JOURNAL OF THE
ANUS, RECTUM AND COLON
dx.doi.org/10.23922/jarc.2025-001
http://journal-arc.jp

Review Article

Hereditary Colorectal Cancer: Clinical Implications of Genomic Medicine and Precision Oncology

Atsushi Yamada[1)] and Tomohiro Kondo[1)2)]

1) Department of Clinical Oncology, Kyoto University Hospital, Kyoto, Japan
2) Department of Real-World Data Research and Development, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Abstract

Approximately 10% of colorectal cancer (CRC) cases occur in the context of hereditary cancer-predisposing conditions caused by germline pathogenic variants (PVs) in cancer predisposition genes, with Lynch syndrome and familial adenomatous polyposis at the top of the list. Although the identification of hereditary CRC has traditionally relied on clinical characteristics, including familial accumulation, multiple and early onset of CRC and other related cancers, and the presence of gastrointestinal polyposis, more comprehensive approaches, such as universal tumor screening and universal germline testing, have recently been employed. From a technical standpoint, next-generation sequencing has enabled genome-wide analysis of genetic alterations in germline and somatic settings. Taking advantage of this technology, germline multigene panel testing has been utilized in genetic testing, which leads to the identification of PVs, not only in well-known hereditary CRC genes but also in rare causal genes, moderate-risk genes, and high-risk genes previously not linked to CRC predisposition. In addition, comprehensive genomic profiling and companion diagnostics for solid tumors occasionally yield unexpected hereditary CRC diagnoses. Thus, more hereditary CRCs have been identified not based on clinical phenotypes but rather by comprehensive approaches or as secondary findings of treatment drug testing. In this review, we discuss the impact of recent advances in genomic medicine on the clinical aspects of hereditary CRC, which has promoted an understanding of the entire landscape of genetic predisposition to CRC.

Keywords

comprehensive genomic profiling, genomic medicine, hereditary colorectal cancer, multigene panel testing, universal germline testing, universal tumor screening

J Anus Rectum Colon 2025; 9(2): 167-178

Introduction

Approximately 10% of patients with colorectal cancer (CRC) possess germline pathogenic variants (PVs) or likely PVs (LPVs) in cancer predisposition genes[1,2]. While Lynch syndrome (LS) and familial adenomatous polyposis (FAP) are the most well-known hereditary CRCs, recent advances in genomic medicine have promoted the understanding of the entire landscape of genetic predisposition to CRC, including rare and/or moderate-risk conditions and high-risk hereditary cancer syndromes previously not linked to CRC. The identification of families with hereditary CRC has significant implications as proper management, including surveillance and prophylactic interventions, can lead to better outcomes in affected individuals. For instance, surveillance colonoscopy and removal of adenomatous polyps decrease

Corresponding author: Atsushi Yamada, yatsushi@kuhp.kyoto-u.ac.jp
Received: January 7, 2025, Accepted: January 17, 2025

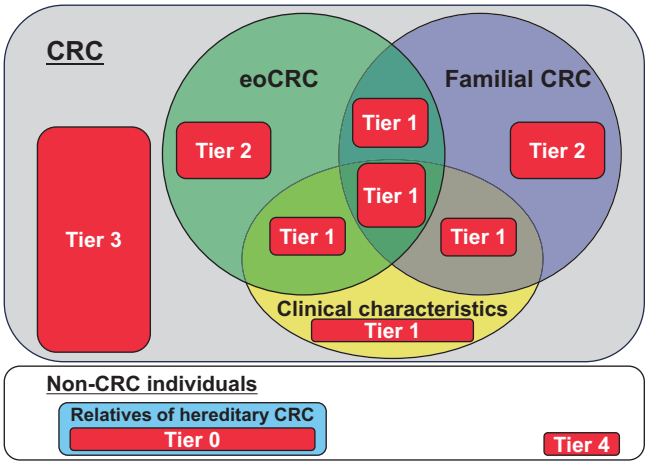

Figure 1. Identification of individuals with hereditary CRC. Red bars indicate individuals affected by hereditary CRC among those with and without the occurrence of CRC. Patients with hereditary CRC in "Tier 1" can be detected using traditional diagnostic criteria specific for each hereditary CRC syndrome or the Amsterdam criteria, whereas the revised Bethesda guidelines enable the screening of individuals in "Tier 2." In contrast, universal approaches (i.e., universal tumor testing and universal genetic testing) and population-based screening are required to identify individuals with hereditary CRC in "Tiers 3 and 4," respectively. Relatives of patients with hereditary CRC can be diagnosed by cascade testing ("Tier 0"). CRC, colorectal cancer; eoCRC, early-onset colorectal cancer

the incidence of CRC and prolong overall survival in individuals with LS[3].

