Characterization of candidate genes in unexplained polyposis and colorectal cancer

Abayzeed Elsayed Osman, F.

Chapter 1

General introduction and outline of this thesis

General introduction

Colorectal cancer

Colorectal cancer (CRC; MIM 114500) is the third most commonly diagnosed cancer and the second leading cause of cancer death worldwide [1-3], with almost 1.9 million new cases and 1 million deaths in 2020. CRC accounts for 10% of all cancer diagnoses and 9.4% of all cancer deaths [2, 4, 5]. CRC results from the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma [6]. In the progression from colorectal adenoma to carcinoma, three major pathways are distinguished: chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) [7]. CIN is the most common type of genomic instability observed in CRC and occurs in 80%-85% of colorectal tumors [6]. While the majority of CRCs occur sporadically, an estimated 35% of CRCs are due to heritable factors [8, 9]. Between 5% and 10% of all CRC cases are associated with well-characterized hereditary polyposis and/or CRC syndromes [9]. The etiology of up to 30% of inherited CRCs is not completely understood, and the underlying genetic factors contributing to the risk of CRC remain undefined [10]. Genome-wide association studies (GWAS) have successfully identified common, low-penetrance single nucleotide polymorphisms (SNPs) associated with the risk of CRC [11-17]. In recent years, major efforts have been made to identify the genetic causes, as the identification of germline pathogenic variants substantially facilitates the clinical management of patients and their families.

Hereditary colorectal cancer syndromes

Hereditary CRC syndromes (Table 1), characterized by dramatic increases in the risk of colorectal neoplasia, are phenotypically divided into polyposis and nonpolyposis syndromes, based largely on the number and histology of the colorectal polyps. The polyposis syndromes can be further divided into adenomatous, hamartomatous, serrated and mixed polyposis syndromes according to the predominant type of polyps, e.g., adenomatous polyps, hamartomatous polyps or serrated polyps. Polyposis is defined by the constitutive development of multiple polyps in the colon and rectum. Polyps are benign outgrowths of tissue into the lumen of the colorectum, but they have the potential to evolve into an in situ carcinoma by the accumulation of additional somatic mutations [18]. This phenomenon is known as the adenoma-to-carcinoma sequence, and it is accepted that more than 95% of colorectal cancers arise from adenomas. Syndromic nonpolyposis CRC is subdivided on the basis of molecular tumor phenotype as DNA mismatch repair-deficient (MMRD) or mismatch

repair-proficient (MMRP) CRC [19-21]. The development of polyps in patients with a nonpolyposis CRC predisposition syndrome is rare, but these polyps evolve rapidly into carcinomas since the polyp-to-carcinoma sequence appears to be accelerated in these patients [22]. Several high-penetrance genes with inherited germline variants, such as *APC* (MIM 611731), *BMPR1A* (MIM 601299), *GREM1* (MIM 603054), *MLH1* (MIM 120436), *MSH2* (MIM 609309), *MSH3* (MIM 600887), *MSH6* (MIM 600678), *MUTYH* (MIM 604933), *NTHL1* (MIM 602656), *PMS2* (MIM 600259), *POLD1* (MIM 174761), *POLE* (MIM 174762), *PTEN* (MIM 601728), *RNF43* (MIM 612482), *SMAD4* (MIM 600993) and *STK11* (MIM 602216), are known to be associated with CRC syndromes [23].

Table 1. CRC predisposition syndromes

Syndrome	Genes	Pattern of inheritance	Prevalence
Lynch syndrome	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*	Dominant	2% - 4%
Familial adenomatous polyposis	*APC*	Dominant	< 1%
MUTYH-associated polyposis	*MUTYH*	Recessive	< 1%
Polymerase proofreading-associated polyposis	*POLE*, *POLD1*	Dominant	Unknown
NTHL1-associated polyposis	*NTHL1*	Recessive	Unknown
MSH3-associated polyposis	*MSH3*	Recessive	Unknown
Serrated polyposis syndrome	*RNF43*	Dominant	Unknown
Constitutional MMR deficiency syndrome	*MLH1*, *MSH2*, *MSH6*, *PMS2*	Recessive	Unknown
Hereditary mixed polyposis syndrome	*GREM1*	Dominant	Unknown
Juvenile polyposis	*SMAD4*, *BMPR1A*	Dominant	< 1%
Peutz-Jeghers Syndrome	*STK11*	Dominant	< 1%
PTEN hamartoma tumor syndrome	*PTEN*	Dominant	< 1%

