Review

Advances in Identification of Susceptibility Gene Defects of Hereditary Colorectal Cancer

Qiang Liu[1,2,3], Yue-Qiu Tan[2,3]✉

1. Hunan Key Laboratory of Translational Radiation Oncology, Hunan cancer Hospital and The Affiliated Cancer of Xiangya School of Medicine, Central South University, Changsha, China
2. Institute of Reproductive and Stem Cell Engineering, School of Basic Medical Science, Central South University, Changsha, China
3. Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China

✉ Corresponding author: Dr. Yue-Qiu Tan, Institute of Reproduction and Stem Cell Engineering, School of Basic Medical Science, Central South University, Changsha, Hunan, 410078, China. E-mail: tanyueqiu@csu.edu.cn; Tel: 86-731-82355301

Abstract

Colorectal cancer (CRC) is a common malignant tumor of the digestive system worldwide, associated with hereditary genetic features. CRC with a Mendelian genetic predisposition accounts for approximately 5–10% of total CRC cases, mainly caused by a single germline mutation of a CRC susceptibility gene. The main subtypes of hereditary CRC are hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). With the rapid development of genetic testing methods, especially next-generation sequencing technology, multiple genes have now been confirmed to be pathogenic, including DNA repair or DNA mismatch repair genes such as *APC, MLH1,* and *MSH2*. Since familial CRC patients have poor clinical outcomes, timely clinical diagnosis and mutation screening of susceptibility genes will aid clinicians in establishing appropriate risk assessment and treatment interventions at a personal level. Here, we systematically summarize the susceptibility genes identified to date and the potential pathogenic mechanism of HNPCC and FAP development. Moreover, clinical recommendations for susceptibility gene screening, diagnosis, and treatment of HNPCC and FAP are discussed.

Key words: hereditary colorectal cancer; hereditary non-polyposis colorectal cancer (HNPCC); familial adenomatous polyposis (FAP); susceptibility genes

Introduction

Colorectal cancer (CRC) is the third most common malignant tumor among all cancer types worldwide, accounting for more than 8% of the annual global cancer-related death rate [1]. The incidence of CRC in men is second only to lung and prostate cancer, while that in women is second only to lung and breast cancer [2]. Although the majority of CRC cases are considered to be sporadic, approximately 30% of CRCs develop from genetic factors [3], and approximately 5% are associated with known susceptibility gene mutations [4]. The most common hereditary CRCs are hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP), followed by MutY homolog (MUTYH)-associated polyposis (MAP),

Peutz-Jeghers syndrome, juvenile polyposis, Cowden/PTEN hamartoma syndrome, and other subtypes [5, 6]. Harboring susceptibility gene defects increase the possibility of developing CRC owing to genetic instability. The major HNPCC susceptibility genes discovered to date include DNA mismatch repair (MMR) genes such as *MLH1, MSH2, MSH6,* and *PMS2* [7-10], whereas the main FAP susceptibility gene is adenomatous polyposis coli gene (*APC*) [11]. Moreover, recent progress in sequencing technology has accelerated the discovery of new susceptibility genes involved in CRC [9, 12-14].

At present, approximately 40 susceptibility genes have been identified to be associated with hereditary CRC [15, 16]. In this review, we searched

for susceptibility genes of CRC to obtain a documented evidence (clinical, functional, or experimental) of the genes causing HNPCC and FAP in humans using PubMed and other computer-based internet websites. The key words included hereditary colorectal cancer (or CRC), hereditary nonpolyposis colorectal cancer (or HNPCC), Lynch syndrome, familial adenomatous polyposis (or FAP), susceptibility genes, gene mutations or variants, genetics, and genetic detection. For the susceptibility genes in HNPCC, we mainly concentrated on the genes related to MMR. In addition, we focused our attention on recent studies conducted in the last five years and highlighted the susceptibility genes with validated evidence. Other informative websites such as Online Mendelian Inheritance in Man (www.OMIM.org) and Gene Cards (www.genecards.org) were then used to compile an updated list of genes from these major sources. We firmly believe that the detection and mechanism elucidation of CRC susceptibility genes are of great significance toward gaining a comprehensive understanding of the molecular mechanism of hereditary CRC. In turn, these efforts will lead to improved accuracy in the clinical judgment of each case, which will allow for more appropriate monitoring or intervention at a personal level to improve patient care and outcome. Overall, this review summarizes the recent progress in identifying hereditary CRC susceptibility genes and elucidation of the underlying mechanism, along with associated recommendations for targeted clinical guidance and screening.