Traditionally, the identification of hereditary CRC has relied on clinical information, including familial accumulation, multiple and early-onset CRC and other related cancers, the presence of polyposis or multiple polyps, and other specific features characterizing specific conditions. More comprehensive approaches to identifying hereditary CRCs have been developed in recent years. In this context, universal tumor screening using microsatellite instability (MSI) test and/or immunohistochemistry (IHC) for mismatch repair (MMR) proteins is recommended in all patients with CRC[4] and endometrial cancer[5] aiming to detect individuals with LS. In addition, MSI testing and IHC for MMR proteins have been widely performed as companion diagnostics to determine the indication of immune checkpoint inhibitors (ICIs) in solid tumors[6], further facilitating LS diagnosis. From a technical standpoint, the invention of next-generation sequencing (NGS) has enabled the genome-wide analysis of genetic alterations in germline and somatic settings. Using NGS, comprehensive genomic profiling (CGP) to sequence dozens to hundreds of cancer-related genes in cancer cells has been introduced into clinical practice for patients with solid tumors. Although its primary purpose is to explore potential treatment drugs, CGP occasionally yields unexpected diagnoses of hereditary cancer-predisposing conditions[7]. NGS technology has also enabled germline multigene panel testing (MGPT), which is utilized in the genetic testing of suspected hereditary CRC cases. Notably, germline MGPT identified PVs/LPVs in well-known hereditary CRC genes, including MMR and polyposis-related genes, and in genes traditionally not linked to CRC predisposition[8,9]. More recently, some authors have proposed the use of universal germline testing to perform germline MGPT in all patients with CRC[1,2] and in those with all solid tumors[10]. Thus, more hereditary CRCs have been identified not based on clinical phenotypes but rather by comprehensive approaches or as secondary findings of companion diagnostics and CGP. In this review, we discuss the impact of recent advances in genomic medicine on the clinical aspects of hereditary CRC.

Overview of Hereditary CRC in Genomic Medicine

Typically, families carrying hereditary cancers possess two key features: familial accumulation and early-onset of specific types of cancer. In addition, it is common for affected individuals to develop multiple tumors; some hereditary CRCs manifest characteristic clinical phenotypes leading to their diagnosis, such as adenomatous or hamartomatous polyposis in the gastrointestinal tract (Figure 1). Although familial accumulation and early-onset tumors are hallmarks of hereditary cancers, none of them are prerequisite conditions. In families carrying hereditary CRCs, a lack of familial accumulation of multiple cancers can be observed because of low penetrance, nuclear family unavailability of medical information on extended family members, and/or de novo occurrence of causal variants. Meanwhile, a non-negligible proportion of familial CRC cases have no detectable PVs on germline testing[9]; this could be attributed to high-risk PVs outside the genes tested or those that conventional methods could not detect. Alternatively, combinations of hundreds of common low-risk variants associated with environmental factors may result in a significantly high risk of CRC in a family[11]. Although early-onset tumors raise the possibility of hereditary cancer, most patients with early-onset cancer do not harbor detectable germline PVs in cancer predisposition genes[12-14]. Conversely, cancer occurrence in elderly patients does not necessarily exclude hereditary cancer.

In the era of genomic medicine, approaches for diagnosing hereditary cancer have changed. By adopting comprehensive screening approaches, including universal tumor screening and universal germline testing, and as secondary findings of companion diagnostics and CGP performed for cancer treatment, the identification of germline PVs predisposing to CRC has increased among those without typical

Table 1. Impact of Genomic Medicine on Non-Polyposis Hereditary CRC.

Causal genes	Inheritance	Comments
LS		
MLH1 *MSH2* *MSH6* *PMS2* *EPCAM*	AD	Universal tumor screening and universal germline testing increased the yield of LS detection among patients with CRC and endometrial cancer. Screening for other solid cancers should also be considered. The prevalence of LS in the general population is estimated to be 1 in 432 to 1 in 550, and it is most frequently caused by PVs in *PMS2* and *MSH6*.
Constitutional *MLH1* epimutation		
MLH1	Non-mendelian	Constitutional *MLH1* epimutation analysis for patients with *MLH1* methylated CRCs in patients with early-onset and/or multiple tumors should be considered. No remarkable family history was observed in most cases.
CMMRD		
MLH1 *MSH2* *MSH6* *PMS2*	AR	Typically, patients develop brain tumors, gastrointestinal cancers, and hematological malignancies showing dMMR by the age of 18. Café-au lait maculae mimicking neurofibromatosis type 1 are commonly seen. May present with colorectal adenomatous polyposis. The parents of the CMMRD probands are likely to carry LS, whereas the siblings are presumably either CMMRD or LS.
FCCTX		
Various genes	AD?	Heterogeneous conditions with putative causal genes, including *BMPR1A*, *BRCA2*, *FAN1*, *MUTYH*, *OGG1*, *RNF43*, *RSP20*, *SEMA4A*, and *SETD6*. The detection of rare causal genes may be facilitated by the use of germline MGPT. Colonoscopic surveillance reduced CRC incidence and improved survival in families with FCCTX.

