CRC follows a multistep tumorigenesis process, in which *TP53* mutations play an important role in the adenoma–carcinoma transition [58]. The higher proportion of C[C>T]G in the present study and a mutation pattern associated with age [24] highlight the importance of early CRC screening in Rwanda. Although aging is an inevitable process, its effects can be mitigated. Promoting

Table 3 *TP53* mutational status according to clinicopathological features in patients with colorectal cancer (CRC) from Rwanda

Clinicopathological factors	Total cases	Cases based on *TP53* mutation status		*p* value
	(*n* = 51)	Pathogenic *TP53* mutation-positive cases (*n* = 23)	Pathogenic *TP53* mutation-negative cases (*n* = 28)	
Age (years old)				0.868
Mean ± SD	61.2 ± 12.5	61.5 ± 11.7	60.9 ± 13.3	
Range	31–89	31–81	32–89	
Age group, ***n*(%)**				0.434
< 50	9	3 (13)	6 (21.4)	
≥ 50	42	20 (87)	22 (78.6)	
Sex, ***n*(%)**				0.212
Female	33	17 (73.9)	16 (57.1)	
Male	18	6 (26.1)	12 (42.9)	
Anatomic site, ***n*(%)**				0.302
Cecum	1	0 (0)	1 (3.6)	
Ascending colon	3	2 (8.7)	1 (3.6)	
Transverse colon	1	1 (4.3)	0 (0)	
Descending colon	2	2 (8.7)	0 (0)	
Sigmoid colon	1	0 (0)	1 (3.6)	
Rectum	43	18 (78.3)	25 (89.3)	
Grade, ***n*(%)**				0.603
Low-grade	18	9 (39.1)	9 (32.1)	
High-grade	33	14 (60.9)	19 (67.9)	
MMR, ***n*(%)**				0.797
MMR-D	6	3 (13)	3 (10.7)	
MMR-P	45	20 (87)	25 (89.3)	

