Hereditary Colorectal Cancer

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HEREDITARY COLORECTAL CANCER BLUES

”You are old father William,” the young surgeon said,
”And your colon from polyps is free”
”Yet most of your siblings are known to be dead-
A really bad family tree.”

”In my youth,” Father William replied with a grin,
I was told that a gene had mutated,
That all who carried this dominant gene
To polyps and cancer fated”.

”I sought advice from a surgical friend,
Who sighed and said-”Without doubt
Your only escape from an untimely death
Is to have your intestine right out.”

”It seemed rather bad luck – I was only nineteen –
So I went and consulted a quack,
Who took a firm grip on my dominant gene
And promptly mutated it back”

”This,” said the surgeon, ”is something quite new
And before we ascribe any merit
We must see if the claims of this fellow are true,
And observe what your children inherit!”

Dukes C, Ann Royal Coll Surg Eng 1952

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1 PREFACE

The work presented in this thesis was carried out between 1999 and 2006 and originates from the Section for Inherited Cancer, Rikshospitalet-Radiumhospitalet Medical Center.

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Astrid Tenden Stormorken

2 GENERAL INTRODUCTION

Abbreviations

AMS	Amsterdam criteria
AMS I	Amsterdam criteria I
AMS II	Amsterdam criteria II
BRCA1/2	Breast cancer gene 1/2
CRC	Colorectal cancer
AFAP	Attenuated familial adenomatous polyposis
FAP	Familial adenomatous polyposis
FS	Flexible sigmoidoscopy
HBOC	Hereditary breast- and breast-ovarian cancer
HNPCC	Hereditary non polyposis colon cancer
IHC	Immunohistochemical analysis
LOCRC	Late onset colorectal cancer
MLH1	Mutator L homologue gene, MMR gene
MSH2	Mutator S homologue gene 2, MMR gene
MSH6	Mutator L homologue gene 6, MMR gene
PMS1-2	Human homologue of yeast postmeiotic segregation gene 1 and 2, MMR gene
MMR	Mismatch repair
MSI	Microsatellite instability
MSI-H	MSI-high
MSL-L	MSI-low
PCR	Polymerase chain reaction
OMIM	(NCBI.nlm.nih.gov/entrez//)
	* Gene of known sequence
	# Descripitive entry - phenotype - does not represent a unique locus
	+ Contains description of known gene and phenotype

Definitions I

Allele: Alternative forms of a gene at the same locus on homologous chromosomes (Hodgson, et al., 1999).

Constitutive mutation: A mutation that leads to the permanent expression of a gene which is normally tightly regulated (Kahl, 1995).

DNA: A nucleotide polymer that carries the genetic information of viruses, bacteria and all higher organisms. DNA may occur single-stranded (as in some viral genomes) or double-stranded (as in organelles and chromosomes of all higher organisms). In double-stranded DNA two complementary strands are wound around each other in opposite orientations. The two strands are held together by hydrogen bonds between complementary bases (A=T; G≡C). The sequence of each strand encodes the genetic information (genetic code) (Kahl, 1995).

Deletion mutation: Any mutation that is generated by the removal of one or more basepairs from a particular genome (Kahl, 1995).

Frameshift mutation: An alteration of the reading frame caused by addition or deletion of nucleotides in a number not divisible by three. It leads to the synthesis of a missense mRNA whose translation usually results in a non-functional protein because the message will be read in triplets specifying wrong aminoacids (Kahl, 1995).

Gene: The fundamental physical and functional unit of heredity that carries information from one generation to the next. A gene is a specific sequence of nucleotides in DNA (Kahl, 1995).

Genotype: The individual's genetic constitution. It describes the alleles at a specific genetic locus (Hodgson, et al., 1999).

Index case: The patient whose disease first directed attention to a particular pedigree (Passarge, 2001).

Locus: A specific position on a chromosome (Hodgson, et al., 1999).

Missense mutation: Any gene mutation in which one or more codon triplets are changed so that they direct the incorporation of aminoacids into the encoded protein, which differ from the wild type (e.g. UUU, encoding phenylalanine, mutates to UGU, encoding cysteine). The replacement of a wild type amino acid by a missense aminoacid in the mutant potentially produces an unstable or inactive protein (Kahl, 1995).

Mutation: The process by which genetic material undergoes a detectable and heritable structural change, or the result of such a change (Oxford dictionary, 1997). There are several definitions of mutations. In this thesis rare genetic variants causing illness are denoted mutations, and genetic variants not causing illness denoted polymorphisms.

Mutation testing: Testing for DNA sequence variation (instability).

Nonsense mutation: Any mutation in a coding sequence that converts a sense codon into a nonsense codon (stop codon) or a stop codon into a sense codon. As a consequence, the encoded protein will be either truncated (premature termination) or too long which in turn hampers or abolishes protein function (Kahl, 1995).

Pedigree: The genetic relationship of individuals based on their kinship presented in a figure (Passarge, 2001).

Penetrance: A quantitive assessment of the proportion of heterozygotes (gene carriers) who express the phenotype (Hodgson, et al., 1999).

Phenotype: The observed result (physical, biochemical or physiological) of the interaction between the genotype and the environment (Hodgson, et al., 1999).

Point mutation: A mutation involving a chemical change in only one single nucleotide (Kahl, 1995).

Rearrangement: Any structural change in a nucleotide sequence, a gene, or a chromosome (Kahl, 1995).

TNM classification: Staging of cancers according to tumour size (T), lymph node involvement (N) and metastases (M).

Wild type: The most frequently occurring phenotype in natural breeding populations. Any gene that is commonly occurring in nature (Kahl, 1995).

Definitions II

Incidence: The number of new cases of cancer in a defined population within a specific period of time (Cancer in Norway, 2003).

Incidence rate: The number of cases per 100 000 persons per year (Cancer in Norway, 2003).

Positive predictive value: The proportion of truly diseased persons out of all those with positive results in the screening tests (Council of Europe, 1974).

Prevalence: Expresses the number of persons in a defined population who are still alive at a specific point in time, and that at some time have been diagnosed with cancer (Cancer in Norway, 2003).

Sensitivity: The ability of a test to give a positive result in people who truly have the disease; a highly sensitive test gives few false negative results (Council of Europe, 1974).

Specificity: The ability of a test to give negative result in people who are free of the diseaes; a highly specific test gives few false positive results (Council of Europe, 1974).

Validity: An expression of the relationship between the results of the test and the true situation as determined by definitive diagnostic examination (Council of Europe, 1974).

2.1.0 Epidemiology of cancer in Norway

2.1.1 All cancers

The Cancer Registry of Norway is a population-based registry that has systematically collected notifications of cancer since 1952. The registry is for practical purposes complete from 1953 (Cancer in Norway, 2003).

In 2003, The Cancer Registry of Norway reported 23 307 new cases of cancer, of these 12 176 were men and 11 131 were women. Cancer incidence (Age adjusted incidence rates) has increased about 80% from 1953-1957 till the last complete 5-year period of 1999-2003 (Cancer in Norway, 2003). Cancer strikes all age groups, but especially the elderly; 85.4 percent of the cases among men and 77.3 per cent of the cases among women are found in people over 55 years of age (Cancer in Norway, 2003).