AD, autosomal dominant; AR, autosomal recessive; CRC, colorectal cancer; CMMRD, constitutional mismatch repair deficiency; dMMR, deficient mismatch repair; FCCTX, familial colorectal cancer type X; LS, Lynch syndrome; MGPT, multigene panel testing; PVs, pathogenic variants

clinical manifestations. Moreover, the use of germline MGPT in patients with suspected hereditary CRC revealed the involvement of high-risk hereditary cancer genes previously not linked to CRC predisposition, such as *BRCA1* and *BRCA2,* as well as moderate-risk genes[1,2,15]. The frequent use of MGPT inevitably increases the detection of variance of uncertain significance (VUS), especially when using a larger panel[15]. It should be noted that the interpretation of variants can be troublesome and can be updated over time[16]. Nonetheless, the recurrent use of MGPT may improve variant interpretation and reduce those categorized as VUSs. Furthermore, recent studies have estimated the prevalence of LS in the general population by utilizing whole-exome sequencing (WES) data from biobank participants[17,18]. Compared with conventional studies focused on probands diagnosed from patients with CRC and/or those meeting clinical criteria, this approach may contribute to elucidating the entire picture of hereditary CRC in terms of prevalence, cancer risk, and other clinical manifestations.

Impact of Genomic Medicine on Hereditary CRC (Table 1)

LS

LS is an autosomal dominant hereditary cancer syndrome caused by germline PVs in one of the MMR genes (*MSH2*, *MLH1*, *MSH6*, and *PMS2*), or *EPCAM*; it represents the most frequent hereditary CRC, accounting for 0.7%-4.0% of all CRC cases[19,20]. Individuals with LS have higher risks of CRC, endometrial cancer, and various other cancers, with the vast majority of tumors showing deficient MMR (dMMR) as a result of impaired function of the responsible MMR protein. Familial accumulation and early onset of related cancer are the hallmarks of LS; clinical criteria incorporating this information, such as the Amsterdam criteria II[21] and revised Bethesda guidelines[22], have been used to screen suspected LS cases. Since it has become evident that the use of these criteria will miss a significant number of affected individuals, universal tumor screening by MSI and/or IHC for MMR proteins has been introduced and widely performed in patients with CRC and endometrial cancer[4,5]. Since MSI testing and IHC for MMR proteins have been widely performed as companion diagnostics for ICIs[6], and CGP[7] has been clinically implemented in daily practice, opportunities for identifying LS in patients with various cancer types are increasing, including cancers that have not been previously linked to LS[23]. Latham et al. evaluated the MSI status determined by targeted NGS in more than 15,000 tumors, including more than 50 cancer types, and showed that the prevalence of LS in patients with MSI-high, MSI-indeterminate, and microsatellite stable tu-

mors was 16.3% (53 of 326), 1.9% (13 of 699), and 0.3% (37 of 14,020), respectively. They reported that among patients diagnosed with LS, half (33 of 66) in the MSI-high and MSI-indeterminate groups and the predominant proportion in the microsatellite stable group were cancers other than CRC or endometrial cancer[23]. Thus, their data indicate the need for LS screening targeting a broader spectrum of solid tumors as well as proficient MMR (pMMR)/non-MSI-high tumors. Indeed, the utility of LS screening incorporating multiple types of solid tumor has been reported[24]. In addition, it has been reported that universal tumor screening could have missed 6.3% of LS probands among patients with CRC, suggesting the need for universal germline testing[2]. In clinical practice, the yield of universal tumor screening may be lower because a significant proportion of possible LS patients do not opt to undergo genetic testing[25]; therefore, the advantages of universal germline testing over universal tumor screening in identifying more probands with LS could be substantial. By analyzing data on biobank participants, the prevalence of LS in the general population was estimated to be 1 in 432-550[17,18]. In a study by Rosenblum et al., most carriers of PVs/LPVs in MMR genes had previously no documented diagnosis of LS, further supporting the need for a universal approach in LS screening[17]. Interestingly, despite *MSH2* and *MLH1* being known as the predominant causal genes of LS, PVs/LPVs were most frequent in *PMS2*, followed by *MSH6* among biobank participants, presumably reflecting the lower penetrance in carriers of *PMS2* and *MSH6*[17,18]. In the treatment of dMMR tumors, including those occurring in patients with LS, the efficacy of ICIs has been demonstrated[26]. Despite the hope that ICIs may also be useful for cancer prevention in individuals with LS, Harrold et al. recently showed that the risk of developing new cancers did not decrease after ICIs, including anti-PD-1/PD-L1 antibodies. However, the incidence of visceral neoplasms may decrease after ICI exposure in the subgroup analysis[27].

