Genetics and tumor genomics in familial colorectal cancer

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Chapter 1

General Introduction

Colorectal cancer (CRC) is one of the most common cancers in the Western world.[1] In the Netherlands, each year around 11,000 patients are diagnosed with colorectal cancer.[2] The lifetime risk in the Netherlands for developing colorectal cancer is approximately 6%, with a five-year survival of about 55%.[1] The age distribution is wide; however, over half of the patients are diagnosed with colorectal cancer above 70 years of age.

The risk of CRC is influenced by both genetic and environmental factors. Although most colorectal cancer arises on a sporadic basis, in 10-30% of the cases inherited predisposition plays a role, as was estimated in twin studies.[3] First-degree relatives of CRC patients are at increased risk of developing colorectal cancer, with a relative risk of about 2.2. This risk is strongly correlated with the number of affected family members and an early onset of disease.[4] With two or more affected family members, the relative risk increases to about 4.0.[5,6] Individuals with a first-degree relative affected with colorectal adenomas are also at increased risk of CRC, with a relative risk of about 2.0.[6] The risk increases when the age at diagnosis of colorectal adenomas of the first-degree relative decreases.[7,8] About 6% of the CRC cases can be explained by several colorectal cancer syndromes, including Lynch syndrome, familial adenomatous polyposis (FAP), and *MUTYH*-associated polyposis (MAP). However, for the other familial cases, the underlying genetics remain elusive. High or moderate risk factors could play a role in families affected with colorectal cancer. Moreover, co-inheritance of several low risk factors could explain the excess risk in such cases.

Tumorigenesis

The development of colorectal cancer is a multistep process that involves somatic genetic and epigenetic changes. Several genes acquire a mutation that provides the cell with a growth advantage. The transcription of genes and microRNAs is dysregulated in cancers; tumor suppressor genes are shut down and oncogenes are activated.

Colorectal cancer generally develops from normal epithelium through different adenoma stages with increasing dysplasia into carcinoma.[9] A genetic model for the development of sporadic CRC was first postulated by Fearon and Vogelstein in 1990 (Figure 1).[10] They described the genetic changes of colorectal tumors along their development from adenoma to carcinoma and they assumed that – although the accumulation of mutations seems most important - the genetic changes occur in a specific order in most CRCs. Later, more genes and genetic aberrations were added to this model.[11,12]

Recent advances have provided more insight in the biology of the colonic crypts and their relation to tumorigenesis (reviewed by [13]). Colonic crypts are finger-like invaginations of the colonic epithelial layer in the underlying connective tissue of the lamina propria. At the base of each crypt stem cells are located that are capable of regenerating all intestinal cell types. When a stem cell acquires a mutation it can, through a selective advantage or genetic drift, fill

[1] http://www.ikcnet.nl

up the whole crypt. Subsequently, crypt fission can lead to spreading of the mutation in neighboring epithelium. Such aberrant crypt foci (ACF), consisting of a cluster of a small number of abnormal crypts, are thought to be an early step in the formation of adenomatous polyps.[13] Colorectal cancer development is often initiated by mutations in *APC*, which leads to an increased proliferation of the cell. *APC* mutations and/or loss of heterozygosity at the *APC* locus are found in over 80% of all colorectal cancers.[14-16] Mutations in *CTNNB1*, encoding β-catenin, have also been found to occur in CRC.[17] *KRAS* mutations are seen with progression of the adenoma. Later, in the progression from adenoma to carcinoma, additional mutations are acquired in for example *SMAD2* and *SMAD4*, and *p53*, often accompanied by loss of heterozygosity (LOH) of 17p and 18q.[11,18]
Recent studies show that the majority of mutations in a tumor occur in genes other than these commonly mutated genes, leading to a unique mutational signature for each tumor.[19,20] However, the role of these other mutations in tumorigenesis still needs to be elucidated.
The carcinogenesis model described above applies mainly to sporadic CRC. In hereditary syndromes, the carcinogenesis model is different depending on the germ-line defect.

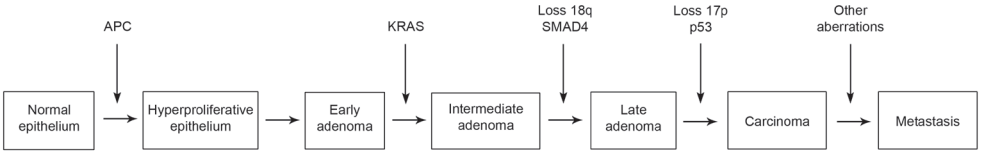

Figure 1. Genetic model of colorectal tumorigenesis as proposed by Fearon and Vogelstein [10]

Signaling Pathways

Several signaling pathways are involved in colorectal tumorigenesis. These pathways become dysregulated via mutational activation or inactivation of one of its proteins or via other ways of (in)activation like methylation.

Wnt signaling pathway
Activation of the Wnt signaling cascade is considered to be the initiating event in colorectal cancer. In the Wnt signaling cascade, β-catenin functions as a transcription factor upon binding to nuclear proteins of the Tcf family and regulates genes involved in cellular activation. APC is a key regulator of β-catenin because it regulates the levels of cytoplasmic β-catenin. APC forms a complex with GSK3β, axin, and β-catenin, that modulates the cytoplasmic β-catenin levels by degradation. Mutational inactivation of APC is observed in over 80% of the CRCs and leads to accumulation of β-catenin in the cytoplasm.[14-16] This accumulation leads to

dysregulation of the transcriptional targets of β-catenin/Tcf.[21] Fifty percent of the tumors that lack mutations in *APC*, display mutations in the β-catenin gene *CTNNB1*.[22] Examples of genes that are regulated by β-catenin/Tcf include cell cycle regulator *c-MYC*, G1/S-regulating *cyclin D1*, and *MMP-7* (matrilysin), a matrix-degrading metalloproteinase.[23-25] Germ-line mutations in the *APC* gene give rise to an inherited cancer predisposing syndrome, familial adenomatous polyposis (discussed below).