2.1.2 Hereditary cancer

High incidence and uneven geographical distribution of hereditary cancer is seen in Nordic countries due to founder mutations (Møller, Heimdal et al., 2001; Arason et al., 1998; Johannesdottir et al., 1996; Nyström-Lathi et al., 1995; Nyström-Lathi et al., 1996; Holmberg et al., 1998). Prevalences of hereditary cancers depend on the clinical criteria employed as well as differences between populations. Families with accumulated cancer due to incidental clustering or shared environmental factors will influence the estimations. Self referred families may have more extreme features and contribute to an overestimation of prevalences. Small families may be missed because numbers of affected relatives are low when numbers of relatives are low.

2.2.0 Management of hereditary cancer

2.2.1 Familial cancer

Individuals in families where cancers have occurred previously, have themselves an increased risk of developing cancer. They may develop cancer at a young age (30-40 years) and have an increased risk of multiple primaries (Watson et al., 1994; Claus et al.,1996; Aarnio et

al.,1999; Breast Cancer Linkage Consortium, 1999; Lynch et al.,1999; Møller, Borg et al., 2001). A family history of cancer may give a rationale for surveillance aiming at early diagnosis and improved prognosis, and it may initiate mutation testing (Syngal et al., 1998; Järvinen et al., 2000; Møller, Evans et al., 1999; Møller et al., 2002). This has implications for the individual persons and financial/capacity implications for the national health system (Douglas et al., 1999).

2.2.2 Patients

Since 1989 patients are either self-referred or referred by their physician to the Section for inherited cancer at Rikshospitalt-Radiumhospitalet. They are included according to their family history of cancer applying wide clinical criteria (Møller, 1993). According to Norwegian legislation (www.Lovdata.no), patients' relatives have to be informed and invited by the index cases to contact the section. Information to the patients is given through genetic counselling. Information is also promoted to colleagues in the medical field and through the media. Surveillance of patients at risk is coordinated by the section in collaboration with relevant specialists. Mutation analyses and testing of tissue is done in collaboration with laboratories or pathology departments, respectively. All activity complies with national legislation. National and international collaboration and research plays an important part in the section's activities.

2.2.3 Family history

Patients are mailed a family information questionnaire to fill in information about their closest relatives, including names, date of birth, cancer diagnoses, year of diagnosis and hospital treatment, and date of death for the deceased. The pedigrees are traced backward and laterally. Information on the site and classification of all cancers and polyps and age at diagnosis are obtained and verified in the medical files and/or in The Norwegian Cancer Registry whenever possible.

2.2.4 Genetic counselling

During the counselling session the family's pedigree is commented and the importance of having exact information on diagnoses explained. Modes of inheritance and biological insight in the mechanisms including Knudson's two hit hypothesis (Knudson, 1986) are explained and information is given on the specific type of inherited cancer running in the family. Risk assessments and surveillance options are discussed.

Regarding genetic testing, legislation requires pre-, under- and post-test counselling in predictive testing. "Diagnostic"testing will often also be predictive testing as most of the hereditary cancers are part of cancer syndromes. A diagnostic test for MMR mutations in a female CRC patient, is at the same time a predictive test regarding her risk for endometrial cancer. Just as doing a diagnostic test for *BRCA1/2* mutations in a patient with breast cancer is a predictive test for ovarian cancer.

2.2.5 Mutation testing

All genetic testing is based on informed consent confirmed in writing. Predictive testing requires genetic counselling. Under Norwegian legislation, immunohistochemistry is considered as genetic testing, and so is extensive family history to determine risk for inherited disorders.

The process of mutation testing is time consuming, costly and there are capacity problems. Modes of testing depend on the types of mutations expected. Mutation screening is normally first undertaken in one affected family member. If a mutation is found, all other available affected members/obligate carriers should be tested to make sure that it is the same mutation that causes cancer in the family and that there is not more than one mutation running in the family. After this, predictive testing can be offered to unaffected family members. In the absence of living affecteds, predictive testing of unaffecteds is inconclusive, except in populations with high frequencies of founder mutations. An example is the Norwegian founder mutations in the *BRCA* genes. In a family with a known mutation all test results should be verified in a second test from a new blood sample.

2.2.6 Ethics and informed consent

Under Norwegian legislation, persons at risk should make the initial contact with the proper health personnel, and not vice versa. Permission to access the medical files for information is obtained in writing from all living persons, and from relatives of the dead ones. Written informed consent underlies all requests for tumour specimens and genetic testing. All activity reported is approved by the Government as health care. All information is kept in our medical files. No results identifying any individual patient are exported from the medical files. No research registry is erected. All activity complies with national legislation and includes genetic counselling of every person included.

2.2.7 Secondary prevention

Major screening programs are offered as public health service to groups of the population thought to be at risk, for example breast cancer screening and screening for cervical cancer. Some persons may be at higher risk for cancer than the general population and may require closer examinations, this approach is referred to as surveillance (Ponz de Leon, 2002). Surveillance should meet the standard requirements for screening, as recommended by WHO: There should be a safe, precise and validated screening test. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcome than late treatment (Wilson, et al., 1968).

Some screening methods are proven to reduce the risk of cancer (CRC) (Järvinen et al., 2000), while others are unproven or debatable (cancers of the ovary, endometrium and uroepithelial tract) (Aarnio et al., 1999; Dove-Edwin et al., 2002). The surveillance programs are often life long and continuity and patient compliance is essential. Results of the surveillance are reported back to the medical departments which also monitor the continuity of the program. Surveillance guidelines are often a result of national and international collaboration and agreements.

2.2.8 Primary prevention

"Prevention is better than cure" (Ponz de Leon, 2002). For cancers closely related to environmental factors (diet/lifestyle), for example lung cancer, prevention is possible by removing the causative agent (tobacco smoking). This is primary prevention. Prophylactic surgery, like oophorectomy and mastectomy in *BRCA* mutation carriers, is another example of primary prevention. The removal of adenomatous polyps would be secondary prevention, while it may be debateable whether a colectomy in a person producing multiple adenomas is primary or secondary prevention.

Chemoprevention is one of the most recent strategies in cancer prevention (Wattenberg et al., 1985), and can be defined as the use of natural compounds or drugs to block, reverse or prevent the development of benign or malignant tumours (Shureiqi et al., 2000).

2.3.0 Colorectal cancer

2.3.1 Epidemiology

In 2003, The Cancer Registry of Norway reported 1035 new cases of colon cancer and 608 cases of rectal cancer among men. Corresponding numbers were 1261 and 462 for women. Colorectal cancer is the most prevalent cancer site for men and women combined and it is the second most common cause of death from cancer in Western countries (Cancer in Norway, 2003). The lifetime risk of developing colorectal cancer is about 4-5%. The incidence of the disease is rapidly increasing after the age of 50 (Cancer in Norway, 2003).

The prevalence of polyps reported in the literature varies widely. Polyp incidence increases with age, with 34% of those over the age of 50 having a single polyp (Lanspa et al., 1990). Adenomatous polyps account for 30 to 50 percent of all polyps and are premalignant lesions (Williams et al., 1982). Autopsy studies show a prevalence of adenomas of about 20% at the age of 50 years increasing to about 50% after the age of 70 years (Clark et al., 1985; Eide et al., 1978; Vatn et al., 1982).