1. Susceptibility genes and mechanism of HNPCC

HNPCC is the most common form of hereditary CRC susceptibility syndrome, also known as Lynch syndrome [17, 18]. HNPCC is inherited in an autosomal dominant manner with high penetrance [19]. Compared with adenoma polyps, the number of adenomas in HNPCC is smaller (<3) [20]. Patients with HNPCC have a higher risk of developing CRC than the general population; approximately 2-4% of all CRC cases are caused by HNPCC [21]. In addition, HNPCC increases the risk of developing other types of cancers such as endometrial cancer, gastric cancer, ovarian cancer, and small intestine cancer [22]. Breast cancer, prostate cancer, and pancreatic cancer have also been associated with HNPCC [23-25].

The diagnosis of HNPCC is based on the Amsterdam II criteria [26] and revised Bethesda criteria [27]. According to the Amsterdam II criteria, patients diagnosed with Lynch syndrome should meet the following conditions: 1) at least three relatives suffered from HNPCC-related tumors (colorectal, endometrial, gastric, ovarian, ureteral, renal, pelvic, small bowel, hepatobiliary, or skin), and one of the relatives is a first-degree relative of the other two; 2) at least two consecutive generations were affected; 3) at least one HNPCC-related tumor was diagnosed before 50 years old; 4) FAP was ruled out; and 5) tumor tissue was confirmed by a pathology method [26]. According to the revised Bethesda criteria, DNA microsatellite instability (MSI) detection should be performed for patients who meet the following conditions: 1) diagnosed with CRC before the age of 50 years; 2) simultaneous or heterochrony colorectal or other HNPCC-related tumors without limit of the diagnosis age; 3) CRC patients under 60 years old who showed histological features of high DNA microsatellite instability (MSI-H, known as MSI of greater than 30% of unstable MSI loci [28]); 4) CRC and another kind of HNPCC-related tumor were diagnosed in one or more first-degree relatives, one of whom was diagnosed before 50 years old; and 5) two or more first- or second-degree relatives were diagnosed with CRC or HNPCC-related tumors without limit of diagnosis age [27].

1.1 Susceptibility genes of HNPCC

HNPCC-related susceptibility genes mainly belong to the families of DNA MMR genes such as *MLH1, MSH2, MSH6*, and *PMS2*. To date, more than 400 mutations have been detected in over 748 families [7, 29-32], with *MLH1* and *MSH2* mutations accounting for up to 90% of the total (Fig. 1A, B). The common susceptibility genes and some of the mutation sites identified in the past five years have been shown in Table 1. *MLH1* is located on chromosome 3p21-23 and contains 19 exons. The most common mutations detected in this gene are missense and splice-site mutations [33, 34]. *MSH2* is located on chromosome 2p21 and contains 16 exons. The main type of *MSH2* mutation detected is frameshift mutations due to small deletions and insertions. Schneider et al. analyzed the mutational profile of MMR genes in 60 unrelated probands diagnosed with colorectal cancer or Lynch syndrome and identified pathogenic or likely pathogenic variants in *MLH1* or *MSH2* in 21 probands (35%) [34]. Another MMR gene, *MSH6*, accounts for about 7-10% of all mutations detected in HNPCC [35], with c.39593962delCAAG and c.39843987dupGTCA being the two most common mutations [36, 37]. *PMS2* mutations are relatively rare, accounting for only about 5% of all mutations in HNPCC [5, 38, 39]. Recent studies found novel deleterious *PMS2* mutations such as c.1492del11 [40]. Importantly, a substantial proportion of CRCs

with solitary loss of PMS2 expression are associated with a deleterious *MLH1* germline mutation, which supports the screening for *MLH1* in patients with tumors of this immunophenotype [41]. Overall, the cumulative risk of developing CRC in *MLH1, MSH2,* and *MSH6* mutation carriers above 70 years old is 41%, 48%, and 12%, respectively [42]. Engel et al. [5] found that the risk of CRC for HNPCC patients with *MLH1* and *MSH2* deletions was 22-74%, while that for patients with *MSH6* or *PMS2* mutations was 10-22% and 15-20%, respectively. Moreover, Chan et al. [43]