TGF-β signaling pathway

The transforming growth factor β (TGF-β) signaling pathway regulates the proliferation and differentiation of cells, embryonic development, wound healing, and angiogenesis.[26] TGF- β can bind three high-affinity cell-surface receptors (type I, II, and III). The intra-cellular domains of receptor type I and II contain serine-threonine protein kinases that initiate phosphorylation of SMAD transcription factors. Receptor type III binds TGF-β and transfers it to the signaling receptors type II. Upon binding of TGF-β, receptor type II forms a complex with receptor type I and phosphorylates the receptor, thereby stimulating the kinase-activity of the receptor.[27] The activated receptor type I phosphorylates SMAD2 and SMAD3 which then bind to SMAD4. This SMAD-complex then translocates to the nucleus, where it acts as a transcription factor. In epithelial cells, TGF-β inhibits cellular proliferation, which explains its tumor suppressor function.

Mutation inactivation of the TGF-β pathway is frequent in colorectal tumors. About 30% of the sporadic CRCs have a mutation in the *SMAD4* gene.[28] Moreover, the TGF-β receptor type II (TGFRB2) is frequently mutated in tumors with DNA mismatch repair deficiency (discussed below) and about 15% of DNA mismatch repair proficient colorectal carcinomas display mutational inactivation of TGFBR2.[29,30] Germ-line mutations in *SMAD4* are found in patients with Juvenile Polyposis (discussed below).[31]

Bone morphogenetic proteins (BMPs) also act in the TGF-β signaling pathway. Similar to TGF-β, BMPs bind to serine-threonine kinase receptors type 1 and 2 (BMPR1 and BMPR2). BMPR2 then phosphorylates and activates receptor type I. The activated BMPR1 subsequently phosphorylates SMAD1, SMAD5, and SMAD8, which associate with SMAD4 and translocate to the nucleus (reviewed by [32]). In addition to *SMAD4* mutations, germ-line mutations in *BMPR1A* are also found in Juvenile Polyposis patients.[33]

p53 signaling pathway

The p53 pathway is an important pathway that is involved in cell-cycle arrest upon cellular stress. It thereby acts as a tumor suppressor.[34] When cellular stresses occur, including DNA damage or hypoxia, p53 is stabilized and binds to the DNA to act as a transcription regulator. Genes regulated by p53 regulates are involved in important cellular processes like DNA repair, cell-cycle arrest, senescence, and apoptosis. Genes that are transcriptionally activated by p53 include *p21*, *MDM2*, *GADD45*, and *Bax*.[35] The pathway is often inactivated in CRC by an inactivating (missense) mutation in the p53 gene and subsequent deletion of the second

allele of the *p53* gene through physical loss of chromosome arm 17p.[36,37] Inactivation of *p53* coincides with the transition of an adenoma to the carcinoma stage.[38]

MAPK signaling pathway

Mitogen-activated protein kinases (MAPK) cascade is an important signaling pathway involved in cellular proliferation. Three major subfamilies of MAP kinases include the extra-cellular-signal-regulated kinases, stress-activated protein kinases, and MAPK14. The subfamily of extracellular-signal-regulated kinases (ERK MAPK), including Ras, Raf, MEK, and ERK, is important for intestinal epithelial differentiation.[39] Upon binding of extracellular signal proteins the Ras/Raf/MEK/ERK cascade transmits the signal to the nucleus where it regulates the transcription of genes involved in proliferation and differentiation. The cascade includes several proto-oncogenes and is dysregulated in about 30% of all cancers. Activating mutations in *KRAS* are found in approximately 38% of colorectal cancer.[40] *BRAF* mutations are observed in about 10% of colorectal cancers and are particularly observed in tumors with *MLH1* promoter hypermethylation.[41,42] Mutations in *KRAS* and *BRAF* appear to be mutually exclusive.[42,43]

Activation of the epidermal growth factor receptor (EGFR) stimulates the MAPK signaling pathway and is a target for cancer treatment. Oncogenic activation of the Ras/Raf/MEK/ERK cascade in colorectal cancer is strongly correlated with impaired response to anti-EGFR treatments like panitumumab and cetuximab.[44]

PI3K signaling pathway

Phosphatidylinositol 3-kinase (PI3K) is an enzyme that phosphorylates inositol phospholipids (IPs). The phosphorylated IPs (PIPs) subsequently activate the downstream AKT pathway. Activation of AKT leads to increased cell survival, growth and cellular proliferation (reviewed by [45]). Protein targets of AKT include mTor, Bad, Caspase 9, Tuberin, and GSK3β.[46] About 32% of colorectal tumors carry an activating mutation in *PIK3CA*, encoding the catalytic subunit.[47] PTEN functions as an inhibitor of the PI3K pathway because it dephosphorylates the inositol phospholipids. The PI3K pathway is interlinked with the MAPK pathway and is also stimulated upon EGFR activation. Consequently, inactivating PTEN mutations or activating PI3K mutations have been associated with reduced response to anti-EGFR treatment.[48,49]

Genetic instability

In addition to acquiring mutations in genes, colorectal tumors become genetically instable. The different forms of genetic instability involved in colorectal tumorigenesis are discussed below.