Constitutional MLH1 epimutation

Most CRCs exhibiting high MSI levels and loss of MLH1 expression are sporadic and are associated with aberrant *MLH1* promoter methylation in tumor cells. On rare occasions, methylation of a single allele of the promoter of the *MLH1* gene is observed in normal cells, which causes transcriptional silencing of the affected allele and is referred to as constitutional *MLH1* epimutation[28]. Similar to LS caused by PVs in MMR genes, constitutional *MLH1* epimutation is linked to multiple and early-onset tumors within the LS spectrum. Although *MLH1* epimutation can be inherited, it is reversible between generations and tends to have no remarkable family history[28,29]. In clinical practice, tumors with positive *MLH1* methylation are considered sporadic and are generally excluded from genetic testing in universal tumor screening for LS. Among *MLH1* methylated CRCs, constitutional *MLH1* epimutation was overrepresented in early-onset patients[29] and in those who met the revised Bethesda guidelines[29,30]. Therefore, constitutional *MLH1* epimutation analysis should be considered for *MLH1* methylated CRCs associated with clinical features resembling LS.

Constitutional mismatch repair deficiency (CMMRD)

CMMRD is a rare cancer predisposition syndrome caused by biallelic germline PVs in one of the four MMR genes[31]. Most patients with CMMRD develop malignant neoplasms, including brain tumors, gastrointestinal cancers, and hematological malignancies, by the age of 18. Non-neoplastic features, such as café-au-lait maculae, are also common in CMMRD, mimicking neurofibromatosis type 1[31,32]. The vast majority of tumors occurring in CMMRD demonstrate dMMR and increased tumor mutational burden (TMB)[31]; therefore, MSI testing, IHC for MMR proteins, and CGP can trigger the detection of CMMRD cases. Although an optimal protocol has not been established, surveillance, including clinical examination, brain magnetic resonance imaging, and colonoscopy, has been reported to improve the survival of individuals with CMMRD[31,33]. Because the parents of patients with CMMRD are likely LS carriers, and the siblings of patients with CMMRD are presumably either LS or CMMRD, it is extremely important to offer cascade genetic testing to the relatives of patients with CMMRD[31].

Familial colorectal cancer type X (FCCTX)

FCCTX is defined as families that fulfill the Amsterdam criteria, and their tumors show pMMR with no germline PVs in MMR genes[34,35]. FCCTX has been linked to an increased risk of CRC; however, unlike LS, most studies observed no association with an increased risk of extracolonic cancer in FCCTX[34,36,37]. Compared with LS, CRCs in FCCTX have characteristic clinicopathological features, including older age at diagnosis, more likely left-sided location, and not linked to poorly differentiated or mucinous histology[34,35,38]. Notably, members of FCCTX families had a lower risk of developing CRC than those of LS[39], whereas cancer-related mortality was higher in FCCTX than in LS[38]. Importantly, colonoscopic surveillance significantly reduces CRC incidence and improves survival among asymptomatic members of FCCTX families[40].

The genetic causes of FCCTX are heterogeneous and have not yet been fully elucidated. Thus far, germline PVs in *BRCA2*[41], *BMPR1A*[42], *FAN1*[43], *MUTYH*[44], *OGG1*[44], *RSP20*[45], *SEMA4A*[46], and *SETD6*[47] have been reported as putative causes of FCCTX. In addition, the co-segregation of germline variants in the *BRCA1* and *RNF 43* genes with CRC in an FCCTX family has been reported[48]. It is expected that the use of MGPT comprising

Table 2. Impact of Genomic Medicine on Adenomatous Polyposis Syndromes.

Causal genes	Inheritance	Comments
FAP		
APC	AD	Genotype-phenotype correlation has been reported to be beneficial in patient management. A part of the de novo cases are due to somatic *APC* mosaicism, which can be detected by next-generation sequencing.
MAP		
MUTYH	AR	Often presents as attenuated adenomatous polyposis but also exhibits classical adenomatous polyposis or SPS. Tumors associated with MAP were enriched for G:C to T:A transversions and *KRAS* G12C (c.34 G>T) variants.
NTHL1-associated tumor syndrome		
NTHL1	AR	Linked to the broad spectrum of tumors enriched from C:G to T:A transitions.
PPAP		
POLE	AD	The tumors developed in patients with PPAP exhibited a very high tumor mutational burden and a good response to treatment with immune checkpoint inhibitors.
POLD1	AD	
Other adenomatous polyposis		
AXIN2	AD	The detection of rare causal genes may be facilitated by the use of germline MGPT. Whole-exome and whole-genome sequencing may promote the future identification of novel causal genes.
MBD4	AD, AR	
MLH3	AR	
MSH3	AR	