found that *MSH1* and *MSH2* germline allele-specific hypermethylation and somatic hypermethylation resulted in loss of MSH1 and MSH2 proteins, and patients with these alterations showed early CRC symptoms. In addition, germline deletions of the epithelial cell adhesion molecule (*EPCAM*) gene result in hypermethylation of the *MSH2* promoter, leading to *MSH2* silencing. *EPCAM* is also considered to be a susceptibility gene that induces HNPCC [44], although the identified mutations of *EPCAM* account for only 1-3% of HNPCC cases [45].
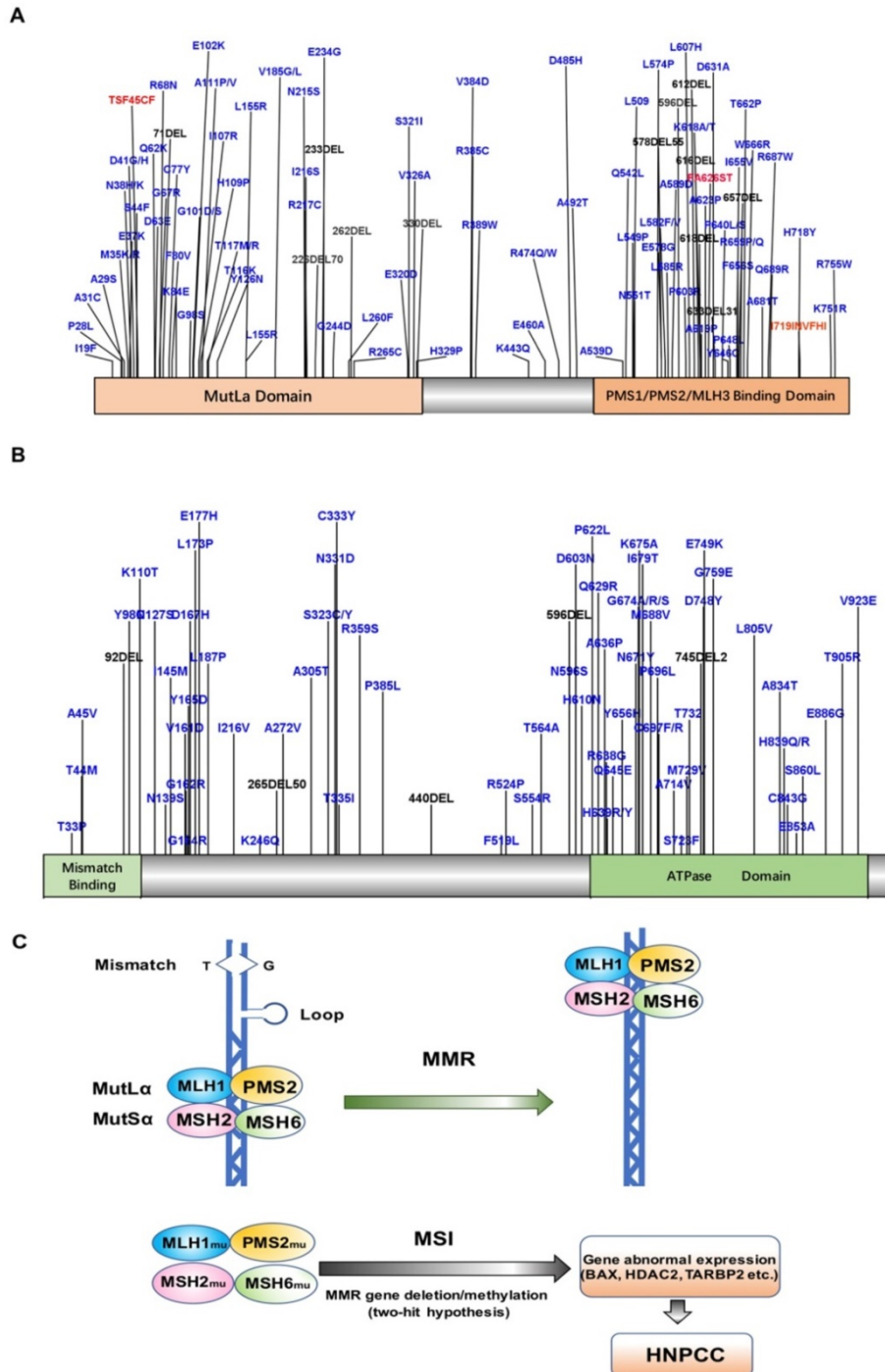

Figure 1. A. Map of MLH1 protein mutation sites B. Map of MSH2 protein mutation sites C. Model of MLH1/MSH2 in HNPCC.

Table 1. Common Susceptibility Genes of HNPCC and FAP and related hot mutation sites identified in the past five years.