2.3.2 Pathology

Hyperplastic polyps

A frequently occurring lesion in the colon is the hyperplastic polyp. If no cellular dysplasia is visible in the microscope, a polyp is scored as a hyperpastic polyp (Rex et al., 1992). It is commonly believed that hyperplastic polyps, especially those located in the rectum and sigmoid colon are benign lesions lacking malignant potential (Ponz de Leon, 2002).

Adenomas

Colorectal adenomatous polyps are well demarcated lumps of epithelial dysplasia that can be classified into three major histological types: tubular, villous and tubulovillous (Ponz de Leon, 2002). The adenomas are classified according to the grade of dysplasia as mild, moderate or severe. Dysplasia predisposes an organ to cancer development. Adenomas are known to be the precursors of sporadic and hereditary colorectal cancer (Vogelstein et al., 1988; Kinzler et al., 1996). Most CRCs are adenocarcinomas which have developed from adenomas. Only about 5% of adenomas become malignant (Wilcox et al., 1987). In sporadic cancer the progression from adenoma to cancer takes approximately 10-15 years (Stryker et

al., 1987). Several studies have shown that removal of adenomas may reduce the incidence of colorectal cancer (Järvinen et al., 2000; Winawer et al., 1993; Thiis-Evensen et al., 1999).

Hamartomas

Hamartomas are uncommon polypoid lesions observed in Peutz-Jeghers syndrome and Juvenile polyposis. Histologically they show a complex branching pattern of smoothe muscle supporting normal lamina propria and glands. These polyps are basically composed of normal elements indigenous to the site in which they appear, although their general architecture is markedly abnormal (Ponz de Leon, 2000).

Cancers

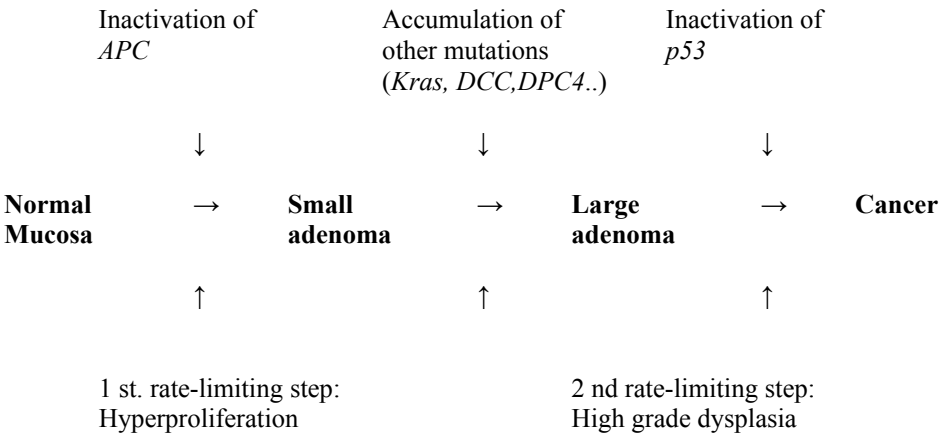
Most colorectal carcinomas (95%) are adenocarcinomas and in many cases (10%-20%) may show a mucinous component (Bosman, 1995). They may be graded into well, moderately and poorly differentiated lesions (Bosman, 1995). Metastasis to regional lymph nodes can be detected in 30%-50% of the patients at the time of diagnosis (Sanchez-Cespedes et al., 1999). Haematogen metastasis to the liver can be seen in 10%-30% of the patients at diagnosis (Gatta et al., 2000). Duke's staging is commonly used, but the TNM system is now increasingly being adapted. Prognosis depends on the stage of disease.

Distal cancers are suggested to be more related to environmental factors and proximal cancers more genetically caused (Bonithon-Kopp et al., 1999). There seems to be increasing prevalence of proximal cancers with increasing age (Eide et al., 1978; Thiis-Evensen et al., 1999; Vatn et al., 1982).

2.3.3 Molecular biology

The majority of CRCs develop from adenomas. The progression from an adenoma to a cancer passes through a series of defined histological stages referred to as the adenoma-carcinoma sequence (Vogelstein et al., 1988). The colorectal tumour initiation requires several different somatic changes before a cell can develop into a carcinoma (Kinzler et al., 1996).

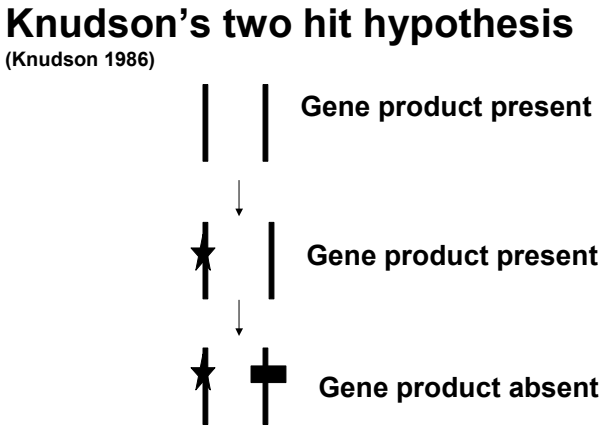
Main events in the adenoma-carcinoma sequence
(Vogelstein et al., 1988; Boland et al., 1995)



Two major mechanisms of genomic instability have been identified in sporadic tumour progression. The first, known as chromosomal instability (CIN), results from a series of genetic changes involving activation of oncogenes such as ki-ras and inactivation of tumour-suppressor genes such as *p53, DCC/SMAD4*, and *APC* (Vogelstein et al., 1988; Fearon et al., 1990). Large chromosomal alterations such as loss of heterozygosity (LOH), is the consequence. The second major mechanism, known as microsatellite instability (MSI) is seen in association with HNPCC, but is also found in 12-15% of sporadic CRC (Thibodeau et al., 1993; Aaltonen et al., 1993). The hallmark of this pathway is a widespread mutation of short repetitive DNA sequences known as microsatellites (Gryfe et al., 2001).

Knudson's two hit hypothesis

The mismatch repair genes encode protein products recognizing and correcting errors that arise when DNA is replicated (Leach et al., 1993, Dunlop et al., 1997). According to the two-hit model of carcinogenesis, the second event in addition to the inherited mutation is expected to be an acquired mutation in the normal allele (Knudson, 1986; Hemminki et al., 1994). If both the first and second hits are truncating mutations, the gene product for the gene in question should be absent in the tumour cells. This has been demonstrated by immunohistochemistry (Thibodeau et al., 1996; de Leeuw et al., 2000).



2.3.4 Screening methods

Faecal occult blood testing (FOBT)

The test is cheap, non-invasive and considered to be cost-effective (Lieberman, 1995). The major drawback is that the test frequently fails to detect precancerous lesions (Rex et al., 2000).

Barium enema

Single contrast barium enema is inadequate for detecting polyps, but the results improve with double-contrast technique. The main limitation of this technique is the impossibility to carry out biopsies or polypectomy.