AD, autosomal dominant; AR, autosomal recessive; FAP, familial adenomatous polyposis; MAP, MUTYH-associated polyposis; MGPT, multigene panel testing; PPAP, polymerase proofreading-associated polyposis; SPS, serrated polyposis syndrome

the above-mentioned genes or more comprehensive approaches, such as WES and whole-genome sequencing (WGS), will facilitate the identification of rare causal genes in more FCCTX families.

Adenomatous polyposis (Table 2)

FAP is a hereditary cancer syndrome characterized by the presence of ≥100 adenomatous polyps in the colon and rectum. Virtually all patients with FAP develop CRC if they do not receive prophylactic treatment; in addition, they are also predisposed to extra-colorectal tumors, including duodenal cancer, gastric cancer, thyroid cancer, pancreatic cancer, hepatoblastoma, and desmoid tumor[20,49,50]. The primary cause of FAP is monoallelic germline PVs in the *APC* gene, which is found in approximately 58%-78% of classical FAP (i.e., ≥100 adenomatous polyps)[51,52] and leads to an autosomal dominant pattern of inheritance. Although germline PVs in *APC* are more common in classical FAP, they are also found in attenuated FAP harboring fewer than 100 adenomatous polyps[51-53]. Interestingly, a genotype-phenotype correlation has been reported. PVs located between codons 1250-1464 in *APC* were associated with profuse polyposis, whereas those in codons 78-157, 312-412, and 1595-2843 were associated with attenuated polyposis[54]. Approximately 15%-25% of FAPs arise de novo without a family history[20,49], and approximately 15%-20% of them are reportedly due to somatic mosaicism for the *APC* gene[20,49,54]. Because NGS can detect low-level variants, it can facilitate the diagnosis of somatic *APC* mosaicism among previously undiagnosed patients with FAP[52,55]. In addition, Young et al. recently reported that paired germline DNA-RNA MGPT can detect deep-intronic DNA variants leading to aberrant RNA splicing that were unidentified by DNA-only genetic testing[56].

MUTYH-associated polyposis (MAP) is the second most frequent type of adenomatous polyposis, which is caused by biallelic germline PVs in the *MUTYH* gene and shows an autosomal recessive inheritance pattern[57,58]. MAP presents as attenuated polyposis more often; however, some patients may exhibit classical polyposis with ≥100 adenomatous polyps. It has been reported that among patients with biallelic germline *MUTYH* PVs, 22.7% had at least 100 adenomatous polyps, whereas 68.5% had fewer than 100[51]. In addition to colorectal adenomatous polyposis and CRC, carriers of biallelic germline PVs in *MUTYH* have a higher risk of developing tumors, including duodenal adenoma and cancer, ovarian cancer, and bladder cancer[57,58]. Notably, patients with MAP commonly harbor serrated polyps, such as sessile serrated lesions and hyperplastic polyps, and they occasionally meet the criteria for serrated polyposis syndrome (SPS)[59]. *MUTYH* is involved in base excision repair (BER), and its impaired function results in an excess of G:C to T:A transversions. Consequently, MAP-associated CRCs show a predominance of the *KRAS* G12C (c.34G>T) variant among *KRAS* alterations[60].

More recently, an association between *NTHL1*, another

Table 3. Impact of Genomic Medicine on Nonadenomatous Polyposis Syndromes.

	Causal genes	Inheritance	Comments
JPS			
	BMPR1A	AD	Juvenile polyps occur throughout the gastrointestinal tract but predominantly in the colorectum. Clinical use of CGP and germline MGPT may promote the diagnosis of more probands, including those lacking typical features.
	SMAD4	AD	
PJS			
	STK11	AD	Characterized by mucocutaneous pigmentation and Peutz-Jeghers hamartomatous polyps predominantly in the small intestine. Clinical use of CGP and germline MGPT may promote the diagnosis of more probands, including those lacking typical features.
PHTS			
	PTEN	AD	Characterized by various extragastrointestinal manifestations, such as macrocephaly, autism spectrum disorders, and mucocutaneous lesions. Clinical use of CGP and germline MGPT may promote the diagnosis of more probands, including those lacking typical features.
HMPS			
	BMPR1A	AD	Germline MGPT, WES, and WGS may contribute in disclosing unknown causal genes and the spectrum of the syndrome.
	upstream of *GREM1*	AD	
SPS			
	MUTYH	AR	Most cases are not hereditary syndromes. Germline MGPT detected PVs/LPVs in various cancer-predisposing genes in ~10% of patients.
	RNF43	AD	