Disease	Gene	OMIM	Transcript	New hot mutation sites
HNPCC	*BUB1*	602452	NM_004336.4	c.67C>G[53]
				c.3005C>G[54]
	EPCAM	185535	NM_002354.2	Exon 8–9 deletion[55]
	EXO1	606063	NM_003686.4	c.2212-1G>C[56]
	MLH1	120436	NM_000249.3	c.-63_-58delins18[57]
				c.888_890delAGAinsC[34]
				c.1681dupT[34]
				c.1863delG[34]
				c.225delT[34]
				c.113A>G[41]
				c.677+3A>G[41]
				c.1833dup[58]
				c.2059C > T[59]
	MLH3	604395	NM_001040108.1	c.2152C>T [60]
				c.2615C>G [60]
	MSH2	609309	NM_000251.2	c.965G>A [53]
				c.1676_1679 delTAAA [61]
				c.212-1G>A[62]
	MSH3	600887	NM_002439.4	c.1035del[63]
				c.2732T>G[63]
	MSH6	600678	NM_000179.2	c.2300_2302delCTC[53]
				c.431G>T [53]
				c.3103C>T[64]
				c.3261dupC[64]
	MUTYH	604933	NM_001128425.1	c.1075C>A [53]
	PMS2	600259	NM_000535.6	c.1492del11[40]
	POLD2	600815	NM_001127218.1	c.203G>T [56]
	SMAD4	600993	NM_005359.5	c.1217C>T [53]
				c.1106A>G [53]
				c.1573A>G [53]
FAP	*APC*	611731	NM_000038.5	c.1219delC[65]
				c.3331G>T [65]
				c.3418delC [66]
				c.2971G>T [67]
				c.510_511insA[68]
				c.1317delA[69]
	MSH3	617100	NM_002439.4	c.1148delA[70]
				c.2319-1G>A[70]
				c.2760delC[70]
				c.3001-2A>C
	MUTYH	604933	NM_001128425.1	c.325C>G [65]
				IVS10-2A>G[71]
				c.1187G>A[51]
				c.536A>G[51]
				c.1214C>T[51]
				c.1437_1439delGGA[51]
	NTHL1	602656	NM_001318193.1	c.268C>T[72]
				c.709+1G>A[73]
	POLD1	612591	NM_002691	c.1421T>C[74]
	POLE	615083	NM_006231	c.1270C>G[74]
	STK11	175200	NM_000455	c.167 G>C[75]
				c.1062C>G[75]

HNPCC: hereditary non-polyposis colorectal cancer; FAP: familial adenomatous polyposis

Apart from MMR genes, other genes have also been detected to be responsible for HNPCC. In particular, *BRCA1/2, APC*, and *MUTYH* biallelic mutations have been detected in HNPCC patients [46-48]. In addition, *PMS1, MLH3, BRAF*, and *TGFBR2* have been shown to directly or indirectly affect the development of HNPCC [24]. The chromosome 11q24 region has also been found to be associated with increased susceptibility of CRC. A study by Pinheiro et al. showed a different mutation frequency in *MSH3* and *TGFBR2* between Lynch syndrome and sporadic MSI CRC regarding tumor location, indicating different pathways of carcinogenesis [49]. Moreover, Rudkjobing et al. [50] found that *ALKBH8* in this

region may be associated with HNPCC in a Danish family, although mutations in this gene were also detected in unaffected family members, suggesting the need for further investigation. DeRycke et al. [51] found missense mutations in *BUB1, MSH2, MSH6, MUTYH*, and *SMAD4* in HNPCC patients by targeting 36 known CRC susceptibility genes in 1231 CRC patients. However, a recent study by Mur et al. suggested that the contribution of *NUDT1* germline mutations to hereditary CRC and to polyposis is negligible as well as *OGG1* mutations, indicating that further investigations are still needed to validate this [52].

1.2 Mechanism of action of HNPCC susceptibility genes

The main cause of HNPCC is mutation of MMR genes [76], which have been implicated in a variety of cellular functions essential for maintaining the basic integrity of genetic material and regulation of the cell cycle, including repair of DNA mis-synthesis or DNA double-strand breaks, and in resistance to DNA recombination and DNA destabilization [77]. A heterodimeric MutSα loop consisting of MSH2 and MSH6 (or MSH2 and MSH3) forms a sliding clamp structure that surrounds DNA, which is responsible for recognizing DNA mismatches and recruiting MLH1-PMS1, MLH1-MLH2, or MLH1-MLH3 heterodimers [78]. In particular, the heterodimer consisting of MLH1 and PMS2 is responsible for recruiting the remaining proteins required for MMR [79, 80]. Thus, when MLH1 and MLH2 are mutated, the MMR function is completely lost, and when MSH6 is mutated, the basal function of MMR is lost. However, mutated PMS2, MLH2, and MSH3 rarely affect MMR function [81]. HNPCC patients typically harbor germline mutations in MMR genes. MSI is a condition of genetic hypermutability (predisposition to mutation) that results from impaired DNA repair, the presence of which represents phenotypic evidence of MMR dysfunction [82]. Therefore, when the normal allele in the targeted organ undergoes a somatic mutation or deletion, the associated MMR protein will lose its function, and DNA repair cannot be completed. Subsequently, the wrong nucleotide insertion and/or deletion in the genome will gradually accumulate, and, in turn, increase the state of MSI, leading to MSI-H. MSI-H tumors are often accompanied by methylation of the *MLH1* promoter, resulting in epigenetic silencing of the gene and sustained loss of MMR protein. This lack of repair decreases the rate of apoptosis of cancer cells to increase their survival, which, in turn, is more likely to translate into the invasion and development of tissue-specific cancers (Figure 1C) [83].