SD, standard deviation

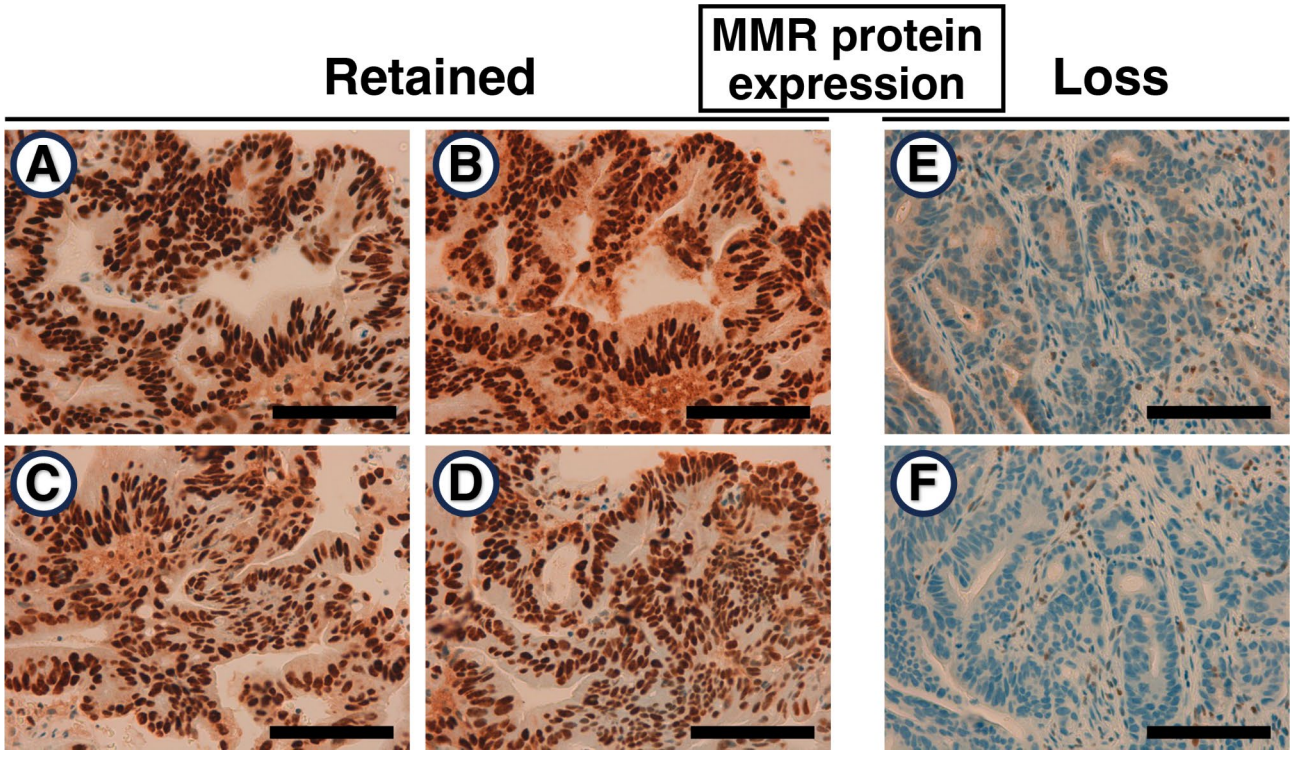

Fig. 2 Immunohistochemical staining of mismatch repair (MMR) protein markers for colorectal cancer in patients from Rwanda. **A-D**: Representative images of retained expression (A: MLH1, B: PMS2, C: MSH2, D: MSH6). **E**, **F**: Representative images of loss of expression (E: PMS2, F: MSH6). Scale bar = 100 µm