Nonpolyposis syndromes

Lynch syndrome

Lynch syndrome (LS; MIM 120435), previously referred to as hereditary nonpolyposis colorectal cancer (HNPCC), is the most common cause of hereditary CRC, accounting for approximately 2% - 4% of all CRCs [10, 24, 25]. The lifetime CRC risk is estimated to be 50%-80% [10, 24]. This syndrome also predisposes patients to extracolonic cancers, such as cancers of the endometrium, small bowel, ureter and renal pelvis, stomach, hepatobiliary tract and ovary [26-32]. LS is inherited in an autosomal dominant pattern and is caused by germline pathogenic variants in one of the MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or 3' end deletion of the *EPCAM* gene, leading to transcriptional read-through into and subsequent epigenetic silencing of MSH2 [33-38]. For LS, the lifetime risk for CRC is highly variable and dependent on the gene involved. The risk for CRC-associated *MLH1* and *MSH2*

mutations is generally higher than the risks associated with mutations in the other LS-related genes [32, 39]. The MMR system consists of several proteins that repair DNA damage during replication and maintain genome stability mainly by correcting base-base and small insertion–deletion mismatches that are generated during DNA replication. MMR proteins function as heterodimers in two main complexes, MutS heterodimers (MSH2/MSH6 and MSH2/MSH3) and MutL heterodimers (MLH1/PMS2, MLH1/PMS1 and MLH1/MLH3). The MutS heterodimers recognize mismatches and small insertions/deletions (indels). The MutL heterodimers form a MutS/MutL/DNA complex for exonuclease activity and termination of mismatch-provoked excision [40, 41]. MMR defects lead to genomic instability and the accumulation of secondary mutations, resulting in a strong mutator phenotype. Mutations occur especially in simple repetitive DNA sequences and microsatellites, resulting in microsatellite instability (MSI). MSI is a hallmark of MMRD cancers and is found in > 90% of LS colorectal cancers [42-44]. In up to 15% of sporadic CRCs, MSI is caused by somatic hypermethylation of the *MLH1* promoter and associated silencing of *MLH1*. These patients frequently also exhibit specific mutations in *BRAF* (V600E) [32].

Familial colorectal cancer type X

In a fraction of families fulfilling the Amsterdam 1 criteria for HNPCC [45], CRCs are microsatellite stable and without MMR gene mutations. These families are defined as having familial colorectal cancer type X (FCCTX) [46, 47]. This heterogeneous group of families has an increased risk of developing CRC and other related tumors [48]. Although the clinical identification of FCCTX has improved in recent years, its genetic etiology remains unknown [47, 49]. Some genes, such as *BMPR1A* [50], *BRCA2* [51], *FAN1* [52], *OGG1* [53], *RPS20* [54], *SEMA4A* [55] and *SETD6* [56], have already been reported to be potentially associated with FCCXT. In addition, a review suggested a possible association with *BCR*, *BMP4*, *CENPE*, *CDH18*, *GABBR2*, *GALNT12*, *GREM1*, *HABP4*, *KIF24* and *ZNF367* [57]. Moreover, a review by Nejadtaghi et al. [58] identified *APC*, *BMPR1A*, *BRAF*, *BRCA2*, *KRAS*, *MGMT*, *RPS20*, *SEMA4A,* and hypermethylation of at least one gene of the MMR system as potentially related to FCCTX. Despite these studies, no defined set of genes is conclusively associated with FCCTX.

Polyposis syndromes

Familial adenomatous polyposis

Less than 1% of all CRCs occur due to familial adenomatous polyposis (FAP; MIM 175100). FAP represents the most common gastrointestinal polyposis syndrome