Microsatellite instability

Microsatellites are short repetitive sequences within the DNA. An accumulation of mutations in microsatellites, that make them shorter or longer, is called microsatellite instability (MSI or MIN). This microsatellite instability is caused by a defective DNA mismatch repair system, which fails to repair errors during DNA replication. MSI-H (MSI-high) is typically seen in tumors from patients with Lynch syndrome, which carry mutations in DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, or *PMS2*. However, MSI is also observed in sporadic colorectal cancers due to hypermethylation of the promoter region of *MLH1*.[50-52] This is observed in about 15% of all colorectal cancers.[53,54] *BRAF* mutations are common in sporadic MSI-H tumors, whereas no *BRAF* mutations are observed in MSI-H Lynch tumors.[42]

Mutation rates are at least 100-fold increased in MMR deficient cells as compared to MMR proficient cells.[55,56] Via this pathway several genes that contain repeat sequences are targeted. Frequently targeted genes include growth factor receptors *TGFB-RII* and *IGFRII*, pro-apoptotic gene *BAX*, and *Caspase 5*.[30,57-59] MSI tumors are generally diploid or near-diploid and gross chromosomal gains and losses are absent.[60-62] However, some studies reported the presence of copy number aberrations in a minor fraction of MSI-H tumors, including gains of chromosomes 4, 8, 12, 13, and 20 and losses of chromosomes 1, 9, 11, and 15.[63,64]

Chromosomal instability

Chromosomal instability (CIN) is seen in the majority of CRCs and is characterized by numerous chromosomal gains and losses and copy neutral loss of heterozygosity (cnLOH). Many studies analyzed the genomic profile of tumors for gains and losses of chromosomes or chromosome arms using array comparative genomic hybridization. With recent advances in technology, genome-wide LOH studies have become feasible using SNP arrays. These SNP arrays not only provide genome-wide LOH information, but can also detect LOH in the absence of copy number aberrations (copy neutral LOH) which for example can arise via mitotic recombination or a physical loss followed by reduplication. CIN tumors are mostly aneuploid, reflecting their chromosomal instability.

Based on a comprehensive meta-analysis of tumor genome profiling studies, a model of genetic colorectal tumor progression has been established.[65] Losses of chromosomes 17p and 18 and gains of chromosomes 8q, 13q, and 20 occur early during tumor development. Losses of chromosomes 4p and 8p, and gains of 7p and 17q are correlated with the transition from primary tumor to liver metastasis. Loss of chromosome 14q and gains of chromosomes 1q, 11, 12p, and 19 are considered to be late events in tumor progression.[65]

Table 2 provides an overview of genomic profiling studies that have been performed since this meta-analysis was published. Most studies used metaphase-based CGH or array CGH to study genomic aberrations and only a minority of the studies used SNP arrays, which also provide information on LOH. Moreover, in most studies sporadic colorectal tumors were analyzed. Specific subgroups of colorectal cancer – for example MSS familial tumors or tumours

from Lynch patients - were studied to a much lesser extent.
Although the molecular basis for CIN is still unknown, several genes have been associated with the CIN pathway. Mitotic checkpoint genes *hBUB1* and *hBUBR1* have been described to contribute to chromosomal instability.[66,67] Tumor suppressor gene *p53*, involved in G1 arrest and apoptosis, is mutated in about 50% of sporadic colorectal cancers.[36] Inactivation of *p53* has been associated with the development of aneuploidy in cancers, because of loss of the arrest at the G1 checkpoint.[68] Mutations in the adenomatous polyposis coli gene *APC*, a member of the Wnt signal transduction pathway, occur early in tumorigenesis and have been linked to chromosomal instability.[69] Other genes that are frequently targeted by point mutations in CIN tumors are oncogene *KRAS*, whose activation leads to growth promotion, and *SMAD4*, a component of the TGF-β pathway.

CpG Island Methylator Phenotype
A third type of genetic instability observed in colorectal cancers is the occurrence of aberrant methylation of CpG islands, leading to the CpG island methylator phenotypes (CIMP).[70] CpG islands are short DNA sequences that are rich in CpG dinucleotides. Such islands are found in the 5' region of about half of all human genes.[71]. Hypermethylation of the cytosines in these CpG islands leads to transcriptional inactivation of such genes. Simultaneous hypermethylation of CpG islands of several genes has been termed the CIMP phenotype. Two subclasses can be distinguished: CIMP1 or CIMP-high with intense methylation of multiple genes and CIMP2 or CIMP-low with methylation of a limited number of genes. CIMP1 tumors are generally MSI-H and have a high frequency of *BRAF* mutations, whereas CIMP2 tumors are generally MSS and have a high frequency of *KRAS* mutations.[72] The cause of CIMP is currently unknown.

Hereditary Colorectal Cancer Syndromes

Several high penetrance colorectal cancer syndromes have been identified in the past two decades. The genes responsible for these syndromes have been identified using linkage analysis, deletion mapping, positional cloning, and by exploring tumor characteristics (Figure 2). The different CRC syndromes are briefly described below. An overview of all CRC syndromes as well as of polyposis syndromes is provided in Table 1. For several polyposis syndromes, including for example Cowden syndrome and hereditary mixed polyposis syndrome, a firm association with CRC has not yet been identified.

Lynch syndrome
Lynch syndrome, formerly called hereditary non-polyposis colorectal cancer or HNPCC, is the most prevalent CRC syndrome. It is estimated that Lynch syndrome accounts for at least 3% of all colorectal cancers.[73,74] Familial clustering of colorectal cancer, stomach, and uterine

cancer was already described by Warthin et al. in 1913.[75] Later, Lynch et al. described two additional families with clustering of colorectal cancer and uterine cancer.[76] In the early nineties, the genes predisposing to Lynch syndrome were identified. The syndrome is caused by germ-line mutations the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*.[77-82] In addition, germ-line epigenetic inactivation of *MLH1*, by hypermethylation of the promoter of *MLH1*, leads to Lynch syndrome.[83] Recently, it was shown that a deletion of the last exons of *TACSTD1*, upstream of *MSH2*, also predisposes individuals to Lynch syndrome, because this deletion leads to epigenetic inactivation of *MSH2* in *TACSTD1*-expressing tissues.[84]
Lynch syndrome is characterized by the development of colorectal carcinomas at a mean age of 42 years for men and 47 years for women. The mean age at diagnosis of endometrial carcinomas in Lynch syndrome patients is approximately 47
years.[85] The colorectal cancers predominantly develop at the right side of the colon.[86] Other manifestations include cancers of the stomach, small bowel, ovaries, and the urinary tract.[87,88] The lifetime risk for developing CRC for carriers of amutation in one of the mismatch repair genes is approximately 66% for men and approximately 43% for women. The cumulative risk of endometrial cancer or CRC in women is approximately 73%.[85]