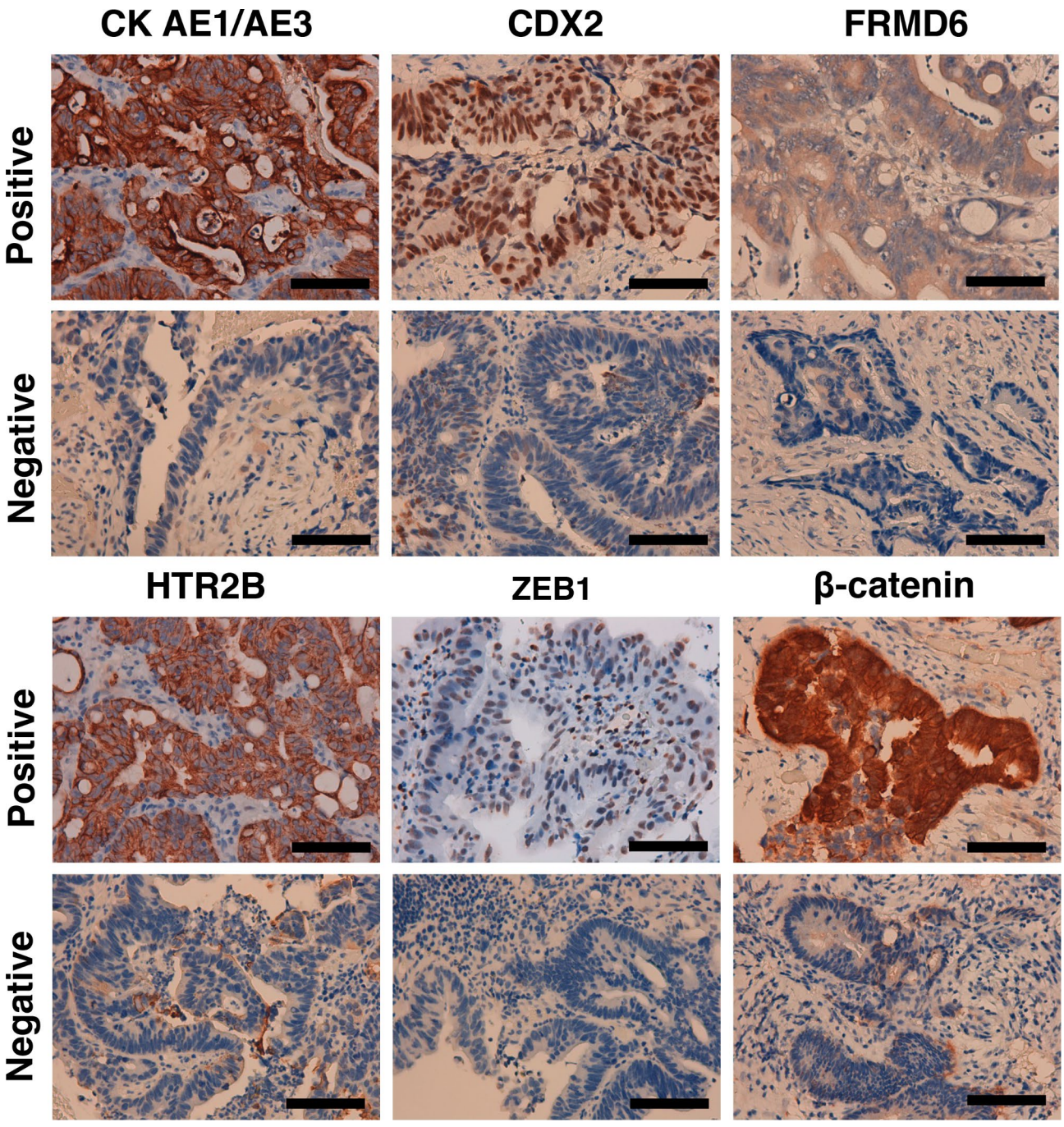

Fig. 3 Representative positive and negative immunohistochemistry staining results for the consensus molecular subtype (CMS) protein markers of CK (AE1/AE3, CDX2, FRMD6, HTR2B, ZEB1, and β-catenin). The photomicrograph on the positive side indicates representative images of CMS protein expression in our colorectal cancer (CRC) cohort. The panels on the negative side indicate low or a lack of CMS protein expression in our CRC cohort. Scale bar = 100 μm

a healthy lifestyle through improved healthcare access, a balanced diet, and regular physical activity may help to prevent some age-related pathologies, including chronic inflammation [64–67]. Moreover, the CMS data enabled us to stratify our patients into four distinct molecular subtypes (CMS1, CMS2, CMS3, and CMS4). These subgroups are biologically different and may predict which patients could benefit from a particular treatment regimen [26]. For example, the CMS1 group responds well to chemotherapy combined with bevacizumab [anti-vascular endothelial growth factor A (VEGF-A) therapy] [68–70]. Conversely, patients in the CMS2 subgroup have shown an improved response to cetuximab [anti-epidermal growth factor receptor (anti-EGFR)] therapy