Flexible sigmoidoscopy and colonoscopy

These tests have the advantage of both detection and removal of premalignant lesions in the same procedure. The test has good sensitivity and and high specificity for detection of both premalignant lesions and cancers. Colonoscopy screens the whole colon, but requires a larger degree of bowel preparation than flexible sigmoidoscopy.

2.3.5 Risk factors

Age, dietary factors and family history are probably the most important causes of high incidence of CRC in the western world. In inflammatory bowel disease, for example patients affected by ulcerative colitis are at risk of CRC.

Family members of CRC patients are facing a higher risk than the general population. About 10% of colorectal cancers may be inherited (Houlston et al., 1992), but no more than 2-6 % have been demonstrated to be caused by inherited mutations (Houlston et al., 2001).

It is unclear to what extent hereditary factors, shared environment and diet contribute to elevated risk. In some cases there might be a combination of genetic and environmental factors, as shown with gastric cancer and HNPCC. Gastric cancer has become less frequent in MMR mutation carriers, just as it has decreased in the population in general. This is thought to be caused by the widespread use of refrigeration of food instead of salting, smoking and soaking in alcohol. A dicrease in Helicobacter Pylori infections might also contribute (Palli et al., 2000).

2.3.6 Prevention

Primary prevention of CRC is focused on modifying diet (reduced fat and calorie intake and increased fruit and vegetable and fibre intake) and increasing exercise. There is some evidence of a protective effect of certain nutrients like calcium, selenium and starch (NHMRC, 1999). Several studies have demonstrated that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may give a protection against polyps and cancer (NHMRC, 1999; Jänne et al., 2000). NSAIDs block cyclooxygenase 1 and 2, which are catalytic enzymes involved in the prostaglandin synthesis. Prostaglandines are markedly elevated in colorectal cancer tumours (Eberhart et al., 1994). There are several ongoing chemoprevention trials. The CAPP1 and CAPP2 studies test whether aspirin and resistant starch make any difference to polyp growth in *APC* mutation carriers who have not had a bowel operation and in MMR mutation carriers, respectively (CAPP1/2. www.ncl.ac.uk/capp).

2.4.0 Hereditary colorectal cancer syndromes

The main focus of this thesis is on HNPCC. As a consequence the hamartomatous polyposes are only briefly described.

2.4.1 Hamartomatous polyposes

These syndromes are rare. They are associated with substantially increased lifetime risks of both intestinal and extra-intestinal malignancies. They account for less than 1 percent of annual incidence of CRC. They are dominantly inherited with variable penetrance.

Cowden disease

Gene: *PTEN* 10q22.3-23.31 OMIM # 158350

Hamartomas involve the skin, intestine, breast and thyroid gland. There may be CNS involvement and increased risk of thyroid and breast cancers. (Eng et al., 1997)

Juvenile Polyposis

Gene: *SMAD4* 18q21.1 OMIM # 17490

Most commonly associated birth defects include malrotation of the midgut, genitourinary defects and cardiac defects. There is an increased risk of gastrointestinal cancer (Lalloo et al., 2005).

Peutz-Jeghers syndrome

Gene: *STK11* 19p13.3 OMIM # 175200

Hamartomatous polyposis of the gastrointestinal tract and melanin pigmentation of the orofacial region. There is an increased risk of gastrointestinal and extragastrointestinal malignancies (Lalloo et al., 2005).

2.4.2 Familial adenomatous polyposis (FAP)

Gene: *APC* 5q21-22 (8535 bp) OMIM + 175100

FAP is the best known of colorectal polyposes and was already described in the 1880s. It is an autosomal dominant disorder with a population frequency of 1 in 10.000 (Bisgaard et al., 1994). One third is caused by new mutations. Penetrance is almost 100 percent at the age of 40. FAP is characterized by hundreds to thousands of adenomas through out the colon. Ninety percent of the mutation carriers will have adenomas by the age of 20 (Lalloo et al., 2005). Progression of one or more of these adenomas into cancer is inevitable.

FAP patients are at risk of extra colonic manifestations such as adenomas and adenocarcinomas of the stomach, the small bowel and the biliary tree, desmoids, osteomas, liver tumours, dental abnormalities, epidermal cysts and congenital hypertrophy of the retinal pigmeny epithelium (CHRPE). There is an excess of adrenal-, brain- and and papillary thyroid cancers. There seems to be a genotype phenotype correlation.

Surveillance: Annual/biannual sigmoidoscopy/colonoscopy from age 11-12 until adenomas appear. Optimal timing for colectomy depends on several factors, but in most cases between the ages of 16 and 20 years (Ponz de Leon et al., 1999).

2.4.3 *MUTYH* associated polyposis (MAP)

Gene:*MUTYH* 1p34.3-p32.1 (7100bp) OMIM * 604933

Recently, homozygous carriers of mutations in the base excision repair gene, *MUTYH* at 1p34.1, have been demonstrated to have a predisposition for multiple adenomas and colorectal cancer (CRC) causing autosomal recessive inherited disease (Jones et al., 2002). The gene encodes a glycolase which is involved in repair of DNA damage caused by reactive oxygen species generated during aerobic metabolism (Ames et al., 1991; Halford et al., 2003). In variant forms this ability is reduced (Al-Tassan et al., 2002).

Sequence variants in *MUTYH* are found in different ethnic groups (Jones et al., 2000). Two common *MUTYH* mutations, Y165C and G382D, account for approximately 85% of the mutations occurring in individuals of Caucasian ancestry (Jones et al., 2000). Combined data from previous studies suggest that about 1% of colorectal cancer cases can be attributed to biallelic *MUTYH* mutations (Halford et al., 2003; Enholm et al., 2003; Fleischman et al., 2004; Kambara et al., 2004).

Phenotypic expression of the *MUTYH* mutation in the heterozygous form is not clear. The mismatch repair genes also have roles in the removal of oxidative damage (Gu et al., 2002). It is demonstrated that the MSH2/MSH6 complex is physically associated with *MUTYH* at an MSH6 binding site and is required to stimulate *MUTYH* activity (Gu et al., 2002; Bai et al., 2005). Disruption of the MSH2/MSH6 complex causes HNPCC, and it has been reported that *MUTYH* mutations are associated with colorectal cancer risk and can be associated with the clinical features of HNPCC (Kairupan et al., 2005; Ashton et al., 2005).

2.5.0 Hereditary non polyposis colon cancer (HNPCC) (= Lynch syndrome)

HNPCC was originally called the “Cancer Family Syndrome”. The first family published, was “Family G” by Aldred Warthin in 1913 (Warthin AS, 1913). The pedigree of the family demonstrated an aggregation of cancers of the colon, endometrium and stomach. The family

was revisited by Henry Lynch in 1971 (Lynch et al., 1971) who found that the family members still were producing an excessive number of cancers and that the cancers appeared to be inherited in an autosomal dominant manner. Distinctions were made between two hereditary colon cancer syndromes, one including colorectal cancers only and the other including colorectal cancer and cancer of the endometrium, ovary, stomach and cutaneous sebaceous neoplasms. The syndromes were named Lynch syndrome I and Lynch syndrome II respectively, but are now most often referred to as HNPCC.