Autosomal dominant inheritable CRC without polyps	**Genes**	**MIM No**
Lynch syndrome	*MLH1, MSH2, MSH6, PMS2*	120435
Autosomal dominant inheritable CRC with adenomatous polyps		
Familial adenomatous polyposis (FAP)	*APC*	175100
Attenuated FAP (AFAP)	*APC*	175100
Autosomal dominant inheritable CRC with hamartomatous/mixed/ hyperplastic polyps		
Peutz-Jeghers syndrome (PJS)	*STK11 (LKB1)*	175200
Juvenile polyposis syndrome (JPS)	*SMAD4, BMPR1A*	174900
Hereditary hemorrhagic telangiectasia syndrome (HHT) *	*ENG, ACVRL1*	187300
Hyperplastic polyposis syndrome (HPT) *	*MUTYH, MBD4*	unassigned
Hereditary mixed polyposis syndrome (HMPS) *		601228
Cowden disease (CD) *	*PTEN*	158350
Birt-Hogg-Dube syndrome (BHT) *	*FLCN*	135150
Autosomal recessive inheritable CRC with adenomatous, serrated adenomas and hyperplastic polyps		
MUTYH-associated polyposis (MAP)	*MUTYH*	608456

Table 1. Overview of colorectal cancer syndromes and polyposis syndromes
* CRC risk unknown

Mutations in a DNA mismatch repair gene lead to a failure to repair errors during DNA replication, especially concerning mismatches and insertion/deletion loops. As a consequence, the hallmark of tumors from Lynch patients is an accumulation of errors in short repetitive sequences, so-called microsatellite instability (MSI).[54,89-91]

In 1990, clinical guidelines were established to identify Lynch syndrome families for research purposes. These so-called Amsterdam Criteria were based on family characteristics, age at diagnosis and the type of cancer.[92] However, because these criteria did not account for extracolonic cancers, new criteria (Amsterdam Criteria II) were established in 1999.[93] The Amsterdam Criteria have a high specificity, but a lower sensitivity to detect Lynch syndrome families. About 40% of the tumors from families that fulfill the Amsterdam Criteria II do not show an MSI phenotype.[94] Therefore, in 1997 the Bethesda guidelines were developed to identify tumors that should be tested for microsatellite instability, as a marker of patients that should be screened for germ-line mutations in one of the DNA mismatch repair genes.[95] These guidelines also select smaller families. In 2004, the Bethesda guidelines were revised to further improve the identification of Lynch syndrome patients.[96] Other criteria, which do not take family history into account, but select patients only on basis of early onset of colorectal adenomas and carcinomas, or recurrent disease, have been tested as well. These criteria prove a sensitive strategy to identify Lynch syndrome patients.[97] Additionally, immunohistochemical screening of all CRC patients for MLH1, MSH2, MSH6, and PMS2 has also been proposed and has so far only been implemented in Denmark. Using this approach, Lynch patients in small families and patients diagnosed at older age or with de novo mutations can also be identified.[98,99]

When a MMR gene mutation is identified in a family, colonoscopic surveillance is offered to the family. This surveillance facilitates early detection of precursor lesions, thereby preventing it from developing into a malignant lesion. In the Netherlands, large scale surveillance of Lynch syndrome families was introduced in the late 1980s.[100] It has been shown that the mortality because of CRC has decreased since the introduction of colonoscopic surveillance.[101]

Recently, two genetic factors have been found to modify the colorectal cancer risk in Lynch Syndrome families. It has been shown that two common low penetrance risk alleles (discussed below), located on 8q23.3 and 11q23.1, are associated with increased colorectal cancers risks. The latter association (11q23.1) was found in female Lynch syndrome patients only.[102]

Bi-allelic MMR mutations

Heterozygous mutations in the mismatch repair genes give rise to Lynch syndrome; however, rare cases of bi-allelic MMR mutations have also been described. Patients with homozygous or compound heterozygous mutations in *MLH1*, *MSH2*, *PMS2*, and *MSH6* develop juvenile leukemia and/or lymphoma associated with neurofibromatosis type 1.[103-109]

Muir-Torre syndrome

The Muir-Torre syndrome (MTS) is characterized by a combination of cutaneous lesions (multiple keratoacanthomas and sebaceous gland tumors) and colorectal, endometrial, urological, and upper gastrointestinal tumors.[110] Most MTS patients carry a germ-line mutation in *MSH2* or *MLH1*. Therefore, the syndrome is considered to be a clinical variant of the Lynch syndrome. In MTS families, mutations in *MSH2* are more frequent than mutations in *MLH1*, as compared to Lynch syndrome families.[111] Recently it was shown that sebaceous gland tumors can also occur in *MUTYH*-associated polyposis.[112]

Turcot's syndrome

Turcot's syndrome (TS) is a rare disorder that is clinically characterized by primary tumors of the central nervous system and colorectal polyposis. The phenotypic spectrum is broad, including various types of central nervous tumors and single adenomas are observed as well as adenomatous polyposis. In patients affected with this syndrome, germ-line mutations were identified in the *APC* gene or in one of the mismatch repair genes. The disease phenotype for patients with an *APC* mutation differs from that of patients with a mutation in one if the mismatch repair genes.[113]

Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited disease which hallmark is the development of a hundred to thousands of adenomatous polyps. FAP accounts for approximately 1% of all colorectal cancers.[114] The polyps develop already during adolescence, but symptoms usually present in the third decade of life.[115-117] The risk of developing carcinomas is nearly 100% if patients are not treated.[115] Extracolonic manifestations include epidermoid cysts, facial osteomas, thyroid carcinoma, duodenal carcinoma, and malignancies of the biliary tract.[115,116] Moreover, approximately 10% of FAP patients develop desmoid tumors, also referred to as aggressive fibromatoses.[118,119]

