Colorectal Cancer

Colorectal Cancer

Colorectal cancer (CRC) is a malignancy caused by uncontrolled division of abnormal cells in the colon or rectum. In the U.S., colorectal cancer accounts for 8.2% (136,830) of new cancer cases and 8.6% (50,310) of cancer-related deaths [1]. The risk of developing CRC over an individual’s lifetime is approximately 4.7% [2].

While most colorectal cancer cases arise sporadically, a family history of the disease is present in a quarter of the patients and about 5% of cases are associated with highly penetrant, inherited conditions, such as Lynch syndrome and familial adenomatous polyposis (FAP) [3, 4] (Figure 1).

![Distribution of colorectal cancer cases between sporadic, familial and inherited cancer syndromes including Lynch syndrome](image)

**Figure 1:** Distribution of colorectal cancer cases between sporadic, familial and inherited cancer syndromes including Lynch syndrome [3, 4].

Other less common inheritable cancer syndromes include attenuated FAP (AFAP), hereditary diffuse gastric cancer (HDGC), juvenile polyposis syndrome (JPS), Li-Fraumeni syndrome (LFS), **MUTYH**-associated polyposis (MAP), Peutz-Jeghers syndrome (PJS), **PTEN** hamartoma tumor syndrome (PHTS). The cause of these cancer syndromes can be partially defined by germline variants in a small set of genes required for proper cell proliferation [4, 5] (Table 1).
### Table 1: List of genes associated with colorectal and other cancer syndromes

<table>
<thead>
<tr>
<th>Colorectal and Other Cancer Syndromes</th>
<th>Associated Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated Familial Adenomatous Polyposis (AFAP)</td>
<td>APC</td>
</tr>
<tr>
<td>Familial Adenomatous Polyposis (FAP)</td>
<td>APC</td>
</tr>
<tr>
<td>Hereditary Diffuse Gastric Cancer (HDGC)</td>
<td>CDH1</td>
</tr>
<tr>
<td>Juvenile Polyposis Syndrome (JPS)</td>
<td>SMAD4, BMPR1A</td>
</tr>
<tr>
<td>Li-Fraumeni Syndrome (LFS)</td>
<td>TP53</td>
</tr>
<tr>
<td>Lynch Syndrome</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
</tr>
<tr>
<td>MUTYH Associated Polypsis (MAP)</td>
<td>MUTYH</td>
</tr>
<tr>
<td>Peutz-Jeghers Syndrome (PJS)</td>
<td>STK11</td>
</tr>
<tr>
<td>PTEN Hamartoma Tumor Syndrome (PHTS)</td>
<td>PTEN</td>
</tr>
<tr>
<td>CHEK2 Associated Cancer Risk</td>
<td>CHEK2</td>
</tr>
</tbody>
</table>

1. **Lynch Syndrome**

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant cancer syndrome that accounts for 2-4% of all colorectal cancer cases, with a population incidence of ~1 in 400-500 [3, 6-11]. Lynch syndrome also accounts for 2-5% of all diagnosed endometrial cancer cases [8, 12-14]. In the U.S., endometrial cancer accounts for approximately 3.2% (52,630) of new cancer cases and about 1.5% (8,590) of cancer-related deaths [3]. In addition, Lynch syndrome increases the risk for other malignancies including ovarian, stomach/gastric, small bowel, hepatobiliary, pancreatic, brain, urothelial and sebaceous skin cancer [6, 10, 15, 16] (Figure 2). Recent literature also suggests an increased incidence of breast and prostate cancer among individuals with Lynch syndrome [17-22].

The average age of onset for colorectal cancer in Lynch syndrome is 61 years, for endometrial cancer it is 46-62 years, while for ovarian cancer it is ~42 years [9]. In addition, tumors typically have a high level of microsatellite instability (MSI-H) [3, 18], an indication of functional defects in the mismatch repair (MMR) machinery responsible for correcting DNA replication errors [3].
Lynch syndrome is typically caused by germline variants in one of the four DNA mismatch repair (MMR) genes, namely, MLH1, MSH2, MSH6 and PMS2. Variants in MLH1 and MSH2 account for 80-90% of identified variants associated with Lynch syndrome, while variants in MSH6 and PMS2 account for approximately 10% and less than 5% of the variants respectively. In addition, 1-3% of individuals with Lynch syndrome have germline deletions in other genes. (Figure 3). A deficiency in the MMR machinery can lead to microsatellite instability and increased mutability of oncogenes and tumor suppressor genes.