Thus, MMR deficiency, a feature of advanced adenomas, is considered to reflect the early stage of transformation from adenoma to cancer. However, MMR deficiency has also been detected in a small subset of low-grade adenomas associated with HNPCC. Moreover, novel MMR deficiency can be identified in non-crypt foci with MSI, suggesting that in some adenomas, the development of MMR dysfunction follows the progression of MSI and that other HNPCC-associated adenomas may develop independently of MMR deficiency [84]. Furthermore, mutations in genes that cause loss of MMR function do not necessarily increase HNPCC susceptibility. For example, *BRAF* (V600E) is a common mutation that promotes the hypermethylation-induced silencing of the *MLH1* promoter and is also one of the hallmarks of sporadic CRC with MSI; however, *BRAF* mutations have neither been detected in HNPCC patients with *MLH1* and *MSH2* germline mutations, nor in tumors of *MSH6* germline mutations and MMR-negative families, indicating that *BRAF* is not involved in the process of HNPCC transformation into hereditary CRC [85].

2. FAP susceptibility genes and mechanism of action

FAP is a common autosomal dominant genetic disorder that typically manifests in adolescence and is classified as a hereditary CRC syndrome caused by endodermal mutations of APC genes [86]. FAP accounts for about 1% of all cases of CRC and the clinical manifestation is multiple adenomatous polyps in the large intestine [87]. Specifically, FAP is characterized by the appearance of hundreds to thousands of adenomatous polyps from late childhood or early adolescence, with most of the adenomas originating from dysplastic aberrant crypt foci, which are considered to be precursor lesions of adenomas or cancers [88]. FAP is also usually accompanied by some other symptoms such as inflammation, osteoma, epidermoid cyst, upper air-intestinal polyps, and congenital hypertrophy of the retinal pigment epithelium [89]. Without intervention, FAP will inevitably transform into CRC after the age of 35 years [90]. Therefore, improving the early diagnosis rate of FAP is of great significance for clinical decision-making, prognosis, and outcome.

FAP can be generally divided into two subcategories according to the number of colorectal adenomas or polyps and the onset age: classical FAP (CFAP) and attenuated FAP (AFAP) [91, 92]. The diagnosis of CFAP is usually based on the presence of more than 100 colorectal adenomatous polyps with an earlier age of onset than polyposis and CRC, whereas AFAP is characterized by 10-100 colorectal adenomatous polyps with an age of onset later than polyposis and CRC. FAP also includes a special subclass of recessive dominant disorders that are mainly caused by mutations in *MUTYH*, known as MUTYH-associated FAP (MFAP). Patients with MFAP generally have greater than 20 colorectal adenomatous polyps, an increased incidence of extraintestinal malignancies, and, rarely, exhibit sebaceous gland tumors, with an age of onset concentrated at 40−70 [93].

2.1 FAP-associated susceptibility genes

APC is one of the currently recognized FAP susceptibility genes, which is located on chromosome 5q21 and encodes a 2843-amino acid protein [94-96]. APC is an important component protein in the Wnt signaling pathway (Figure 2A). FAP-associated *APC* gene mutations are dominated by germline mutations, including small deletions, nonsense or frameshift mutations, and genomic rearrangements caused by splice-site mutations and intron deletions, followed by the formation of truncated APC proteins and loss of APC function, ultimately leading to the development of FAP [97, 98]. A mutation cluster region (MCR) in exon 15 of *APC* [99], covering mainly the β-catenin-binding functional domain of APC, has been identified as a site with higher mutation rates, including gene deletions occurring in c.39273931delAAAGA and c.31833187delACAAA, and site mutations occurring in exon 15, c.1744G>A, or c.1744G>T [97, 100] (Figure 2A). Besides, according to a study by Kojima et al., *APC* and *STK11* mutations found in duodenal adenomas/adenocarcinomas highlight the importance of proteins encoded by these genes in tumor development [75]. Mutations in *MUTYH* have also been associated with FAP in special cases of recessive genetic FAP [101]. *MUTYH*, located on chromosome 1p32, is commonly found as biallelic mutations and is less commonly associated with genomic deletion or duplication. The main mutation sites are located in exon 7 (c.536A>G) and exon 13 (c.1187G>A) [98, 102].