2.5.1 Clinic

Because there is no specific clinical sign in the affected patient to make a diagnosis of inherited colorectal cancer, recognition of the inherited cause was previously limited to family history alone. Features are young age at diagnosis, multiple primaries, synchronous and metachronous cancers, proximal CRC and other HNPCC related cancers. HNPCC related tumours include colorectal-, endometrial-, ovarian-, stomach-, small bowel-, pancreas-, ureter and renal pelvis-, hepatobiliary tract-, and brain tumours (usually glioblastoma), sebaceousgland adenomas and keratoacanthomas.

Some syndromes are associated with mutations in the HNPCC genes;

2.5.1.1 Muir-Torre syndrome

In this syndrome there is an occurrence of skin sebacous tumours with internal malignancy. Sebacous tumours are rare and associated with a high risk of internal cancers. (Rutten et al., 1999).

2.5.1.2 Turcot syndrome

In this syndrome there is a co-occurrence of colorectal adenomatous polyposis and primary brain tumour. Some cases might be due to autosomal recessive inheritance of two mutations in the same HNPCC gene (Hamilton et al., 1995).

2.5.1.3 Neurofibromatosis type 1

A syndrome has been identified in which children with biallelic DNA-MMR gene mutations in *MLH1, MSH2, MSH6* or *PMS2* develop clinical signs of Neurofibromatosis type 1, in particular café au lait spots and early onset neoplasia. The pattern of malignancies is characteristic with brain tumours, haematological malignancies in the first decade and colorectal cancer in the second decade (Trimbath, et al., 2001).

2.5.2 Clinical criteria

For research purposes, a description of families assumed to have HNPCC was adopted (Vasen et al., 1991). The criteria were referred to as the Amsterdam criteria (Amsterdam criteria I) and were designed to identify dominantly inherited colorectal cancer with early onset for research purposes to identify the underlying mutated genes (Vasen et al., 1991).

Similarly, to achieve higher sensitivity to identify affected families, another set of criteria referred to as the Bethesda guidelines were established (Rodriguez-Bigas et al., 1997) and later revised (Umar et al., 2004). The Bethesda guidelines were constructed to determine which tumours should be examined for MSI.

2.5.2.1 The Amsterdam criteria I: (Vasen et al., 1991).
At least three relatives in two successive generations with histologicaly verified colorectal adenocarcinoma, at least one being diagnosed at <50 years of age. FAP excluded. (All criteria must be met)

2.5.2.2 The Amsterdam criteria II: (Vasen et al., 1999).
Extension of the Amsterdam criteria II by including cancers of the endometrium, duodenum, ureter and renal pelvis in addition to colorectalcancers. (All criteria must be met).

2.5.2.3 Bethesda criteria: (Rodrigues-Bigas et al., 1997).
Individuals with cancer in families that meet the Amsterdam criteria I for HNPCC.

Individuals with two HNPCC-related cancers including synchronous or metachronous colorectal cancers or associated extracolonic cancers (endometrial-, ovarian-, gastric-, hepatobilary-, small bowel-, or upper urinary tract urothelial cancer).

Individuals with colorectal cancer and a first degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma, in whom one of the cancers was diagnosed before age of 45 and the adenoma was diagnosed before age 40.

Individuals with colorectal cancer or endometrial cancer before age 45.

Individuals with right-sided colorectal cancer with an undifferentiated pattern of histopathology diagnosed before age 45.

Individuals with signet-ring cell-type colorectal cancer diagnosed before age 45.

Individuals with adenomas diagnosed before age 40 (Familial adenomatous polyposis was excluded).

(Meeting any of the criteria listed is sufficient).

2.5.2.4 Revised Bethesda criteria: (Umar et al., 2004).
Colorectal cancer diagnosed in a patient who is less than 50 years of age.

Presence of synchronous or metachronous colorectal, or other HNPCC – associated tumours, regardless of age.

CRC with MSI-H histology diagnosed in a patient who is less than 60 years of age.

Colorectal cancer diagnosed with one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age fifty.

Colorectal cancer diagnosed with two or more first- or second degree relatives with HNPCC-related tumours, regardless of age.

(Meeting any of the criteria listed is sufficient).

2.5.3 Pathology

Rijcken et al (2003) have shown that it seems unlikely that hyperplastic polyps are precursors for cancers in patients with HNPCC. In HNPCC, the progression from adenoma to carcinoma may take < 3 years (Vasen et al., 1995; Lynch et al., 1996).

CRCs feature mucinous, poorly differentiated tumours and tumours with marked host lymphocytic infiltration and lymphoid aggregation at the tumour margin (Shashidharan et al., 1999). Right sided tumours. Endometrial tumours are of endometroid type (de Leeuw et al.,

2000). The infiltrating cancers may have a better prognosis compared to sporadic colorectal cancers with the same tumour histology (Sankila et al., 1996).

2.5.4 Molecular genetics

Genes: Mismatch repair genes (Bronner et al., 1994; Fishel et al., 1993; Leach et al., 1993; Nicolaides et al., 1994; Papadoupolous et al., 1994; Akiyama et al., 1997; Miyaki et al., 1997).

MSH2	2p16	(3111 bp)	OMIM # 120435
MLH1	3p21.3	(2484 bp)	OMIM # 609310
MSH6	2p16	(4200 bp)	OMIM + 600678
PMS1	2q31-33	(3063 bp)	OMIM + 600258
PMS2	7p22	(2771 bp)	OMIM + 600259

The MMR genes encode protein products which recognize and correct errors that arise when DNA is replicated (Leach et al., 1993; Dunlop et al., 1997). The major function of the DNA mismatch repair system is the elimination of base-base mismatches and insertion-deletion loops. MSH2 complexes with MSH6 and forms the hMutSα heterodimer which recognizes base-base mismatches. MLH1 and PMS2 form the hMutLα heterodimer which plays a role in coordinating the mismatch repair system.

Most of the MMR mutations found in humans have been detected in either *MSH2* or *MLH1* genes. The majority of mutations in *MLH1* and *MSH2* are evenly distributed, with some clustering in *MSH2* exon 12 and *MLH1* exon 16 (Peltomaki et al., 1997). Available evidence suggests that *PMS1, PMS2* and *MSH6* account for less than 5% of constitutional mutations detected in HNPCC families (Peltomaki et al., 1997). All classes of DNA variations may cause HNPCC; nonsense, frameshift, splice, missense, point mutations, exon deletions/duplications and rearrangements. Most *MSH2* alterations consist of frameshift (60%) or missense mutations (31%) (Peltomaki et al., 1997).

Whether or not missense mutations are disease-associated has become a practical clinical problem. Only the availability of a functional test will be able to determine the pathogenicity of the mutation. Functional tests for *MLH1* missense mutations have been done,

demonstrating that the majority of the investigated missense mutations had impaired repair function and were likely to be causative for HNPCC (Shimodaira et al., 1998; Guerette et al., 1999). In one of our papers we demonstrate that an *MSH2* missense mutation is disease associated (Stormorken et al., 2003).

One to two percent of all CRC occurs in MMR mutation carriers (Dunlop et al., 2000; Salovaara et al., 2000).