Figure 2: Lifetime cancer risk by age 70 in individuals with Lynch syndrome, compared to the general population.

Figure 3: Distribution of identified variants within Lynch syndrome causing genes. The risk of cancer may vary depending on the mutated gene.
2. Familial Adenomatous Polyposis (FAP) and Attenuated FAP

FAP is an autosomal dominant cancer syndrome and contributes to approximately 0.5% of diagnosed CRC cases [3, 26]. The classic form of FAP is characterized by the presence of hundreds to thousands of adenomatous polyps (adenomas) in the colon and rectum, beginning in early childhood [3].

FAP is caused by germline variants in the \textit{APC} gene, which encodes a protein important in cell proliferation inhibition and negative regulation of the Wnt signaling pathway [26, 27]. Individuals with FAP who carry an \textit{APC} pathogenic variant have a 95% risk of developing colon cancer and 12% risk of small intestine cancer [2, 3] (Figure 4).

![Figure 4: Lifetime colon and small intestine cancer risk in individuals with FAP, compared to the general population [2, 3].](image)

Without colectomy, colon cancer is inevitable as almost all untreated classic FAP patients develop CRC by age 50. Polyps and cancer development can also be found in the duodenum/small intestine, the periampullary region and the stomach. FAP patients also have increased risk of malignancies in other organs, including the pancreas, thyroid, liver and brain. Other clinical findings of FAP include osteoma, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium (CHRPE) and desmoid tumors.

In the attenuated form of FAP (AFAP), fewer than 100 colorectal adenomatous polyps are present and diagnosis of cancer is, on average, 15 years later than in classic FAP cases. The rate of identifying pathogenic variants in \textit{APC} is lower in patients whose clinical findings are suggestive of AFAP [26, 28, 29].
3. Hereditary Diffuse Gastric Cancer (HDGC)

HDGC is an autosomal dominant cancer syndrome associated with increased risk of diffuse-type gastric cancer as well as lobular breast cancer [30, 31].

HDGC is caused by germline variants in the CDH1 gene, which encodes the E-cadherin protein crucial in calcium-dependent cell-cell adhesion [32]. Individuals who are heterozygous for pathogenic variants in the CDH1 gene have a cumulative risk of diffuse gastric cancer of 70% (95% CI, 23%-68%) in men and 56% (95% CI, 44%-69%) in women, with a mean age at diagnosis of 40 years [30, 33, 34]. In addition, women have a cumulative risk of lobular breast cancer of 42% (95% CI, 23%-68%) [30, 33, 35, 36].

![Figure 5: Lifetime cancer risk by age 80 in individuals with HDGC, compared to the general population [2, 33].](image)

Decreased expression in CDH1 gene or reduced CDH1 function may also lead to an increased risk of colorectal cancer, therefore, enhanced surveillance is recommended for individuals with germline variants in CDH1 and a family history of colorectal cancer [30, 34].

4. Juvenile Polyposis Syndrome (JPS)

JPS is an autosomal dominant disorder characterized by benign hamartomatous polyps in the gastrointestinal (GI) tract, specifically in the stomach, small intestine, colon, and rectum [37]. The “juvenile” in JPS refers to the distinctive morphology of the polyps and not to the age of the patient, since JPS is diagnosed in adults as well as in children. Juvenile polyps are mostly benign; however, if left untreated they may cause bleeding and malignant transformation resulting in CRC [37].

Patients with JPS have an increased risk of colorectal and gastrointestinal cancers [3, 38, 39] (Figure 6).

![Figure 6: Lifetime cancer risk in individuals with JPS, compared to the general population [2, 3, 38, 39].](image)
Approximately 75% of individuals with JPS have an identifiable germline variant [37, 40]. Germline variants in SMAD4 and BMPR1A account for approximately 40% of cases of JPS [40]. BMPR1A and SMAD4 genes encode common core intracellular components of the TGF-beta/BMP pathways [40]. TGF-beta/BMP signaling pathways control numerous biological processes, including growth inhibition, cell migration, invasion, epithelial-mesenchymal transition, extracellular matrix remodeling and immune suppression [41, 42].

5. Li-Fraumeni Syndrome (LFS)
LFS and its variant, Li-Fraumeni-like (LFL) syndrome are autosomal dominant disorders characterized by predisposition to multiple early onset cancers [43]. LFS has high variability in penetrance, age of cancer onset and tumor spectrum [44]. LFL syndrome is associated with incomplete LFS features [45]. The most common cancers associated with LFS are sarcoma, breast cancer, brain tumors and adrenocortical carcinoma. Other cancers include leukemia, choroid plexus papilloma, Wilms tumors, and gastric, colorectal and pancreatic cancer [46].