The role of mutations in the clip excision repair gene Nth-like DNA glycosylase 1 (*NTHL1*) in hereditary CRC diseases has attracted increasing attention in recent years. Weren et al. [72] reported three families carrying the *NTHL1* c.268C>T mutation, in which all affected members presented the phenotype of a base excision repair-associated adenomatous polyposis and CRC inherited in a recessive manner. Using whole-genome sequencing or other validated methods, *POLE* and *POLD1* mutations were suggested to affect the occurrence of inherited polymerase proofreading-associated polyposis or even CRC [74, 103-105], and hot

mutation sites such as c.1270C>G in *POLE* or c.1433G>A in *POLD1* were found. *CD36* encodes a scavenger receptor involved in fatty acid processing in the intestine. A recent study found a substantially lower age of polyposis diagnosis for patients in the severe FAP group and higher age for patients in the attenuated FAP group with *CD36* rs1761667 and rs1984112 SNPs, respectively [106]. However, whether different *CD36* mutations have distinct consequences on FAP need to be further investigated.

In addition, some rare cases of dominant polyposis have also been associated with genetic mutations; for example, mutations in *SMAD4* and *BMPR1A* were detected in juvenile polyposis patients [106-108]. Moreover, mutations in *LKB1/SKT11* were associated with Peutz-Jeghers syndrome; *GREM1* mutations were detected in mixed-type (serrated type and juvenile type) polyposis patients [109]; and Cowden/PTEN hamartoma syndrome was associated with mutations at the G129E site of *PTEN* [110].

Besides, exome sequencing by Adam et al. in 102 unrelated individuals with unexplained adenomatous colorectal polyposis identified biallelic *MSH3* germline mutations as a recessive subtype of colorectal adenomatous polyposis [70]. However, elucidating the mechanisms by which these genes increase the susceptibility to CRC requires more research and data support.

2.2 Mechanism of FAP susceptibility genes

Loss of APC protein function is currently considered to be the key factor in increasing FAP susceptibility [94]. Wild-type APC protein and glycogen synthesis kinase 3β (GSK-3β) combine with axin to form a so-called "destruction complex", promoting β-catenin degradation and preventing its accumulation in the cytoplasm [111, 112]. Upon mutations of *APC*, the Wnt signaling pathway is activated, and β-catenin is transferred to the nucleus and activates the transcription factor TCF/LEF, which
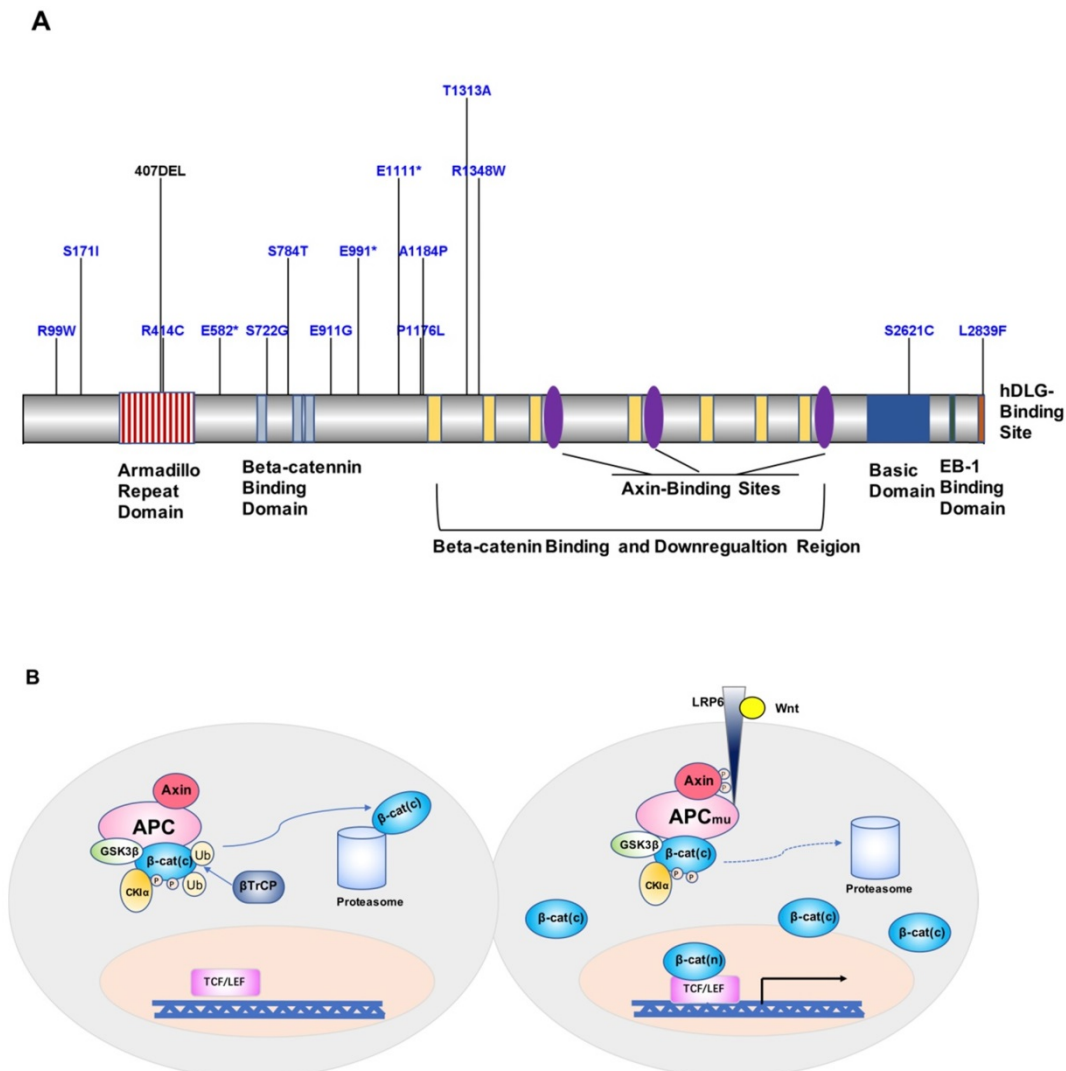