2.5.4.1 Probability for MMR mutations

Fulfillment of Amsterdam criteria I, number and mean age of colorectal cancers in the family and presence of endometrial cancer were entered into a multivariate logistic regression model to calculate probability for the affected patient to carry a *MLH1* or *MSH2* mutation (Wijnen, Vasen et al., 1998). The probability for *MSH6* mutation was not calculated. A family with a high mean age of diagnosis and without any cases of endometrial cancer has a low probability of harbouring an MMR mutation.

2.5.5 Microsatellite instability (MSI)

MSI is defined as presence of extra alleles at a microsatellite when compared with normal DNA from the same individual and results from frameshift mutations at repeats (Lalloo et al., 2005).

MMR genes correct mispairing that occurs during DNA replication. If the MMR system is dysfunctional, numerous replication errors (RER) accumulate in the genome. The majority of HNPCC tumours show MSI.

About 15-20 % of sporadic cancers demonstrate MSI. The figure is even higher in early-onset sporadic colon malignancy. This is usually due to hypermethylation of the *MLH1* promotors, thus MSI alone in colon cancer is a poor predictor of HNPCC. MSI in sporadic rectal cancers is rare, and is a good marker of HNPCC (Cunningham et al., 1998). MSI is also seen in about 15% of sporadic endometrial and gastric cancers.

2.5.6 Immunohistochemistry

The presence of MMR gene products may be analysed by using antibodies against MLH1, MSH2, MSH6 and PMS2 proteins. Staining can be performed on sections from formalin-fixed, paraffin embedded tissue blocks containing tumour tissue and normal adjacent mucosa.

The MLH1, MSH2, MSH6 and PMS2 staining patterns in normal cells are nuclear. Expression is particularly prominent in the epithelium of the digestive tract as well as in testis and ovary. In the intestine, expression is confined to the replicating compartment, i.e the bottom half of the crypts (Hamilton et al., 2000). Staining is evaluated using normal epithelial cells, stromal cells or lymphocytes in the same slide as controls. Loss of nuclear staining in tumour cells and normal staining of the internal control indicates loss of expression of MMR protein.

Tumours lacking normal MSH2 protein may also lack MSH6 protein (de Leeuw et al., 2000; Wagner et al., 2001; Schweizer et al., 2001; Stormorken et al., 2001; 2003; 2005). This is most likely the result of abrogation of the MutSα complex formed by the MSH2 and MSH6 proteins and acting as a mismatch recognition factor (Schweizer et al., 2001). It has been shown that MSH2 has two interaction regions with MSH6 (Guerette et al., 1998) and that the MSH6 protein is unstable in the absence of MSH2 (Chang et al., 2000; Marra et al., 1998). Thus, absence of MSH6 protein may be expected if no normal MSH2 protein is present.

PMS2 protein forms a heterodimer with the MLH1 protein. Absence of MLH1 protein due to a germline mutation also leads to loss of the PMS2 protein.

Polyps are “normally” seen in adult persons. In MMR mutation carriers, studies have shown that about 75% of adenomas detected in HNPCC families showed MSI as well as loss of protein (Iino et al., 2000). Still, some adenomas may show presence of all three proteins. This may indicate that the adenoma formation is not caused by the inactivation of the second allele (confer the two-hit model), but that they may have an increased propencity to become malignant in MMR mutation carriers. The “second hit” makes the polyp malignant, it may not cause the polyp (Rijcken et al., 2002).

2.5.7 Hypermethylation

MLH1 promotor hypermethylation and lack of expression of this protein has been shown in 10-15 % of sporadic colorectal cancers and occurs in about 15 % of sporadic endometrial cancers as well (Thibodeau et al., 1998; Kuismanen et al., 2000; Esteler et al., 1998). It is strongly associated with increasing age (Schweizer et al., 2001).

Biallelic inactivation of *MLH1* expression is caused by epigenetic inactivation of both parent copies of the gene. It has been shown that *MLH1* promotor methylation is associated with sporadic MSI cancers that lack MMR gene mutation and that methylation is not a common second hit mechanism in inactivation of MMR genes in HNPCC cancers (Veigl et al., 1998; Wheeler et al., 2000).

2.5.8 Mutation testing

Demonstration of disease-predisposing variations in DNA-base sequences is instrumental to identify high risk persons. Due to the heterogeneity of the mutation spectrum in MMR genes, complete mutation analysis is expensive, time consuming and only available in specialized centres. Once a mutation is detected in an affected family member it is possible to offer predictive testing to other family members.

2.5.9 Genotype-phenotype correlations and cancer risk

In a previous study (Vasen et al., 2001) *MSH2* mutation carriers were found to have a significantly higher risk of developing endometrial cancer and cancer of the urinary tract than *MLH1* mutation carriers. The risk of developing cancer of the stomach, ovary and brain was also higher in the *MSH2* mutation carriers than in the *MLH1* mutation carriers, but the difference was not statistically significant in this study.

We have previously shown a predominance of endometrial carcinoma and higher age at diagnosis in families with an *MSH6* mutation compared with families with an *MLH1* or *MSH2* mutation (Wijnen et al., 1999).

Mean percentage lifetime risk (by age 70 years) of developing any HNPCC related tumour was 76% for *MLH1* mutation carriers, 80% for *MSH2* mutation carriers and 73% for *MSH6* mutation carriers (Hendriks et al., 2004). The latter has a higher mean age at onset. For all mutations a higher risk of developing CRC is seen in male mutation carriers.

It has been shown that HNPCC tumours often have a better prognosis compared to sporadic colorectal cancers with the same tumour histology (Lynch et al., 1996; Sankila et al., 1996).

Genotype-phenotype correlations (Wijnen et al., 1999; Vasen et al., 2001; Hendriks et al., 2004).

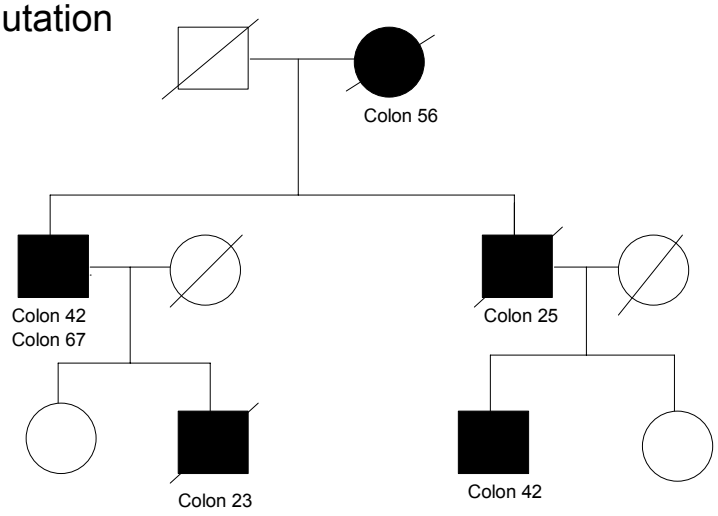
Gene	**Mean percentage cancer risk at 70 yrs**	**Mean age at diagnosis**
All HNPCC related tumours		
MLH1	76 (62-85)	
MSH2	80 (70-86)	
MSH6	73 (60-82)	
CRC in men		
MLH1	65 (39-80)	43 (16-81)*
MSH2	63 (49-73)	44 (16-90)*
MSH6	69 (42-83)	55 (26-84)
CRC in women		
MLH1	53 (33-66)	43 (16-81)*
MSH2	68 (43-82)	44 (16-90)*
MSH6	30 (12-44)	57 (41-81)
Endometrial carcinoma		
MLH1	27 (14-38)	48 (+/-5.5)**
MSH2	40 (21-54)	49 (+/-6.6)**
MSH6	71 (50-83)	54 (43-65)