LFS and LFL are caused by germline variants in the TP53 gene. TP53 variants are the most frequently acquired genetic alteration in human cancers, with greater than 35,000 mutations described in different types of cancer [47]. Individuals with LFS who carry a TP53 pathogenic germline variant have a significant increased lifetime risk of developing cancer [48] (Figure 7) and are usually somatic alterations that are acquired within the cancer itself.

TP53 encodes the p53 transcription factor required for cellular responses to environmental and genotoxic stress [49]. The MDM2 protein, encoded by the MDM2 gene, modulates p53 protein activity [50, 51]. A genetic variant of MDM2 has been associated with an earlier age of cancer onset in patients with Li-Fraumeni syndrome [52-57].

6. MUTYH-Associated Polyposis (MAP)
MAP is an autosomal recessive inherited syndrome characterized by the presence of adenomatous polyposis of the colorectum [58, 59]. MAP is the most important differential diagnosis of APC-associated FAP. The disease phenotype of MAP is relatively mild and mimics AFAP in most cases. Individuals with MAP predominantly have adenomatous polyps, however unlike AFAP, hyperplastic polyps are common [3].

MAP is caused by germline variants in the MUTYH gene, which encodes a DNA repair protein [58-60]. Individuals carrying germline variants in both copies in the MUTYH gene have an increased risk of developing colon and small intestine cancer [58-61] (Figure 8), while those who carry one copy of MUTYH variants and a family history of CRC may also have an increased risk of colorectal, gastric, endometrial and liver cancers [62].

**Figure 7:** Lifetime cancer risk in individuals with LFS, compared to the general population [2, 48].
20-80% of individuals with MAP are diagnosed with CRC between 50 and 80 years of age. CRC was present in half of MAP patients at the time of polyposis diagnosis, though MUTYH variants have also been reported in individuals with early onset CRC with few to no polyps [3, 59].

7. Peutz-Jeghers Syndrome (PJS)

PJS is an autosomal dominant inherited disorder characterized by hamartomatous polyps in the GI tract, pigmented mucocutaneous lesions and cancer predisposition. The hamartomatous polyps of PJS are most common in the small intestine but can also occur in the stomach, large bowel and extraintestinal sites [66]. Gastrointestinal polyps can lead to chronic bleeding resulting in anemia and increased risk of malignant transformation [66].

PJS patients have increased risks for various malignancies, including breast, colon, pancreatic, gastric/stomach, gynecologic, and lung cancers, as well as tumors of the testes [66-68] (Figure 9). The risks among PJS patients for developing any first cancer by ages 20, 30, 40, 50, 60 and 70 years are 2%, 5%, 17%, 31%, 60% and 85%, respectively [69]. In PJS, malignancies appear at an average age of 42 years, an earlier onset compared to the general population [68].

**Figure 8:** Lifetime cancer risk in individuals with MAP, compared to the general population [2, 3, 63-65].

**Figure 9:** Lifetime cancer risk in individuals with PJS, compared to the general population [2, 66-68].
PJS is associated with germline variants in the tumor suppressor gene **STK11**, which encodes a kinase involved in the regulation of metabolism, cell differentiation, proliferation, polarity and apoptosis\[70-72\]. Loss of kinase activity is likely to be responsible for the development of PJS \[73\].

8. **PTEN Hamartoma Tumor Syndrome (PHTS)**

PHTS is a collection of rare autosomal dominant disorders involving disorganized growth of benign tumors called hamartomas in multiple organ systems \[74, 75\]. PHTS disorders include adult Cowden syndrome (CS), pediatric Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS) and Proteus-like syndrome \[75\].

PHTS is associated with deleterious germline variants in the **PTEN** gene, which encodes a phosphatase important in regulating cell growth and survival \[74-76\]. Individuals carrying a deleterious variant in the **PTEN** gene have an increased lifetime risk of breast cancer, thyroid cancer, endometrial cancer, colorectal cancer, kidney cancer and melanoma \[77, 78\] (Figure 10).

![General population risk vs. PHTS risk](image.png)

**Figure 10**: Lifetime cancer risk in individuals with PHTS, compared to the general population \[2, 77, 78\].

CS is a multiple hamartoma syndrome with a high risk of benign and malignant tumors of multiple organ systems. Up to 85% of CS individuals carry a pathogenic **PTEN** variant \[75\]. BRRS is a congenital syndrome characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glans penis. Up to 65% of BRRS individuals carry a pathogenic **PTEN** variant \[75, 79\].