Figure 2. A. Map of APC protein mutation sites. B. Model of the contribution of the *APC* gene to FAP development and progression.

promotes oncogene expression [62]. In addition, APC protein binds to the cellular structure microfilament proteins F-actin and tubulin, which affects intercellular adhesion and plays a tumor-suppressor role in digestive organs such as the stomach, colon, and pancreas [113, 114]. Moreover, *APC* mutations result in increased chromosomal instability, causing reduced fidelity of segregated chromosomes and increased chromosome number (aneuploidy) imbalance or loss of heterozygosity rates, thereby further exacerbating mutations in *APC* (Figure 2B). According to Knudson's two-hit hypothesis, additional somatic *APC* mutations will occur in the diseased tissue and further promote tumor formation [115]. Indeed, Wang et al. [112] found that in FAP patients of a Chinese family, the c.1744-1G>A site mutation in *APC* occurred at the wild-type splice acceptor site at the boundary of exons 14 and 15, resulting in aberrant splicing of the gene to form a differential spliceosome, thus leading to the disease phenotype.

In contrast to the pathogenic mechanism of *APC* mutation, *MUTYH* mutations mainly cause MUTYH-FAP susceptibility by affecting the base repair mechanism [116]. *MUTYH* encodes a 535-amino acid protein and is involved in base-repair mechanisms by binding to MSH2/MSH6 to form covalent intermediates with DNA substrates, recognizing dA:dG and dA:dC mismatches generated during DNA replication, so as to clear erroneous adenines from newly generated daughter strands [117]. When *MUTYH* is mutated in both alleles, its glucokinase activity is lost. Experiments with *Escherichia coli* showed that mutation of Y82C (hY165C) severely impaired the catalytic activity of the MUTYH glycokinase, resulting in decreased mismatch specificity and adenine flip ability. Further, mutation of G253D (hG382D) reduced the rate of adenine removal catalyzed by MUTYH protein by 85%, along with the level of *MUTYH* expression, thereby exacerbating the loss of MUTYH function, leading to inability of repairing incorrect bases and contributing to recessively inherited FAP [118].

3. Genetic testing of hereditary CRC susceptibility genes

There are five main genetic methods available for the detection of hereditary CRC susceptibility genes: (1) judgment according to the clinical history, (2) clinical prediction models, (3) direct sequencing, (4) MSI or MMR immunohistochemistry (IHC), and (5) high-throughput sequencing. The main advantage of focusing on clinical history is that it is an inexpensive approach that can be used for individuals with or without a personal history of cancer, while the

disadvantages are its poor sensitivity and specificity, inconsistent use, and difficulty in applying complex standards [119]. The use of clinical prediction models (such as PREMM5) is a more cost-effective method that can also be used for individuals with or without a personal history of cancer, and the model does not take into account MSI/MMR IHC results; however, the main disadvantage of this approach is that the model performance for the group of patients unaffected by cancer is unclear [120]. Direct sequencing is the most accurate and direct mutation detection method, which mainly involves the use of traditional Sanger sequencing. This method is of relatively low cost, but the workload is large, making it difficult to process large amounts of data. Therefore, this method is mostly suitable in validation studies of a small number of genes [53]. Universal testing with MSI and/or MMR IHC has the main advantage of cost-effectiveness and high sensitivity for HNPCC screening, although the disadvantage is that it cannot be used for individuals without a cancer history, and the results are susceptible to individual effects of non-HNPCC forms of MSI-H/MMR-D; for example, MSH6 levels are artificially reduced in patients with rectal cancer receiving chemotherapy, which would bias the MMR immunohistochemistry results [119, 121]. Finally, high-throughput sequencing technology is suitable for patients with large errors in MSI and/or MMR immunohistochemistry [122] and can be targeted according to the specific research need by selecting the whole genome or key genomic regions for sequencing analysis. This technology can generate a large amount of data in each region of interest with high accuracy, providing great convenience for mutation identification. For example, the combination of exome profiling with high-throughput sequencing has proven to be the most rapid and effective method for identifying the genetic causes of inherited diseases [123].