AD, autosomal dominant; AR, autosomal recessive; CGP, comprehensive genomic profiling; HMPS, hereditary mixed polyposis syndrome; JPS, juvenile polyposis syndrome; LPVs, likely pathogenic variants; MGPT, multigene panel testing; PHTS, PTEN-hamartoma tumor syndrome; PJS, Peutz-Jeghers syndrome; PVs, pathogenic variants; SPS, serrated polyposis syndrome; WES, whole-exome sequencing; WGS, whole-genome sequencing

BER gene, and adenomatous polyposis and CRC was reported[61,62]. Biallelic PV carriers in *NTHL1* possess higher risks for adenomatous polyposis and CRC and are further linked to a broad spectrum of tumors[57,58,61]. Distinct from tumors in patients with MAP, tumors developed in association with *NTHL1* dysfunction demonstrate the enrichment of C:G to T:A transitions[57,58,61,62].

POLE and *POLD1*, which encode DNA polymerase ε and δ, respectively, have also been associated with hereditary CRC. Indeed, monoallelic germline PVs in the proofreading exonuclease domains of *POLE* and *POLD1* are known to cause polymerase proofreading-associated polyposis (PPAP), which is a cancer-predisposing condition that exhibits autosomal dominant inheritance and is associated with multiple colorectal adenomas, CRC, and extracolorectal cancers such as endometrial cancer[63,64]. Because most CRCs with PVs in the exonuclease domains of *POLE* and *POLD1* show an extremely high TMB[65,66], and a high TMB is an established predictive biomarker for a favorable response to immune therapy[67], cancers that occur in patients with PPAP can be good targets for treatment with ICIs. A recent report by Ambrosini et al. showed that patients with metastatic CRCs harboring *POLE* and *POLD1* PVs had a significantly higher overall response rate and superior progression-free survival after treatment with ICIs than those with dMMR/MSI-high metastatic CRCs[66].

Other rare causes of adenomatous polyposis include monoallelic germline PVs in *AXIN2*[68,69], monoallelic[70] and biallelic germline PVs in *MBD4*[71], and biallelic germline PVs in *MSH3*[72] and *MLH3*[73]. In addition, CMMRD can present with adenomatous polyposis[31]. The detection of PVs in these rare causal genes could be promoted by genetic testing using MGPT, WES, or WGS. Germline PVs/LPVs in cancer-predisposing genes other than those previously associated with adenomatous polyposis have been detected by MGPT in a significant proportion of patients with classical and attenuated polyposis with probable adenomatous histology[74].

Nonadenomatous polyposis (Table 3)

Juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), and *PTEN*-hamartoma tumor syndrome (PHTS) are hereditary cancer-predisposing conditions characterized by autosomal dominant inheritance and the presence of multiple hamartomatous polyps in the gastrointestinal tract[75]. JPS is caused by monoallelic germline PVs in *SMAD4* or *BMPR1A* and is associated with an increased risk of CRC and other cancers, including gastric, duodenal, small intestinal, and pancreatic cancers. Hamartomatous polyps observed in JPS are called juvenile polyps and occur throughout the gastrointestinal tract, predominantly in the colon and rectum. JPS may also present with gastrointestinal

bleeding, protein-losing gastroenteropathy, and various extraintestinal manifestations, including telangiectasia, pigmented nevi, and skeletal stigmata. Individuals with germline *SMAD4* PVs frequently present with combined JPS and hereditary hemorrhagic telangiectasia[76,77]. PJS is caused by monoallelic germline PVs in *STK11* and is characterized by mucocutaneous pigmentation and Peutz-Jeghers hamartomatous polyps in the gastrointestinal tract, predominantly in the small intestine. PJS symptoms are mostly caused by hamartomatous polyps, including gastrointestinal bleeding, anemia, and small bowel intussusception. PJS has been associated with the occurrence of various cancer types, including CRC and gastric, small intestinal, pancreatic, breast, lung, ovarian, and uterus cancers[78,79]. PHTS comprises a spectrum of conditions caused by monoallelic germline PVs in *PTEN*, including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndrome. The characteristic clinical features of PHTS include macrocephaly, autism spectrum disorders, mucocutaneous lesions such as trichilemmoma and oral papilloma, hamartomatous and other types of colonic polyps, and glycogenic acanthosis of the esophagus. PHTS is also linked to malignant tumors, including breast, thyroid, endometrial, renal cell cancers, and CRC[80,81]. The above-mentioned clinical presentations of hamartomatous polyposis have been the key to identifying affected individuals; however, the clinical use of CGP and germline MGPT may contribute to the diagnosis of more probands, including those lacking typical features.