Germ-line mutations in the adenomatous polyposis coli (*APC*) gene were identified to be responsible for FAP.[120-124] *APC* is involved in the Wnt signaling pathway and its mutational inactivation leads to activation of the Wnt signaling through reduced degradation of β-catenin. The majority of the *APC* mutations in FAP patients concern nonsense mutations or frameshift mutations that lead to a truncated protein. Mutations at codons 1061 and 1309 account for about a third of all germ-line mutations. The tumors from FAP patients carry an additional somatic mutation in *APC.* The nature of this second-hit appears to be dependent on the type and location of the germ-line mutation.[125] The 'just-right' signaling model has been put forward as an explanation for this phenomenon. This model proposes that selection of *APC* genotypes occurs to retain some downregulation of β-catenin signaling rather than a constitutive activation of β-catenin signaling.[126]

Attenuated FAP

Attenuated FAP is a phenotypic variant of classical FAP, in which patients develop less adenomatous polyps and have a later onset of colorectal cancer. The number of polyps that patients develop can be very variable; within one family some affected members have few polyps while other family members have several hundred polyps at young age.[127-129] Attenuated FAP seems to be associated with mutations in the 3' and 5' end and exon 9 of *APC*.[129-132]

***MUTYH*-associated polyposis**

MUTYH-associated polyposis (MAP) is the only colorectal cancer syndrome described with a recessive mode of inheritance. It is caused by bi-allelic mutations in the base excision repair gene *MUTYH*.[133-135] MAP patients develop multiple polyps and cancer in their colon. However, the number of polyps is generally lower as compared to FAP patients.[134,136,137] The MUTYH protein plays a role in the repair of oxidative DNA damage: upon oxidative DNA damage, MUTYH removes incorrectly incorporated adenines opposite to an 8-oxo-guanine. As a consequence of MUTYH deficiency, somatic G:C>T:A transversions are seen in MAP patients in critical genes such as *APC* and *KRAS*. In *APC*, these transversions occur primarily in GAA DNA sequences.[133,134] In *KRAS*, a specific GGT>TGT mutation (c.34 G>T, p.Gly12Cys) is found in about 64% of MAP carcinomas.[138]

The penetrance of colorectal cancer development in MAP patients is nearly 100% at the age of 60 years.[139]

The clinical relevance of heterozygous mutations in *MUTYH* is still under debate. Recent association studies in large series using different approaches found some evidence for a modest and late onset increase in CRC risk for heterozygous mutation carriers (odds ratios 1.5-1.7).[139-141] This risk is comparable to the risk of CRC that first-degree relatives of individuals with CRC have. Most studies, however, fail to find significant associations between heterozygous *MUTYH* mutations and CRC risk. Similarly, two meta-analyses do not find evidence for such association.[142,143] However, these meta-analyses analyzed only the two hotspot mutations Y179C and G396D.

It has also been hypothesized that *MUTYH* mutations act as phenotypical modifiers in MMR mutation carriers, especially concerning *MSH6* mutation carriers. Enrichment of *MUTYH* mutations in carriers of missense mutations in *MSH6* has been described, but could not be confirmed in a second study. Furthermore, a strikingly mild phenotype was observed in a patient with a bi-allelic mutation in *MUTYH* and a *MSH6* mutation.[144-146]

Juvenile polyposis

Juvenile polyposis syndrome (JPS) is characterized by the development of multiple juvenile hamartomatous polyps in the gastrointestinal tract and an increased risk of cancer. It is a rare autosomal inherited disease with a penetrance of up to 70%.[147] About 1 in 100,000 individuals are affected with JPS and the mean age at diagnosis is 16 years.[148] JPS is caused by germ-line mutations in *SMAD4* and *BMPR1A*.[31,33] Mutations in these genes explain,

however, only about 40% of JPS patients. SMAD4 and BMPR1A are both members of the transforming growth factor-β (TGF-β) superfamily. The BMP receptor type 1A activates the transcription factor SMAD4 to downregulate cellular growth and division.

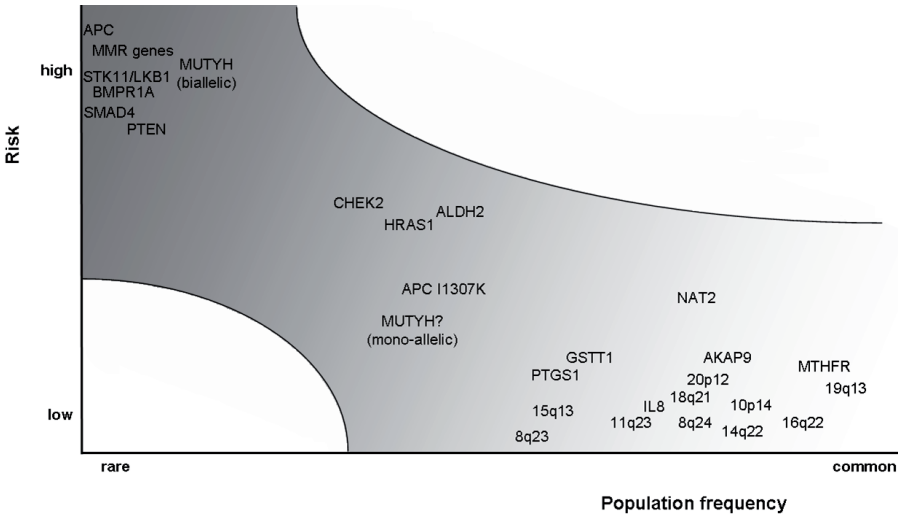

Figure 2. Genetic risk factors for colorectal cancer

This graph shows the known CRC risk factors. Rare variants with a low CRC risk are very difficult to find (lower left white area) and common variants that confer a high CRC risk are believed not to exist (upper right white area).