and the second most common cause of hereditary CRC [59, 60], with an estimated incidence varying from 1:8000 to 1:37600 [61]. FAP is an autosomal dominant precancerous condition characterized by the development of colorectal adenomas, which inevitably progress to colorectal carcinoma unless detected early [10]. In the classic form of FAP, patients develop hundreds to thousands of colorectal adenomas during adolescence or the third decade of life, and the lifetime risk of CRC is almost 100%. Attenuated FAP (AFAP) is a phenotypically distinct form of FAP in which patients have a milder manifestation than classic FAP. AFAP is characterized by fewer colorectal adenoma polyps (less than 100 polyps), a later age of adenoma development and a lower lifetime risk of CRC (70%) [62, 63]. FAP is caused by germline variants in the tumor suppressor gene *APC* [64-67]. *APC* is located on chromosome 5q21-q22 and consists of 15 exons encoding a protein of 2845 amino acids (310 kDa). *APC* plays a major role in the Wnt signaling pathway by negatively regulating the β-catenin oncoprotein [68-70]. Germline *APC* variants lead to the development of multiple adenomas as a result of inactivation of the remaining wild-type *APC* allele in the tumor, either through somatic mutations or through loss of heterozygosity of *APC* [59, 70, 71]. Correlations between the FAP phenotype and the site of mutation in the *APC* gene have been reported; patients with AFAP generally have a mutation in the 5′ or 3′ region of the *APC* gene, whereas individuals with FAP carry mutations elsewhere in this gene [72]. De novo variants are responsible for approximately 25% of FAP cases who lack a family history of the disease, and approximately 20% of these have somatic mosaicism [73-77].

MUTYH-associated polyposis

In 2002, Al Tassan et al. reported for the first time that inherited defects of the base excision repair gene *MUTYH* predispose patients to multiple colorectal adenomas and carcinoma [78], causing MUTYH-associated polyposis (MAP; MIM 608456) [78-81]. MAP is an autosomal recessive inherited syndrome caused by biallelic germline variants in the base excision repair gene *MUTYH*, characterized by a greatly increased lifetime risk of CRC (80%) [82] and accounting for less than 1% of CRC cases [60, 83]. An estimated 1 in every 20,000 European individuals have biallelic *MUTYH* variants [61]. *MUTYH* encodes a DNA glycosylase involved in oxidative DNA damage repair, is located on chromosome 1 between p32.1 and p34.3 and consists of 16 exons [84]. The enzyme excises adenine bases from the DNA backbone at sites where adenine is inappropriately paired with guanine, cytosine, or 8-oxo-7,8-dihydroguanine, a major oxidatively damaged DNA lesion [85-87]. Consequently, tumors from MAP patients with dysfunctional MUTYH protein display an excess of somatic mutations with a strong bias toward C:G > to A:T

transversions at NpCpA or NpCpT sites in multiple genes, including *APC* and *KRAS* [88-90]. A molecular hallmark of cancers caused by MAP is the presence of the somatic *KRAS* c.34G>T mutation [91, 92]. MAP patients show substantial variability in clinical features but usually present with an attenuated polyposis phenotype, showing fewer than 100 adenomas, although a few MAP patients with CRC without polyps have also been reported. The evidence that monoallelic variants confer an elevated CRC risk is somewhat controversial. In a large population-based series, biallelic *MUTYH* variant carriers showed a 28-fold increased risk for CRC, while monoallelic *MUTYH* variants were not associated with an increased CRC risk [93]. However, in other studies, a small increased risk for CRC was reported for *MUTYH* monoallelic variant carriers [94, 95]. Win et al. reported that the CRC risk for monoallelic variant carriers depends on family history and can be sufficiently high to warrant consideration of more intensive CRC screening than for the general population. CRC risk is higher for monoallelic carriers of Y179C than for G396D [96]. A previous study reported that biallelic *MUTYH* carriers have an increased risk of bladder and ovarian cancers, while *MUTYH* monoallelic carriers have an increased risk of gastric, liver, breast and endometrial cancers [97].