Hereditary mixed polyposis syndrome (HMPS) is characterized by multiple colorectal polyps, including Peutz-Jeghers polyps, juvenile polyps, serrated polyps, and conventional adenomas; it is associated with the development of CRC. Although the genetic causes of HMPS have not been fully elucidated, germline PVs in *BMPR1A* and duplications in a region upstream of the *GREM1* gene have been reported in HMPS[82].

SPS is the most frequent polyposis syndrome of the colorectum; it is characterized by the presence of multiple and/or large serrated polyps throughout the colon, with a high risk of CRC[83,84]. The majority of SPS cases do not have relatives with polyposis and are thus considered to be non-inherited conditions associated with environmental exposures, such as cigarette smoking. Conversely, first-degree relatives of patients with SPS reportedly have a five-fold relative risk of CRC, suggesting some genetic susceptibility[83]. Monoallelic germline PVs in *RNF43*[85] and biallelic germline PVs in *MUTYH*[59] have been reported in a small proportion of SPSs. More recently, genetic testing using MGPT revealed PVs/LPVs in *CHEK2*, *MUTYH*, *POLD1*, *RNF43*, and *SMAD4* in 9.6% of the tested patients with SPS[86], verifying the role of genetic predisposition in some patients with SPS.

Impact of the CGP Test on Hereditary CRC Identification

Although its primary purpose is to explore potential treatment drugs, germline PVs or presumed germline PVs (PGPVs) may be detected by CGP, which is often referred to as germline or secondary findings[87,88]. The CGP test includes tumor-normal paired and tumor-only testing, which requires tumor specimens. More recently, CGP using circulating tumor DNA, commonly known as liquid biopsy, has also been implemented in clinical settings[89]. In tumor-normal paired testing, DNA analysis derived from normal tissues allows determination of germline PVs. In contrast, tumor-only testing and liquid biopsy cannot clearly distinguish germline and somatic variants[88]. PVs detected by tumor-only testing and liquid biopsy, possibly of germline origin, are called PGPVs and require confirmatory germline testing[7]. For tumor-only testing, the European Society for Medical Oncology Precision Medicine Working Group (ESMO-PMWG) proposed a filtering strategy for PGPVs to select PVs that warrant confirmatory germline testing, considering factors such as age at tumor diagnosis, cancer type, clinical actionability of genes, and variant allele frequency in tumor tissue[7]. As an indicator of the probability that the PVs detected by tumor-only testing being the germline PVs for each gene, the ESMO-PMWG defines the germline conversion rate (GCR), calculated as the ratio of the number of tumor-detected germline PVs to the total number of tumor-detected PVs[7].

Data from the Memorial Sloan Kettering Cancer Center using the MSK-IMPACT® assay (n = 45,472, encompassing 47 cancer types and limited to non-hypermutated samples) indicated that among hereditary CRC-associated genes, MMR genes such as *MSH2* (22/37, 59.5%), *MSH6* (43/78, 55.1%), and *MLH1* (22/43, 51.2%) showed high GCR. In contrast, *APC* exhibits a very low GCR (27/2369, 1.1%); therefore, confirmatory germline testing for PGPVs in *APC* is recommended only for patients diagnosed before the age of 30 to enhance the yield of true germline PVs[7]. When applying the ESMO-PMWG criteria to the MSK-IMPACT® dataset, >50% of the PGPVs selected for confirmatory germline testing were confirmed to be true germline PVs[7]. Our previous study analyzing data from another CGP test, FoundationOne CDx®, showed the validity of the ESMO-PMWG criteria in selecting PGPVs with a high likelihood of germline origin detected by tumor-only CGP in clinical practice[90].

CGP leads to germline PV identification in hereditary CRC genes in patients with CRC and other solid tumors. PVs in the *APC* gene detected in *APC*-related tumors ("on-tumor"), including CRC, demonstrated a significantly lower GCR (15/2083, 0.7%) compared with those in non-*APC*-related tumors ("off-tumor") (12/286, 4.2%)[7], presumably

because of the high rate of somatic *APC* PVs in CRC. Furthermore, in the MSK-IMPACT® CRC dataset (n = 4370), the GCR of on-tumor genes (e.g., *APC*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, and *PMS2*) was lower than that of off-tumor genes (e.g., *BRCA1*, *BRCA2*, *RET*, and *VHL*) ("on-tumor": 118/3767, 3.1% vs. "off-tumor": 122/347, 35.2%)[7]. Taken together, the opportunities for diagnosing hereditary CRC can be expanded by carefully interpreting the CGP results.