The MMR genes include *MLH1*, *MSH2*, *MSH6*, and *PMS2*. For the risk variant identified in genome-wide association studies their respective chromosomal locations are depicted in the graph: 8q23, rs16892766; 8q24, rs6983267; 10p14, rs10795668; 11q23, rs3802842; 14q22, rs4444235; 15q13, rs4779584; 16q22, rs9929218; 18q21, novel 1; 19q13, rs10411210; 20p14, rs961253.

Peutz-Jeghers syndrome

The Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disease that predisposes to hamartomatous polyposis, affecting mostly the small bowel. It affects approximately 1 in 200,000 individuals and has a high penetrance. In around 50% of the patients, the syndrome is caused by germ-line mutations in *STK11* (also named *LKB1*), encoding serine/threonine protein kinase 11.[149] STK11 forms a complex with Ste adaptor protein STRAD and MO25.[150,151] This complex is involved in the regulation of cellular responses tot energy stress and cell polarity.[152] In addition to (benign) hamartomatous polyps, PJS patients develop mucocutaneous pigmentation and have an increased risk of developing malignancies in the gastrointestinal tract.[153]

Research into the characterization of the pathways in which STK11 is involved have not yet

provided a conclusive answer to the question how STK11 exerts its tumor suppressor function.[128] STK11 has been described to have a regulatory role in control of cell-cycle arrest, p53-mediated apoptosis, the Wnt signaling cascade, the ras-pathway, TGF-β signaling, and energy metabolism (reviewed by [154]).

Familial Colorectal Cancer of unknown cause

As briefly mentioned above, heritable factors may play a role in up to 30% of all CRC.[3] Family history is a strong risk factor for the development of colorectal cancer. About 6% of all CRC is explained by the colorectal cancer syndromes described above. However, for the other familial cases the underlying genetics remain elusive. Among these familial cases are large families with a clear positive family history of CRC. Such Amsterdam Criteria positive families without a mismatch repair defect have been termed 'Familial Colorectal Cancer Type X' or mismatch repair proficient CRC.[155] It has been estimated that about forty percent of the families fulfilling the Amsterdam Criteria is not affected with the Lynch syndrome, suggesting that other yet to be identified genetic factors predispose these families to CRC.[94] Moreover, it can be calculated from a Finnish study that only about 12% of the excess CRC risk associated with a family history of mismatch repair proficient CRC is explained by the known genes.[4,156]

In mismatch repair proficient CRC families, the cancer incidence is generally lower than in Lynch families.[4,155] Segregation analysis performed by Lubbe and colleagues suggested that aggregation of CRC in mismatch repair proficient families follows a recessive inheritance model.[4] Jenkins et al. also reported a role for recessively inherited factors in familial CRC.[157] The results of the study of Lindor et al. on the other hand, supported a dominant model of inheritance.[155] These differences in the suggested model of inheritance might be explained by differences in patient ascertainment. Lindor et al. included Amsterdam Criteria positive families, whereas Lubbe et al. included CRC patients diagnosed below 70 years of age. Finally, Jenkins et al. included only CRC patients with an age at diagnosis below 24 years.

High penetrance risk loci

Generally, linkage analysis is adopted to identify rare alleles that confer a high risk of familial colorectal cancer. Several loci have been identified using this approach; however, none of these have yet led to the identification of the genes responsible for the increased CRC risk in FCC families.

Wiesner and colleagues reported genetic linkage of CRC to chromosome 9q22.2-31.2 in a set of 74 affected sibling pairs.[158] Two other studies provided support for the presence of a CRC susceptibility locus in this region.[159,160] Kemp and colleagues described linkage to

colorectal cancer susceptibility of a region located on chromosome 3q21-q24.[161] In a later extension of this study including 34 additional families, linkage to this region was confirmed. However, mutation screening of 30 genes failed to identify pathogenic variants.[162] Further support for this region harboring a CRC susceptibility locus was provided by a Swedish linkage study of Picelli et al.[163] A linkage scan in 18 Swedish colorectal cancer families revealed linkage on chromosome 11, 14 and 22.[164] And finally, linkage to chromosome 7q31 has been identified using an affected sibling approach.[165]
Recently, a 111 kb copy number variable region on 3q26 was proposed to contain a regulatory element for *PPMIL* which could cause CRC susceptibility in *APC* mutation-negative polyposis families.[166] This region was identified using copy number analysis in polyps of these patients and subsequent gene expression analysis in the candidate region.

Low Penetrance Risk Alleles

Two approaches are generally used to identify low penetrance colorectal cancer risk variants. The first method is the candidate gene analysis. In candidate gene approaches, the following groups of genes are most frequently studied: genes involved in carcinogen metabolism, genes involved in methylation, genes encoding DNA repair proteins, microenvironmental modifiers, and oncogenes and tumor suppressor genes.[167,168] Two large meta-analyses of studies using the candidate gene approach, identified several polymorphisms that are associated with an increased or decreased colorectal cancer risk (Figure 2). The C677T polymorphism in *MTHFR* (methylene tetrahydrofolate reductase) is associated with a decreased CRC risk for homozygous carriers of the variant allele. *HRAS1* is a proto-oncogene that contains a variable number of tandem repeats region (VNTR). Rare alleles of this VNTR are associated with a moderately increased colorectal cancer risk. For the *NAT2* gene, only a phenotypic association was identified, i.e. a fast acetylatorship has been associated with an increased CRC risk. However, genotypic associations could not be identified. GSTT1, a detoxification enzyme, is associated with a small increase in colorectal cancer risk for the null genotype (homozygous deletion). ALDH2, a mitochondrial enzyme responsible for oxidation of acetaldehyde, is associated with in increased risk both in heterozygous and homozygous carriers of the variant allele. And finally, the variant I1307K in *APC* is associated with an increased CRC risk.[167,168] Other low risk variants that have been associated with CRC include *CHEK2* 1100delC, *AKAP9* M463I, *PTGS1* G213G, *IL8* c.-352T>A, *MTHFR* A429E.[169-171]
The second approach to identify low risk variants is genome-wide association studies, which have become feasible with the availability of high density single nucleotide polymorphism (SNP) arrays. Recent genome-wide association studies have successfully identified several loci that are associated with an increased risk of developing colorectal cancer. Risk loci were identified on chromosomes 8q24.21 (rs6983267), 18q21.1 (rs4939827, rs12953717 and rs4464148), chromosome 15q13.3 (rs4779584), 11q23.3 (rs3802842), 10p14 (rs10795668),