9. **Other cancer susceptibility genes**

While many of the cancer susceptibility genes are associated with known cancer syndromes, the association is less well-defined for other genes. For example, pathogenic variants in **CHEK2** (checkpoint kinase 2) gene are reported to increase the susceptibility of developing breast cancer \[80-82\], prostate cancer \[83-85\] and colorectal cancer \[84, 86\]. However, the cancer risk estimates and the clinical diagnosis for individuals with **CHEK2** variants are not well-established. The **CHEK2** gene encodes a checkpoint protein kinase important for maintaining genome integrity \[87\]. The **CHEK2** protein interacts with other cancer susceptibility gene products such as the p53 and BRCA1 tumor suppressor proteins \[88, 89\].
Potential Indications for COLOTRUE® Test

The National Comprehensive Cancer Network (NCCN) lists the following criteria to select patients for colorectal cancer testing:

- Meet revised Bethesda guidelines or Amsterdam criteria
- >10 adenomas in the same individual
- Individuals from a family with a known high-risk syndrome associated with CRC
- Individuals with multiple GI hamartomatous polyps

COLOTRUE® Test Technology

COLOTRUE® analyzes the coding and flanking regions (+/- 20 bp) of 13 genes associated with hereditary colorectal cancer (APC, BMPR1A, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53) by next-generation sequencing-based (NGS) and Sanger confirmation of reported gene variants. Additionally, a site-specific analysis is performed to test for the variant defined as SNP309T>G in the MDM2 gene (a TP53 modifier). Sanger fill-in is used for all genes in areas of low coverage, which is defined as a read depth of less than 25x.

Possible Outcomes

Pathway Genomics' classifies variants using a 5-tier system based on the American College of Medical Genetics (ACMG) guidelines. According to this system, variants are classified as “Pathogenic”, “Likely Pathogenic”, “Uncertain Pathogenicity (VUS)”, “Likely Benign” or “Benign”. Pathogenic, Likely Pathogenic and Uncertain Pathogenicity (VUS) are always reported in the COLOTRUE® test. Likely Benign and Benign variants are not reported.

- **Pathogenic**: Variants with known clinical significance and demonstrated to increase the risk of cancer.
- **Likely Pathogenic**: Genetic changes that have some preliminary clinical data suggesting an association with cancer but not sufficient to make a definitive determination of pathogenicity and associated cancer risk.
- **Uncertain Pathogenicity (VUS)**: Genetic changes which may have deleterious effects on protein structure and/or function but which have either no supporting evidence or the data is conflicting, thus a determination of pathogenicity cannot be made.
- **Likely Benign**: Likely benign variants are genetic changes with strong but limited evidence to be classified as benign and are not likely to increase the risk for cancer.
- **Benign**: Benign variants are genetic changes that are previously reported and have sufficient evidence to be classified as benign with no clinical relevance.

Consultation with a health care professional who has training and experience in cancer genetics is highly recommended. Cancer surveillance and prevention options should take into account the patient’s age, personal risk factors, and personal and family health history.
Limitations and Warnings
The etiology of cancer is multifactorial, that is, cancer can occur as a result of various factors, including both inherited and acquired genetic variants, diet, life style choices and age. Pathway’s genetic test for colorectal cancer evaluates inherited genetic variants in thirteen genes (APC, BMPR1A, CDH1, CHEK2, MDM2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11 and TP53) and SNP309T>G in the MDM2 gene. This test does not detect gross genetic rearrangements including most duplications, inversions, or deletions. Gross genetic rearrangements are estimated to account for at least 2-10% of mutations\textsuperscript{[80]}. It is possible that variants in genes and genetic regions not tested in Pathway’s DNA sequencing test may contribute to an individual’s risk for cancer. Therefore, a negative test result where no pathogenic variants are detected does not eliminate the individual’s cancer risk. Further, a positive test result does not guarantee an occurrence of cancer, since the variants in these genes are not 100% penetrant. Rather, pathogenic variants predispose a person to a higher risk of cancer. The results of the test must be interpreted in the context of the individual’s clinical history. Genetic counseling is recommended for the individual and for other at risk family members\textsuperscript{[9]}. Pathway Genomics Corporation strives to provide the most accurate test results. As part of this mission we constantly monitor and refine our data analysis algorithms. If and when an improvement is identified, it is our policy to conduct a “look back” process of previously analyzed patient data. Such re-analysis of patient data may lead to reclassification of patient results. In that event, a corrected report will be issued.
References