Early-onset CRC

The incidence of CRC diagnosed before the age of 50 is referred to as early-onset CRC (eoCRC), and it is increasing worldwide[91]. Risk factors for eoCRC include family history of CRC, hyperlipidemia, obesity, and alcohol consumption[91,92]. The occurrence of cancer(s) at a younger age is a hallmark of hereditary cancer; germline genetic testing using MGPT is recommended for all patients with eoCRC[91]. However, the majority of patients with eoCRC are sporadic, without a family history of CRC or detectable germline PVs in known cancer-predisposing genes. Among patients with eoCRC, only 14%-26% had a first-degree relative with CRC, and up to 20% possessed germline PVs/LPVs associated with cancer predisposition. Although the majority of germline PVs/LPVs were found in high-penetrance CRC genes such as MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) and polyposis-related genes (e.g., *APC*, *MUTYH*, *SMAD4*, and *BMPR1A*), they were also observed in genes not traditionally linked to CRC (e.g., *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, and *TP53*), indicating the utility of MGPT incorporating a broader spectrum of cancer-predisposing genes[12-14].

Universal Germline Testing of CRC and Other Solid Tumors

Recently, a more comprehensive approach for identifying individuals with cancer-predisposing conditions has been proposed. Yurgelun et al. utilized a cohort of >1,000 consecutive patients with CRC without preselection by MSI/MMR status, age at diagnosis, and personal/familial cancer history and performed MGPT to analyze germline alterations in cancer susceptibility genes. They found that 9.9% of patients with CRC had at least one PV/LPV, including 3.1% in LS genes, 1.0% in *BRCA1* and *BRCA2*, 1.1% in other high-penetrance genes (*APC*, biallelic *MUTYH*, *PALB2*, *CDKN2A*, and *TP53*), and 4.7% in moderate-penetrance genes (monoallelic *MUTYH*, *APC* I1307K, *ATM*, *BRIP1*, *CHEK2*, *NBN*, and *BARD1*). Because specific risk-reducing interventions are recommended to most individuals harboring PVs/LPVs and most individuals with non-LS cancer-predisposing conditions lacked the characteristic clinical features of the respective syndromes, the authors argued for the benefit of comprehensive germline testing in unselected patients with CRC[1]. Pearlman et al. reported the outcomes of a prospective study involving >3,000 patients with surgically treated CRC, in which they performed universal tumor screening for LS combined with germline MGPT in patients with dMMR tumors, <50 years of age, multiple tumors, or a family history of CRC and endometrial cancer. They showed that 38.6% of patients with hereditary cancer syndromes, including 6.3% of those with LS, would have been missed if universal tumor screening had been employed without a combination of MGPT[2]. Another study by Coughlin et al. retrospectively analyzed the results of germline MGPT in > 34,000 patients with CRC performed in a commercial laboratory; they found that 11.9% of the patients had clinically actionable PVs, including 3.1% in genes not traditionally associated with CRC[15]. These recent studies support the legitimacy of universal germline testing in patients with CRC, although its clinical implementation should be considered to balance its disadvantages, such as the cost of NGS and the increasing need for genetic counseling. Moreover, universal germline testing has been proposed for other solid tumors[10,93], which may increase the chance of identifying CRC-predisposing individuals from those with other solid tumors.

Future Directions

In the era of genomic medicine, comprehensive approaches and the use of germline MGPT have promoted the identification of individuals with hereditary CRC, which has led to a better understanding of the entire landscape of CRC-predisposing conditions. The broader use of germline MGPT has increased PV detection in rare causal genes, moderate-risk genes, and high-risk genes previously not associated with CRC and among individuals not associated with the characteristic features of known hereditary CRC syndromes. However, the cause of a significant proportion of CRC predispositions remains unexplained. Future studies utilizing genome-wide sequencing approaches, including WES, WGS, and RNA sequencing, may contribute to the discovery of novel mechanisms hidden in the human genome, which may lie in unknown, rare causal genes or within noncoding sequences that lead to the dysregulation of gene expression. In addition, new technologies may facilitate the identification of unknown mechanisms, as long-read sequencing enables the identification of structural variants that are difficult to detect using conventional methods[94,95]. Furthermore, studies focusing on PV carriers in hereditary CRC genes in the general population are needed to elucidate their prevalence and penetrance. The ultimate goal of hereditary CRC practice is to identify affected individuals and families and provide appropriate care to improve quality of

life and prolong survival. To this end, it is crucial to train specialists, including clinical geneticists, genetic counselors, and clinicians, who are well-versed in managing hereditary CRC. As advances in genomic medicine have expanded the opportunity for identifying probands with hereditary CRC, accessibility to requisite medical care should also be universally promoted.

Conflicts of Interest

There are no conflicts of interest.

Author Contributions

Atsushi Yamada and Tomohiro Kondo wrote and approved the final version of the manuscript.

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