With the development of next-generation sequencing technology, large-scale series of products can now reduce the cost of sequencing and experimental reactions by simplifying the sample pool, which can improve the ability of differential information capture and analysis through specific targeted region design; this approach has also been shown to specifically improve the efficiency and accuracy of the detection of hereditary CRC susceptibility genes [124-126]. Moreover, combining high-throughput sequencing technology with other new types of gene test services such as RNA sequencing, multi-gene sequencing panels, and epigenetic high-throughput sequencing will provide great convenience and more options for clinical practice and fundamental scientific research [123, 127,

128]. In practical applications, the most appropriate detection method should be selected according to the requirements of test time, sample characteristics, and the medical cost tolerance of patients.

4. Genetic counseling and fertility guidance for hereditary CRC

Familial CRC is characterized by late onset and rapid progression. Thus, the lesion is typically not detected early enough with traditional surgical detection methods. Accordingly, genetic testing is helpful for the early screening and assessment of cancer risk in patients and their families, facilitating early diagnosis and treatment, allowing the clinicians to provide timely guidance for fertility, and ultimately reducing cancer incidence and mortality [129, 130]. Given the wide variety of germline mutations in CRC patients as reviewed above, many of which lack obvious clinical features of genetic risk, it is recommended that all individuals diagnosed with CRC (especially those diagnosed before the age of 50) should undergo multigene panel testing [120]. In particular, genetic counseling and testing should be performed in HNPCC or FAP probands and their relatives.

Newly diagnosed HNPCC patients can undergo immunohistochemical and MSI tests for the presence of MLH1, MSH2, MSH6, and PMS2 proteins. Approximately 15% of sporadic CRCs also show MSI resulting from hypermethylation of the *MLH1* promoter [131]. The *BRAF* (V600E) mutation is also quite common in sporadic MSI-H tumors (63.5%) [132], but is rare in HNPCC. Therefore, *MLH1* hypermethylation and *BRAF* mutation analysis can be used to distinguish HNPCC-associated CRC from sporadic CRC [133]. Patients with *MLH1*-deleted HNPCC should be further tested for *BRAF* mutation status along with assessment of *MLH1* promoter methylation levels. For HNPCC patients with a history of tumors, genetic evaluation should be performed when there is evidence of MMR defects, to confirm the lack of *BRH* mutation or *MLH1* hypermethylation.

Patients with classical FAP and mild-phenotype FAP should firstly be tested for mutations of *APC*, and their children should undergo preventive genetic testing as of the age of 10 years. In FAP patients with a recessive mode of inheritance, if there is no *APC* mutation, the entire *MUTYH* needs to be tested. The currently emerging family multigene panel test based on next-generation sequencing technology includes many cancer susceptibility genes and different cancer risk profiles, which is practical for HNPCC probands who do not meet the Amsterdam criteria or Bethesda guidelines and for patients with other specific syndromes (such as germline breast cancer).

In cases in which the pathogenesis can be identified, we recommend that genetic counseling be performed for both the patients and their families. Moreover, fertility guidance can be offered as needed during the counseling. For family members carrying the same pathogenic genes, the risk of cancer should be assessed, so that early intervention can be initiated as required. Genetic counseling is also recommended for patients with FAP or HNPCC without identified pathogenic genes because the family members of these patients will also be susceptible to the disease. With the continuing advance of techniques and the deepening study into the etiology of FAP or HNPCC, new potential causes and mechanisms are also expected to be discovered in the future.

Summary and perspectives

The defect in one of the susceptibility genes of HNPCC and FAP is one of the root causes of hereditary CRC. Therefore, testing for HNPCC and FAP susceptibility gene mutations is of great significance for the diagnosis, prognosis, and timely intervention of familial CRC patients and their high-risk relatives. At the same time, identification of these genetic defects can be used as targets for individualized treatment. For example, regular aspirin use was shown to significantly reduce the incidence of HNPCC and colon cancer [134]. In FAP patients with *APC* germline mutations, use of the non-steroidal anti-inflammatory drug sulindac and the COX inhibitor celecoxib reduced the adenoma burden in the rectal mucosa preserved in patients after colectomy [135]. Therefore, further study of HNPCC and FAP susceptibility gene defects will surely open up new directions for the treatment of hereditary CRC.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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