Polymerase proofreading-associated polyposis

Germline pathogenic variants affecting the exonuclease domain of *POLE* and *POLD1* predispose patients to multiple colorectal adenomas and carcinomas, causing so-called polymerase proofreading-associated polyposis (PPAP; MIM 615083, 612591) [98-102]. PPAP is an autosomal dominant disease with a high penetrance [98]. In addition to multiple adenomas and CRC, variant carriers also present with extra colonic cancers, such as endometrial, ovarian, brain, pancreatic, and small intestinal cancer and melanoma [103-106]. A recent study indicated that PPAP constitutes 0.1-0.4% of familial cancer cases, reaching 0.3-0.7% when only CRC and polyposis are considered [107]. Although the precise risk and mean age of CRC development are not clear, a study found patients with variants in *POLE* to have a 28% risk and patients with *POLD1* variants to have an 82% to 90% risk of CRC by the age of 70 years [108]. *POLE* and *POLD1* encode the catalytic subunits of DNA polymerases epsilon and delta, respectively. Polymerase epsilon and delta are involved in DNA replication of the leading and lagging strands and possess an accurate proofreading domain that removes incorrectly inserted nucleotides during DNA replication [109]. While the majority of CRCs from *POLE* or *POLD1* variant carriers are MMR proficient, a subset of CRCs in *POLE* variant carriers showed MMR deficiency without germline MMR gene variants [110]. De novo variants in *POLE* have been identified in several singletons [99], but the prevalence of de novo *POLE* variants remains to be determined. Tumors

from *POLE* and *POLD1* pathogenic variant carriers show an ultrahypermutated phenotype with the number of somatic mutations exceeding 100 mutations/Mb [111, 112]. *POLE* defects are associated with signature SBS10 and show an excess of C:G>A:T and C:G>T:A [113, 114]. Thus far, no clear signature has been described for *POLD1*-mutated CRCs.

NTHL1-associated tumor syndrome

In 2015, a rare recessive inherited form of polyposis and CRC syndrome that is caused by biallelic pathogenic variants in the base excision repair gene *NTHL1* was discovered [115]. After the discovery, several additional families from different ethnic groups with biallelic germline variants in *NTHL1* in a homozygous or compound heterozygous state were reported [116-122]. Different extracolonic malignancies were observed in individuals with biallelic germline *NTHL1* variants, including malignancies of the endometrium, breast and duodenum [115, 116, 119]. Based on the frequency of loss-of-function (LoF) variants in the publicly available database, the incidence of NTHL1 deficiency is estimated to be 1:114,770, approximately fivefold lower than the incidence of MAP (1:19,079) [61]. Endonuclease III-like protein 1, encoded by *NTHL1*, is a bifunctional glycosylase involved in base excision repair that recognizes and removes oxidized pyrimidines [123]. Tumors from biallelic *NTHL1* LoF variant carriers show a bias toward C>T transitions at non-CpG sites [115, 124] with a unique mutational signature referred to as signature SBS30 [124]. Signature 30 has previously been identified in one patient with breast cancer [125]. Retrospective analysis of tumor and germline sequencing data of this breast cancer patient revealed a heterozygous germline *NTHL1* variant with loss of heterozygosity in the tumor [124].

MSH3-associated polyposis

Another polyposis syndrome with a recessive inheritance pattern is referred to as MSH3-associated polyposis (MIM 617100) [126]. After whole-exome sequencing (WES) of leukocyte DNA from 102 unrelated individuals with unexplained adenomatous polyposis, two unrelated individuals with compound heterozygous LoF germline variants in *MSH3* were identified, suggesting that *MSH3* mutations represent an additional recessive subtype of colorectal adenomatous polyposis [126]. The tumors from the carriers demonstrated high microsatellite instability of di- and tetranucleotides (Elevated Microsatellite Alterations at Selected Tetranucleotide repeats (EMAST) [127]) and immunohistochemical loss of MSH3 in normal and tumor tissues [126]. The associated phenotype was characterized by the presence of colorectal and duodenal adenomas, CRC, gastric cancer and early-onset astrocytoma [126].

Constitutional MMR deficiency syndrome

Constitutional MMR deficiency (CMMRD; MIM 276300) syndrome is a rare autosomal recessive childhood cancer predisposition syndrome caused by biallelic pathogenic germline variants in one MMR gene (*MLH1*, *MSH2*, *MSH6* and *PMS2*). CMMRD is characterized by a high risk of developing a broad spectrum of malignancies during childhood and adolescence, including mainly T-cell non-Hodgkin lymphomas, high-grade gliomas and gastrointestinal tumors, mainly CRC tumors. Another characteristic of CMMRD is café-au-lait maculae (CALM) [128, 129]. Remarkably, a large proportion of CMMRD patients develop multiple synchronous adenomas ranging from a few up to > 100 polyps, mimicking attenuated familial adenomatous polyposis [130-132]. Polyps in CMMRD can also histologically resemble those in juvenile polyposis [131].