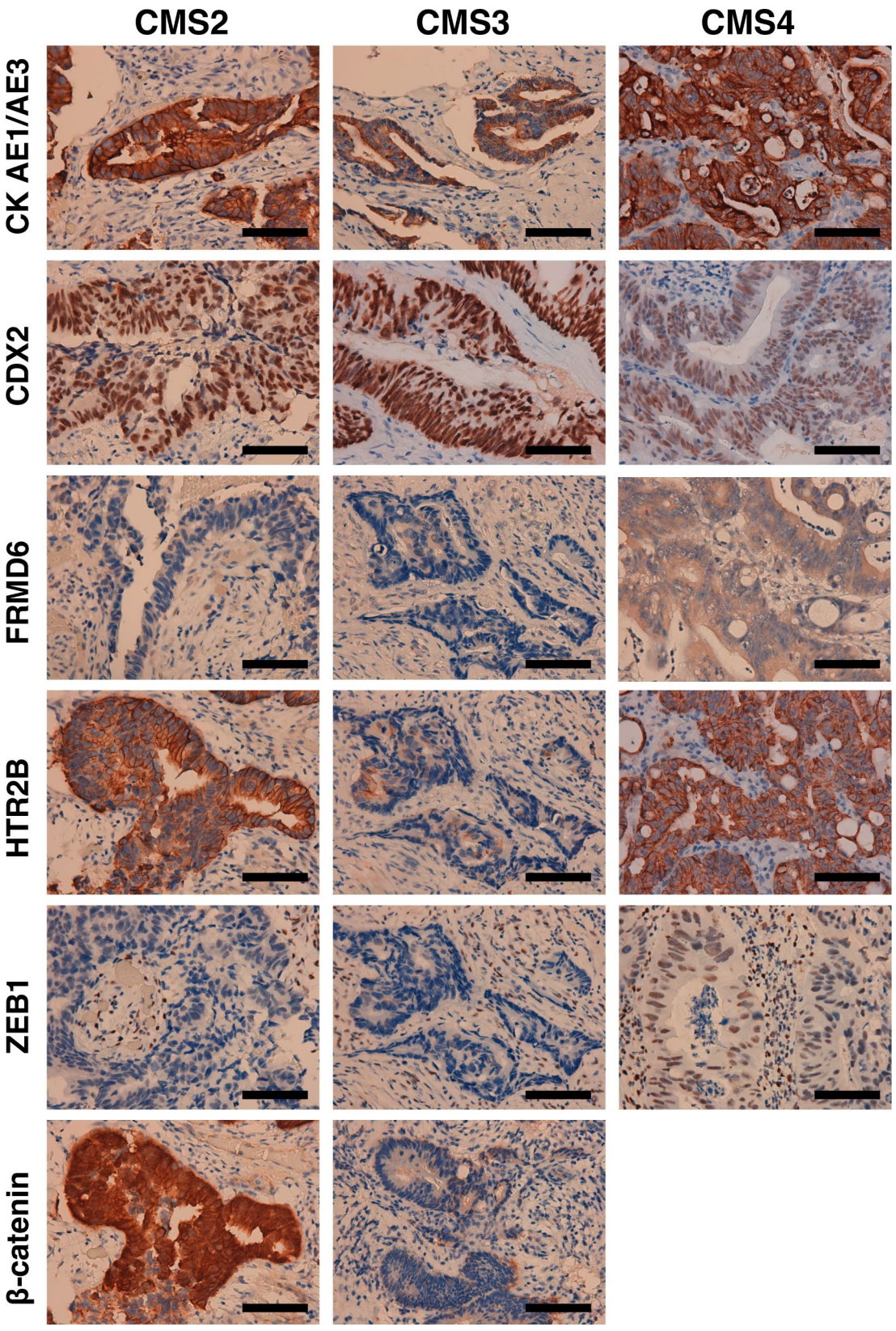

Fig. 4 (See legend on next page.)

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Fig. 4 Immunohistochemical staining of consensus molecular subtype (CMS) protein markers for colorectal cancer in patients from Rwanda. Representative images of protein markers [CK AE1/AE3, CDX2, FRMD6, HTR2B, ZEB1, and β-catenin] used for CMS classification (CMS2, CMS3, and CMS4) are shown. Strong cytoplasmic CK AE1/AE3 and nuclear CDX2 staining in CMS2 and CMS3 highlight their epithelial origin. β-catenin staining serves as a tie-breaker: strong nuclear, cytoplasmatic, and membrane staining is observed in CMS2, while CMS3 is negative. CMS4 (mesenchymal) exhibits higher keratin content with CK AE1/AE3 but weaker nuclear CDX2 expression. Additionally, CMS4 shows strong cytoplasmic staining for HTR2B, weak to moderate staining for FRMD6, and moderate nuclear staining for ZEB1. These features are consistent with mesenchymal-like subtypes ($p > 0.6$), as confirmed by the online CMS classifier (https://crcclassifier.shinyapps.io/appTesting/). Scale bar = 100 µm

compared with mesenchymal-like tumors (CMS4) [31]. In contrast, patients in the CMS4 group show no benefit from adjuvant chemotherapy. These patients have a worse overall and relapse-free survival [28]. Therefore, the information regarding the molecular subtypes of CRC in Rwanda is important for patient triage and selecting individualized therapy.