*men and women in total, numbers from Vasen et al., 2001
** numbers from Wijnen et al., 1999

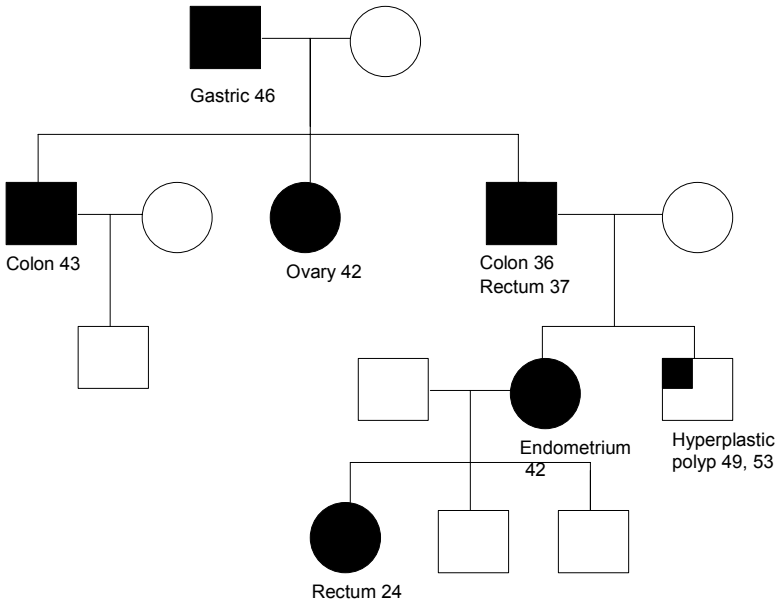
Examples of pedigrees:

○ = female ● = affected female □ = male ■ = affected male ⁄= diseased
Text under the symbol: cancer and age at diagnosis.

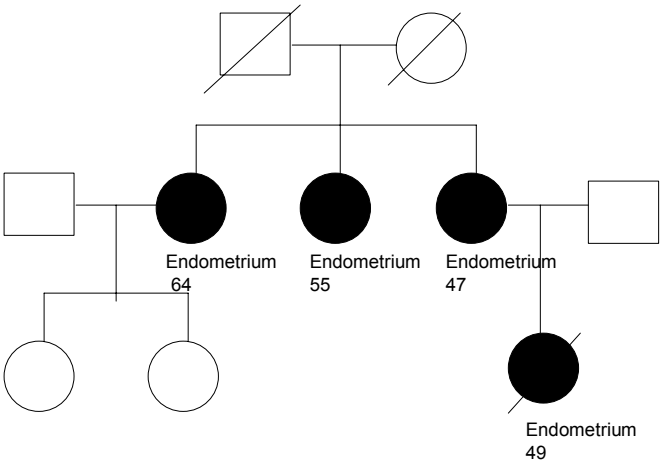
MLH1 mutation



MSH2 mutation



MSH6 mutation



2.5.10 Surveillance

Persons at risk are included in lifelong screening programs. Dedicated registries monitor the surveillance programs in some centres, giving the opportunity of assessing effects of intervention.

In HNPCC families without an identified mutation, assuming autosomal dominant inheritance with high penetrance, all first degree relatives of affecteds are assumed to have about one in three lifetime risk of HNPCC related cancer and are offered surveillance programs. When mutation testing is available, predictive testing should be offered and those not carrying the family mutation excluded from follow-up.

According to the guidelines proposed by the International Collaborative Group on HNPCC (www.insight-group.org), surveillance of the colon and endometrium is recommended for all mutation carriers. Surveillance of other sites is based on the phenotypes of the disease in each individual family. Family members may develop cancers at sights that are not included in the surveillance programs, demanding alertness from the physicians handling the patients.

2.5.11 Screening for colorectal cancer

Early detection and removal of adenomas or carcinomas may prevent colorectal cancer deaths (Järvinen et al., 2000). HNPCC patients appear to develop intestinal polyps at about the same frequency as the general population, but at a younger age (Vasen et al., 1995). The adenomas

are shown to be more likely to undergo malignant transformation and to display an accelerated adenoma to carcinoma transition as compared to the adenomas seen in the general population (Lynch et al., 1977; Jass et al., 1992).

According to the prevention guidelines of the International Collaborative Group on HNPCC (www.insight-group.org) total colonoscopy is the method of choice. Until 1996 the recommended interval between examinations was three years, after this, families suspected of HNPCC are recommended screening every two years from 25-30 years of age. If tubular adenoma(s) are detected or if the examination has not been complete a new colonoscopy should be performed the following year. Polyps detected are removed and evaluated by pathologist.

2.5.12 Extracolonic screening

Endometrium

Annual or biannual transvaginal sonography. The value of this surveillance is unknown
A liberal approach toward prophylactic hysterectomy for women older than 50 years with a truncating *MSH6* mutation has been advocated (Hendriks et al., 2004).

Gastric Cancer

Screening by endoscopy has been suggested, but is unproven and should only be discussed in families with a high incidence of this type of cancer.

Uroepithelial tract

Surveillance of the urinary tract should be considered in *MSH2* mutation carriers. (Vasen et al., 2001). The recommended protocol includes cytologic examination of the urine and sonography starting at the age 30-35 years old at 1-2 years intervals (www.insight-group.org). But one must be aware that sensitivity and specificity of these tests are yet unknown (Sijmons et al., 1998).

2.5.13 Treatment

Demonstration of mutation may give valuable information for selection of treatment modalities of HNPCC related tumours, because MMR deficient tumours seem to be resistant to certain chemotherapeutic agents (Sedwick et al., 1999).

2.6.0 Late onset hereditary gastrointestinal cancer (LOCRC)

The affecteds in the families tend to have onset of cancer at an older age. Inclusion criteria are four or more HNPCC related cancers in one lineage all diagnosed over 50 years of age (NGICG, 2005). The genetic basis of LOCRC is unknown and the adenoma-carcinoma sequence is not thought to be accelerated. They may be well served with colonoscopy every five years from 40 years on (NGICG, 2005).

3 BACKGROUND AND AIMS OF THE STUDY

Hereditary non-polyposis colorectal cancer may be caused by mutations in the mismatch repair genes *MLH1, MSH2, MSH6 or PMS2*. Family history (Amsterdam criteria) and immunohistochemistry in tumour specimens can be used as pre-screening tools to select families for mutation screening. Mutation carriers have an increased risk of developing colorectal- and other HNPCC related cancers. Persons at risk are invited to participate in surveillance programs aiming at early diagnosis and prevention of cancer.

The aim of this thesis was to Ddescribe hereditary colorectal cancer among Norwegian patients by using family history as the initial approach. We compared genotype-phenotype correlations and the cumulative risks for cancer. We wished to Ddevelop and validate methods used to select families at risk and to estimate the prevalences of such families. Finally we wanted to report the findings at prospective colonoscopic follow-up in hereditary CRC kindreds.