Serrated polyposis syndrome

Serrated polyposis syndrome (SPS; MIM 617108) was previously known as hyperplastic polyposis syndrome (HPS). SPS is characterized by the presence of multiple serrated polyps throughout the colon and rectum and is associated with an increased risk of CRC for affected individuals and their first-degree relatives [133-135]. The prevalence of SPS is estimated to be 1:2000 in the general population [134]. In 2014, Gala et al. reported the association between SPS and *RNF43* by identifying a novel germline variant in two individuals with multiple serrated polyps [136]. Subsequently, the role of *RNF43* germline variants as the cause of multiple serrated polyps was supported by several other studies [137-139]. The study by Yan et al. showed loss of the remaining wild-type allele from carriers through somatic mutations or loss of heterozygosity, adding the potential role of *RNF43* in the development of colonic serrated neoplasia [138]. Buchanan et al. proposed that mutations in *RNF43* might account for only a small proportion of SPS, and consequently, there is no need for routine germline testing of *RNF43* in individuals who meet the criteria for SPS [140].

Hereditary mixed polyposis syndrome

Hereditary mixed polyposis syndrome (HMPS1 MIM 601228) is a rare autosomal dominant disorder that is associated with an increased risk of CRC, characterized by polyps of multiple and mixed morphologies, including serrated lesions, Peutz–Jeghers polyps, juvenile polyps and conventional adenomas [141-144]. The genetic etiology for HMPS1 was first described in 2012, when a 40-kb duplication in the 5′ regulatory region of *GREM1* was identified as a causal mutation in families of Ashkenazi Jewish origin and was shown to lead to increased and ectopic expression of *GREM1* in the colonic mucosa [144]. Excess GREM1 protein

levels suppress bone morphogenetic protein [144], allowing epithelial cells to retain stem cell-like properties, form ectopic crypts and ultimately become neoplastic [145]. The 40-Kb duplication has been identified in 1:184 Ashkenazi Jewish individuals with a personal or familial history of polyposis or CRC [146]. In addition to the founder Ashkenazi duplication, several other *GREM1* variants were identified in families with polyposis and CRC [147-149].

Hamartomatous polyposis syndromes

Hamartomatous polyposis syndromes (HPSs) are a rare heterozygous group of disorders that are inherited in an autosomal-dominant manner and are characterized by the development of hamartomatous polyps of the gastrointestinal tract. Hamartomatous polyposis syndromes have malignant potential for the development of CRC as well as extracolonic cancers [63]. These conditions account for less than 1% of CRC cases and occur at approximately one-tenth of the frequency of adenomatous polyposis syndromes [150, 151]. The hamartomatous polyposis syndromes include juvenile polyposis syndrome (JPS), Peutz-Jegher's syndrome (PJS) and PTEN hamartoma tumor syndrome (PHTS).

Juvenile polyposis syndrome (JPS)

JPS is characterized by the development of multiple gastrointestinal polyps, the most common location of which is the colon (98%). Patients with JPS syndrome have a high risk of colon cancer, and there is also an increased risk of gastroduodenal cancer. Pathogenic germline variants in *SMAD4* or *BMPR1A* are found in approximately 20-60% of JPS patients [63].

Peutz–Jeghers syndrome (PJS)

PJS is caused by germline variants in *STK11* (previously known as *LKB1*) and is characterized by multiple characteristic hamartomatous polyps in the gastrointestinal tract associated with mucocutaneous pigmentation. Patients with PJS have an increased risk for CRC and extra colonic cancers [63].

PTEN hamartoma tumor syndrome (PHTS)

Germline variants in the tumor suppressor gene *PTEN* are responsible for a group of phenotypically diverse conditions, which have collectively been called PTEN hamartoma syndrome (PHTS) [63, 134, 152]. PHTS includes Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS), both of which are inherited in an autosomal dominant pattern [151, 153, 154]. CS is rarely identified before adulthood and is characterized by multiple developmentally disorganized benign growths,

or hamartomas, with an increased risk of both benign and malignant tumors [155]. Individuals with CS are at risk for developing breast, thyroid, endometrial, colon, skin and renal cancers [156]. BRRS patients show gastrointestinal hamartomatous polyps, lipomas, macrocephaly and developmental delay [152].