8q23.3 (rs16892766), 20p12.3 (rs961253), 14q22.2 (rs4444235), 16q22.1 (rs9929218), and 19q13.1 (rs10411210).[172-181] The next step is to study the mechanisms that underlie CRC susceptibility associated with the different loci. Some of the loci are in high linkage disequilibrium with genes that are strong candidates for the causal relation with colorectal cancer. However, for other loci no genes are in the direct vicinity, which makes the interpretation of these risk factors much more difficult. For rs6983267 (8q24.21), it has now been shown that it is located in a transcriptional enhancer and that it affects the DNA-binding affinity of Wnt-regulated transcription factor TCF7L2 (or TCF4). The enhancer element interacts with the *MYC* promoter; however, no correlation was observed between the rs6983267 genotype and *MYC* expression.[182,183] Additionally, a role in tumor evolution was suggested for rs6983267; tumor studies showed that the risk allele was favored in about 66% of the tumors with allelic imbalance at the locus of rs6983267.[184] For the locus on 18q21.1, the causal SNP has been identified by resequencing the genomic region. This SNP - named Novel 1 - also maps to a transcription factor binding site and has been associated with reduced expression of *SMAD7*. No relation with copy number changes on chromosome 18q21.1 was identified for Novel 1 in tumors.[185]

Environmental Factors

Several environmental factors can influence the risk of the development of colorectal cancer. First, diet is an important factor that influences the CRC risk. Intake of vegetables and fruits is found to reduce the risk of CRC. The effect of raw vegetables, green vegetables, and cruciferous vegetables has been particularly consistent in different studies.[186] The degree of risk reduction however, varies in the different studies. Fiber intake has also been suggested to decrease colorectal cancer risk, however existing data are inconsistent.[186] Consumption of red meat and processed meat has, on the other hand, been associated with an increased risk of colorectal cancer.[186,187] Moreover, alcohol consumption has been associated with an increased colorectal cancer risk.[186,188] Secondly, physical activity and body mass can influence CRC risk. Physical activity is known to reduce the risk of colon cancer, whereas obesity increases the risk of colon cancer. However, both factors do not seem to influence the risk of rectal cancer.[186,188,189] Thirdly, non-steroidal anti-inflammatory drugs (NSAIDs) and hormone replacement therapy (HRT) are described to lower the risk of colorectal cancer.[186] Fourthly, smoking of cigarettes, cigars, and pipes increases the risk of developing colorectal cancer.[186,188] And finally, recent studies in mice suggest that commensal colonic bacteria can promote colorectal tumorigenesis via inflammation.[190]

The influence of environmental factors differs per individual, based on their individual lifestyle. However, environmental factors can in part be shared by individuals from the same family. For example, dietary habits are part of a shared environment or ‘inherited environment’ within a family. In a study that analyzed cancer risk in monozygotic and dizygotic pairs of twins it was

estimated that shared environmental factors contribute up to 5% to the total colorectal cancer risk.[3]

New fields in CRC research, prevention, and treatment

Recent studies that identified colon cancer tumor-initiating cells, provide support for the cancer stem cell hypothesis.[191-193] The stem cell model proposes that tumorigenesis is initiated by dysregulation of the process of self-renewal of colonic stem cells and that as a consequence tumors contain a subcomponent that retains stem cell properties.[194] These cancer stem cells have been suggested to exhibit a greater plasticity that the stem cells populating the normal crypts. The stem cell properties ("stemness") of certain subpopulations of cancer cells can therefore also be seen as an extreme plasticity of tumors to adapt to and survive variable and constantly changing environmental conditions.[195] The concept of cancer stem cells has several consequences for cancer prevention and treatment. Cancer stem cells are more resistant to cytotoxic chemotherapy, because an intrinsic property of stem cells is that they promote survival and are resistant to apoptosis. Additionally, stem cells express multidrug resistant genes, including multifunctional efflux transporters that have an important role in drug distribution.[196] This indicates that novel treatments should be developed that can eradicate the cancer stem cells. Moreover, it has been proposed that stem cells are capable of metastasizing and can remain quiet until the appropriate signals activate them to develop into a macroscopic metastasis.[194]

Current topic in cancer prevention is the possible installation of population-based screening for colorectal cancer. The fecal occult blood test (FOBT) provides a non-invasive and sensitive method (sensitivity: ~65%) for detecting invasive colorectal cancer.[197] The Health Council of the Netherlands has advised to implement biennial immunochemical FOBT in men and women of 55-75 years of age as a nationwide screening program.[198] Another potential non-invasive screening method is a DNA test in stool, in which a panel of genetic markers is tested.[199]

New strategies in the diagnosis and treatment of colorectal cancer have been developed in recent years. For rectum cancer a more standardized way of treatment by the so-called total mesorectal excision (TME) in combination with preoperative radiotherapy has led to a reduction of local recurrence.[200] Furthermore, transanal endoscopic microsurgery (TEM) was introduced. Initially it was solely used as an approach to curatively remove large sessile adenomas of the rectum.[201] However, trials are now underway to also use the TEM technique in combination with neoadjuvant (preoperative) radio-chemotherapy to treat early rectal cancer. Biomarkers and imaging modules are being further developed to predict and monitor therapy response.

Surgeons more and more use endoscopic techniques to remove colorectal cancer with great benefit for the patients in terms of reduction of morbidity and decrease of hospital stay.