The observed frequency of *TP53* mutations in CRC patients from Rwanda (45.1%) is consistent with the results from a previous study which reported a mutation rate of 45% in distal colon and rectal tumors [71]. Furthermore, the predominance of missense mutations (78.3%) in our cohort is consistent with *TP53* mutation data from publicly available resources: The International Cancer Genome Consortium data portal for Chinese cases and the cBioPortal (https://www.cbioportal.org/datasets) for US cases from Memorial Sloan Kettering Cancer Center [48]. The rate of missense mutations in CRC cases from China is reported as 76.9%, and 64.0% for US rectal cancer cases from cBioPortal platform. Remarkably, the majority of mutations we identified resided within the TP53 DNA-binding domain (residues 102–292), which is known to be functionally critical [50]. Supporting this notion, a review of the literature indicates that proteins harboring mutations at specific residues within the DNA-binding domain, including residues *245*, *248*, 273, and 282 (italics for those identified in the present study) exhibit impaired DNA binding capacity. Furthermore, mutation at residues 157, *175*, and 220 have been documented to disrupt protein folding, consequently impairing DNA binding, a critical process for TP53 function [50, 72]. These findings collectively suggest that *TP53* mutations are frequent events in patients with CRC from Rwanda, and the identified mutations display mutational characteristics similar to those reported in studies from China and the US.

Our study successfully implemented the recently developed immunohistochemistry-based CMS classification method [28] to the patients with CRC from Rwanda. The observed distribution of CMS subtypes (CMS1: 11.8%, CMS2: 54.9%, CMS3: 17.6%, and CMS4: 15.7%) aligns well with frequencies reported in previous studies utilizing both the original transcriptomic method [26] and the immunohistochemistry approach [36]. Intriguingly, *TP53* mutations exhibited a higher prevalence within the CMS2 subtype compared to other CMS groups in patients with CRC from Rwanda. This finding is consistent with previous observations by Thota et al.. (77.5% in CMS2) [73] and Smeby et al.. (79.0%) [55], highlighting a potential association between *TP53* variants and the CMS2 subtype. This study represents the first application of the Li et al.. immunohistochemistry-based CMS classification method [36] to a Rwandan population with CRC. The observed distribution of CMS subtypes aligns well with previous studies utilizing both transcriptomic and immunohistochemistry approaches, suggesting the generalizability of this CMS classification method across diverse populations. Furthermore, considering the established prognostic and predictive value of CMS groups [28, 29, 31], this immunohistochemistry-based classifier holds promise for stratifying patients with CRC according to CMS in resource-limited settings like Rwanda.

Our study design precluded matching tumor and non-tumor tissues for *TP53* sequencing, limiting our ability to definitively differentiate between somatic mutations and germline polymorphisms. Nevertheless, we considered variants with MAF < 1% in 1000 Genomes and gnomAD as putative mutations for analysis, enhancing the confidence in our findings. With Sanger sequencing technique, which is known to be a more specific method, we have identified a substantial number of mutations which were classified as pathogenic. Further studies using next-generation sequencing techniques are recommended not only to increase the yield in mutations but also the number of genes to be analyzed. The present study provides significant findings on feasibility of immunohistochemistry-based CMS classification. However, the small sample size makes it difficult to generalize our findings in Rwandan population as a whole, much bigger studies are encouraged in this regard.

Conclusion

Our data demonstrate a high prevalence of *TP53* mutations in CRC of patients from Rwanda with distinct characteristics including an enrichment within the CMS2 subtype. This study, representing the second investigation into molecular alterations in patients with CRC from Rwanda and the first to explore *TP53* mutations and CMS classification in CRC cases from Rwanda, contributing to a more comprehensive understanding of the molecular landscape of this disease. Applying this approach to understudied populations holds promise for

Table 4 Consensus molecular subtype (CMS) classification in patients with colorectal cancer (CRC) from Rwanda and its association with clinicopathological features