Missing heritable factors in CRC and polyposis

The exact contribution of heritable factors to CRC and polyposis is still not fully understood. Based on Nordic twin and family studies, it has been estimated that 12-35% of all CRCs are linked to genetic factors [8, 157]. Later, estimates for heritability of CRC decreased to approximately 15% of all CRC cases [158, 159]. The currently known high-penetrant Mendelian polyposis and/or CRC syndromes can only explain 5-10% of all CRC cases [8, 60, 160, 161]. In the case of polyposis, the genetic causes remain unexplained in approximately 20% of polyposis cases [162]. In approximately 60% of MMRD CRCs without somatic *MLH1* promoter hypermethylation, no underlying germline MMR variants are known. These patients are referred to as having suspected Lynch syndrome (sLS) or Lynch-like syndrome (LLS) [163]. Studies have shown that patients with double somatic MMR pathogenic variants can still have hereditary CRC caused by genes involved in DNA repair since they can lead to acquired pathogenic variants in the MMR genes [164-166]. The genetic background is unknown for 50-60% of hereditary nonpolyposis colorectal cancer (HNPCC) families who fulfil the Amsterdam criteria [45] but do not have a mutation in one of the MMR genes (MMRP), referred to as familial colorectal cancer type X (FCCTX) [167]. In addition to the identification of rare high-penetrant risk genes contributing to the heredity of CRC, it is estimated that common variants may explain approximately 12% of the relative risk for CRC [14, 16, 161, 168]. In more than approximately one-third of CRC patients with a suspected hereditary cause, the underlying genetic factors remain unexplained [157]. It is important to resolve this issue with heritability, and the identification of genetic factors has important implications for the carriers and their families, as it helps risk assessment, directs clinical management, and guides preventive and therapeutic options [10, 169].

Novel candidate genes for CRC and polyposis

Recently, different candidate genes have been identified but require further evidence to be implemented in routine genetic testing. New candidate genes have been proposed for predisposition to hereditary CRC and polyposis, such as *BUB1* [170], *BUB3* [170], *FAN1* [52], *LRP6* [171], *RPS20* [54], *FOCAD* [172], *PTPN12* [171], *GALTN12* [173, 174], *MIA3* [175] and the constitutional epigenetic silencing of *PTPRJ* [176]. Recently, *MCM8* was proposed for predisposition to CRC with a recessive pattern of

inheritance [177]. In a systematic review performed to validate the association between variants in *RPS20*, *FANCM*, *FAN1*, *TP53*, *BUB1*, *BUB3*, *LRP6* and *PTPN12* and the development of CRC, the evidence supports the association between variants in *RPS20* and CRC but not in the other candidate genes [178].

Outline of this thesis

The aim of this thesis is to study the underlying genetic causes of unexplained polyposis and CRC. In particular, the role of *POLE*, *POLD1*, *APC* and *NTHL1* in unexplained cases was studied.

Chapter 2 describes the assessment of the prevalence of *POLE* p.(Leu424Val) and *POLD1* p.(Ser478Asn) in a Dutch series of index patients with unexplained familial early onset CRC and polyposis. In this study, we analyzed phenotypes and tumor characteristics in *POLE* variant carriers. We proposed that MMR deficiency in the tumors from *POLE* p.(Leu424Val) carriers is due to secondary MMR somatic mutation resulting from the hypermutation phenotype caused by the *POLE* variants.

In **Chapter 3**, the sequencing of the exonuclease domains of *POLE* and *POLD1* in unexplained index patients with multiple colorectal polyps is described in search for novel germline variants in these genes.

Chapter 4 focuses on screening of *APC* for mosaic and deep intronic variants in unexplained colorectal polyposis patients to study their role as predisposing factors for polyposis and CRC in this cohort.

Chapter 5 shows the molecular and clinical characterization of the tumor spectrum of individuals with biallelic LoF germline variants in *NTHL1*. To establish the disease phenotype of individuals with NTHL1 deficiency, we identified individuals with biallelic LoF germline variants in *NTHL1* and performed mutational signature analysis on different tumor types from these individuals to determine the association between NTHL1 deficiency and tumor development.

In **Chapter 6,** the role of monoallelic LoF germline variants in *NTHL1* in the risk of polyposis and/or CRC is investigated. Finally, **Chapter 7** provides a general discussion of the thesis and future perspectives.

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