Whereas 15 years ago a patient with liver metastases would soon die of this condition, nowadays partial liver resections are being performed, sometimes in combination with isolated liver perfusions and/or radiofrequency ablation (RFA).[202,203] These developments have thereby increased the lifespan of such CRC patients.[204] Furthermore, the use of new chemotherapeutic regimens such as the addition of oxaliplatin and irinotecan has led to improved patient outcomes.[205-207] The introduction of orally given 5-fluorouracil (5-FU) derivatives is of major benefit for patients in terms of tolerability.[208] As a second line of therapy the use of targeted drugs i.e. inhibitors of cancer transduction pathways have shown its effects in certain groups of CRC patients.[209] Biomarkers are identified that predict treatment response. An example for the latter is that the presence of a somatic *KRAS* mutation predicts a lack of response upon treatment with epidermal growth factor receptor (EGFR) inhibitors.[210] There is a peculiar subgroup of CRC patients with peritoneal carcinomatosis patterns that benefit from cytoreductive surgery in combination with hyperthermic intraperitoneal chemotherapy (HIPEC). This therapy had shown to increase the overall survival of such patients.[211] More recently, it has been discussed whether HIPEC should be offered as a prophylactic treatment to prevent the progression of carcinomatosis.[212]

Table 2. Overview of published studies with comparative genomic hybridization analysis of colorectal tumors.

Study	Technique/platform	Samples	Conclusions
Diep et al.[65]	Meta-analysis of 31 CGH studies	373 primary CRCs and 102 liver metastases	Results from the combined analyses suggest that losses at 17p and 18 and gains of 8q, 13q, and 20 occur early in the establishment of primary CRCs, whereas loss of 4p is associated with the transition from Dukes' A to B-D. Deletion of 8p and gains of 7p and 17q are correlated with the transition from primary tumor to liver metastasis, whereas losses of 14q and gains of 1q, 11, 12p, and 19 are late events.
Scheffer et al.[213]	Affymetrix GeneChip Human Mapping 50K SNP arrays	130 colorectal tumors at different stages	Amplifications on chromosomes 7, 8q, 13q, 20, and X. Deletions on chromosomes 4, 8p, 14q, 15q, 17p, 18, 20p, and 22q. Samples with simultaneous deletions in 18q, 8p, 4p, and 15q have a particularly poor prognosis.
Darbary et al.[214]	Affymetrix 10K SNP arrays	13 sporadic colorectal cancers	Uniparental disomy was occurring in many colorectal cancers and often coordinately involved chromosome 14 and 18.
Postma et al.[215]	In house printed 30K oligonucleotide-based array	32 primary tumors of patients with advanced CRC (16 with good and 16 with poor response to chemotherapy)	Responders overall had more chromosomal alterations than nonresponders, especially loss of chromosome 18.
Coss et al.[216]	GenoSensor Array 300 (aCGH)	39 primary colorectal cancers	Amplifications: 20q (79%), 7q (46%), 2p (44%), 7p (41%), 13q (36%), 11p (31%). Deletions: 1p (46%), 17p (36%), 6q (31%), 18q (28%).
Nakao et al.[217]	MacArray Karyo4000 (4k BAC array)	77 sporadic colorectal cancers	Gain: 20q (70%), 7p, 8q, 13. Loss: 18q (68%), 8p, 17p. Aberrations on 3q, 10q, 11q, 15q, and Xp were linked to lymph node metastasis.
Melcher et al.[218]	Spectral karyotyping and SNP array	15 primary MSI tumors and 15 primary CIN tumors	The combination of spectral karyotyping and SNP-array analysis permits the detection of uniparental disomy.
Van Puijenbroek et al.[62]	Illumina 6K SNP arrays (Beadarray)	37 MSI-H colorectal tumors (31 from patients with familial MMR deficiency and 10 sporadic MLH1 hypermethylation)	All carcinomas showed few chromosomal aberrations. MSI-H carcinomas of MMR_UV carriers present more aberrations.
Al-Mulla et al.[219]	Metaphase-based CGH	65 MSS colorectal cancers	In MSS CRCs, the number of chromosomal losses is inversely proportional to Raf kinase inhibitor protein (RKIP) expression levels.
Camps et al.[220]	Agilent oligo microarray and chromosome 8 Human BAC microarray	51 primary adenocarcinomas of the colon	Low-level copy number changes of chromosomes 7, 8, 13, 18, and 20. A significant association of chromosomal breakpoints with structural variants in the human genome was observed.
Lips et al.[221]	Illumina 6K SNP arrays (Beadarray)	36 rectal carcinomas, with both an adenoma and a carcinoma fraction	Five specific chromosomal aberrations, in combination with immunohistochemistry for p53 and SMAD4, can predict possible progression of sessile rectal adenoma to early rectal carcinomas.
Derks et al.[222]	Metaphase-based CGH (n=51) and 5K BAC array (n=20)	71 colorectal carcinomas	Promoter methylation of pivotal tumor suppressor and DNA repair genes is associated with specific patterns of chromosomal changes in CRC, which are different from methylation patterns in MSI tumors.

Table 2 continued. Overview of published studies with comparative genomic hybridization analysis of colorectal tumors.

Study	Technique/platform	Samples	Conclusions
Diep et al.[65]	Meta-analysis of 31 CGH studies	373 primary CRCs and 102 liver metastases	Results from the combined analyses suggest that losses at 17p and 18 and gains of 8q, 13q, and 20 occur early in the establishment of primary CRCs, whereas loss of 4p is associated with the transition from Dukes' A to B-D. Deletion of 8p and gains of 7p and 17q are correlated with the transition from primary tumor to liver metastasis, whereas losses of 14q and gains of 1q, 11, 12p, and 19 are late events.
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A PubMed search was performed on 22 June 2009. Search terms: "colorectal neoplasms/genetics[mesh] AND (SNP array OR CGH OR comparative genomic hybridization)". Limits: language: English. Only studies that were published after the comprehensive meta-analysis of Diep et al. were listed.[65] Only studies including human colorectal tumors with genome wide genomic data were included. Case reports and studies including less than 10 tumors were excluded. The study of Lips et al.[221] did not appear in the search results, but was included upon literature review.

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