Characteristics	Total	CMS1	CMS2	CMS3	CMS4	*p* value
Frequency, ***n*(%)**	51	6 (11.8)	28 (54.9)	9 (17.6)	8 (15.7)	
Age (years old)						
Mean ± SD	61.2 ± 12.5	62.3 ± 11.2	60.9 ± 11.8	61.6 ± 13.8	61 ± 16.3	
Range	31–89	48–77	31–81	32–83	38–89	
Age group, ***n*(%)**						0.904
< 50	9	1 (16.7)	5 (17.9)	1 (11.1)	2 (25.0)	
≥ 50	42	5 (83.3)	23 (82.1)	8 (88.9)	6 (75.0)	
Sex, ***n*(%)**						0.304
Female	33	3 (50.0)	18 (64.3)	8 (88.9)	4 (50.0)	
Male	18	3 (50.0)	10 (35.7)	1 (11.1)	4 (50.0)	
Grade, ***n*(%)**						0.288
Low-grade	18	1 (16.7)	12 (42.9)	4 (44.4)	1 (12.5)	
High-grade	33	5 (83.3)	16 (57.1)	5 (55.6)	7 (87.5)	
MMR status, ***n*(%)**						< 0.001
MMR-D	6	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
MMR-P	45	0 (0.0)	28 (100.0)	9 (100.0)	8 (100.0)	
***TP53* mutations**, ***n*(%)**						0.651
Pathogenic variants	23	3 (50.0)	14 (50.0)	4 (44.4)	2 (25.0)	
Others[a]	28	3 (50.0)	14 (50.0)	5 (55.6)	6 (75.0)	

SD: Standard deviation, MMR: mismatch repair, MMR-D: MMR-deficient, MMR-P: MMR-proficient

[a]Non-pathogenic variants and wild-type

Table 5 Comparison of consensus molecular subtype (CMS) classification in colorectal cancer (CRC) between the present study and previous studies

SN	Study	Year	Method	CMS classification, *n* (%)				Total[a]
				CMS1	CMS2	CMS3	CMS4	
1	The present study	2024	IHC-based	6 (11.8%)	28 (54.9%)	9 (17.6%)	8 (15.7%)	51
2	Kagawa et al. [53]	2022	Transcriptomic	173 (13.6)	335 (26.3)	306 (24.0)	461 (36.2)	1275
3	Li et al. [36]	2021	IHC-based	16 (27.1)	18 (30.5)	10 (16.9)	15 (25.4)	59
4	Purcell et al. [54]	2019	Transcriptomic	60 (23.1%)	145 (55.8%)	38 (14.6%)	17 (6.5%)	260
5	Smeby et al. [55]	2019	[b]Transcriptomic	62 (20.0%)	138 (44.0%)	54 (17.0%)	62 (20%)	316
6	Smeby et al. [55]	2019	[c]Transcriptomic	46 (14.0%)	154 (48.0%)	43 (14.0%)	76 (24%)	319
7	Loree et al. [56]	2018	Transcriptomic	104 (17.0%)	281 (46.0%)	78 (13.0%)	144 (24%)	608
Range percentage of CMS groups in the other studies [36, 53, 56]				13.6–23.1	26.3–55.8	13.0–24.0	6.5–36.2	

SN: serial number, IHC: Immunohistochemistry

[a]Total number of classified cases

[b]Oslo series cohort

[c]GSE39582 cohort

uncovering novel insights into the molecular epidemiology of human carcinogenesis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-13009-8.

Supplementary Material 1

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Author contributions

HS, FM, and AN initiated the project with Rwandan people. AN performed experiments and drafted the manuscript. DU provided technical assistance, data curation and also assisted in providing samples needed for this study. HK, JBS provided technical assistance regarding tissue processing. AM, ELN, SU participated in data curation, review and editing of the manuscript. FM performed some experiments, provided technical advice regarding the

interpretation of *TP53* mutations. MCN, GN, BS, VD, ER, PK, FN provided the samples needed for this study. RI, HW involved in data curation, technical assistance and provided technical advice regarding the interpretation of *TP53* mutation patterns. BR helped in writing, review and editing the manuscript. SB, data curation, technical assistance for MMR assessment. KY, YS, HY coordinated the study design, and helped draft the manuscript. HS and KS were involved in study conception, study design, funding acquisition, supervision and project administration. All authors have read and approved the final manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
This study was approved by the institutional review board of the University of Rwanda College of Medicine and Health Sciences (Approval notice: No.295/CMHSIRB/2020 and No.310/CMHSIRB/2021), the Ethical Committee of the University Teaching Hospital of Kigali (Ref.: EC/CHUK/2/064/2020) and the ethics committee of the Hamamatsu University School of Medicine (EC HUSM number: 20 – 011). All study participants were informed of the purpose of the study and voluntarily signed an informed consent form before participating in this study.

Competing interests
The authors declare no competing interests.

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