The clinical phenotypes of mutations in the MLH1, MSH2 and MSH6 genes and consequences for surveillance.

The risk of developing colorectal, endometrial and other cancers between families with various MMR-gene mutations might show genotype-phenotype variation. Survival analysis was used to calculate the cumulative risk of developing cancer in the various subsets of relatives. (Papers I, II)

The role of missense or inframe mutations in HNPCC.

Whether or not missense or inframe mutations are disease-associated has become a practical clinical problem. Demonstration of disease-predisposing mutations is instrumental to select high-risk persons for clinical examinations. One large kindred with an inframe *MSH2* codon N596 deletion was examined with IHC and LOD score was computed to demonstrate that the mutation was linked to disease. (Paper III)

Microsatellite instability and immunohistochemical analysis in endometrial tumours from HNPCC patients.

Instability of microsatellite repeat sequences has been observed in colorectal carcinomas and in extracolonic malignancies, predominantly endometrial tumours, occurring in the context of HNPCC. We wished to investigate whether MSI in endometrial hyperplasia and altered protein staining for the MMR genes indicated that inactivation of MMR genes is an early event in endometrial tumourigenesis. (Paper IV).

The prediction of MMR gene defects using family history and IHC as pre-screening methods.

According to the two-hit model of carcinogenesis, the second event in addition to the inherited mutation is expected to be an acquired mutation in the normal allele, leaving the gene product of the gene in question absent in the tumour cells. This has been demonstrated by immunohistochemistry. We validated the sensitivity, specificity and positive predictive value of immunohistochemistry compared with various clinical criteria, to select HNPCC kindreds for mutation testing. (Papers V, VI).

The role of MUTYH mutations in HNPCC and late onset familial colorectal cancer.

Recently, carriers of biallelic mutations in the base excision repair gene *MUTYH*, have been demonstrated to have a predisposition for multiple adenomas and colorectal cancer. In this paper we investigated the role of *MUTYH* mutations in families having been examined with IHC without showing loss of MMR protein and the role of heterozygous carriers of *MUTYH* mutations. (Paper VII).

Estimated prevalences of hereditary cancer and need for surveillance.

Surveillance of persons at increased risk is economically cost-beneficial if inherited cancers are prevented or cured by health care programs offered. Continued surveillance of an unnecessary high number leads to unjustified cancer worry, is costly and occupies health care facilities. Genetic workup was done in all persons (n=7224) attending the NORwegian Colorectal Cancer Prevention (NORCCAP) trial in Telemark county. Prevalence of hereditary cancer and need for surveillance was estimated. (Paper VIII).

Prevention of colorectal cancer by colonoscopic surveillance in families with hereditary colorectal cancer.

Persons at risk for CRC have the last years been subjected to follow-up by colonoscopy in many centres. Altogether 1133 healthy individuals were included according to their family

history of colorectal cancers and were prospectively followed with colonoscopies. We here report the observed results of our activity the last 15 years. (Paper IX).

4. SUMMARY OF PAPERS

The present thesis is based on the following papers:

I. *MSH2* mutation carriers are at higher risk of cancer than *MLH1* mutation carriers. A study of 138 HNPCC families.

HFA Vasen, A Stormorken, FH Menko, FM Nagengast, JH Kleibuker, G Griffioen, BG Taal, P Møller and JT Wijnen.

Journal of Clinical Oncology 2001; 19 (20):4074-4080.

We compared the risk of developing colorectal, endometrial and other cancers between families with various MMR-gene mutations. Clinical and pathologic data were collected from 138 families with HNPCC. Survival analysis was used to calculate the cumulative risk of developing cancer in the various subsets of relatives.

Mutations were identified in 79 families: 34 in *MLH1*, 40 in *MSH2* and five in *MSH6*. The lifetime risk of developing cancer at any site was significantly higher for *MSH2* mutation carriers than for *MLH1* mutation carriers (P<.01). The risk of developing colorectal or endometrial cancer was higher in *MSH2* mutation carriers than in *MLH1* mutation carriers, but the difference was not significant (P=.13 and P=.057, respectively). *MSH2* mutation carriers were found to have a significantly higher risk of developing cancer of the urinary tract (P<.05). The risk of developing cancer of the ovaries, stomach, and brain was also higher in the *MSH2* mutation carriers, but the difference was not statistically significant.

Pending large prospective studies, an extension of the current surveillance program in *MSH2* mutation carriers with the inclusion of the urinary tract should be considered.

II. Cancer risk in Hereditary nonpolyposis colorectal cancer due to *MSH6* mutations: Impact on counseling and surveillance.

YMC Hendriks, A Wagner, H Morreau, F Menko, A Stormorken, F Quehenberger, L Sandkuijl, P Møller, M Genuardi, H van Houwelingen, C Tops, M van Puijenbroek, P Verkuijlen, G Kenter, A van Mil, H Meijers-Heijboer, GB Tan, MH Breuning, R Fodde, JT Wijnen, AHJT Bröcker-Vriends and H Vasen.

Gastroenterology 2004; 127(1):17-25.

The cumulative cancer risks between *MSH6* and *MLH1/MSH2* mutation carriers were compared. MSI analysis and IHC were performed in the available tumours.

A total of 146 *MSH6* mutation carriers were identified. The cumulative risk for colorectal carcinoma was 69% for men, 30% for women, and 71% for endometrial carcinoma at 70 years of age. The risk for all HNPCC-related tumours was significantly lower in *MSH6* than in *MLH1* or *MSH2* mutation carriers (P = 0.002). In male carriers, the risk for colorectal cancer was lower in *MSH6* than in *MLH1* or *MSH2* mutation carriers, but the difference was not significant (P = 0.0854). MSI analysis in colorectal tumours had a sensitivity of 90% in predicting a mutation in *MSH6*.

We recommend starting colonoscopic surveillance in female *MSH6* mutation carriers from age 30 years. Prophylactic hysterectomy might be considered in carriers older than 50 years.

III. The inframe *MSH2* codon 596 deletion is linked with HNPCC and associated with lack of MSH2 protein in tumours.

A Stormorken, W Müller, A Lindblom, K Heimdal, S Aase, IM Bowitz Lothe, T Norèn, JT Wijnen, G Möslein and P Møller.

Familial Cancer 2003; 2(1): 9-13.

Whether or not missense or inframe mutations are disease-associated has become a practical clinical problem. One large kindred applying for health care had an N596del mutation in the *MSH2* gene.

Information on the site and classification of all cancers and age at diagnosis was obtained and verified. The mutation was tested for in all available affected persons. All available tumour material was examined by IHC for presence of MLH1, MSH2 and MSH6 gene products. Lod scores were computed by the MLINK programme.

All branches met the revised Amsterdam criteria, while only one branch met the original Amsterdam criteria. We demonstrated that the mutation was linked to disease with a lod score of 5.7 in the family, and all but one examined tumours lacked the MSH2 protein. The family demonstrated that the N596del mutation in the *MSH2* gene is highly penetrant and confers the usual expression of the gene. Presence or absence of mutation is now being used for predictive genetic testing in the kindred.

IV. Prediction of a mismatch repair gene defect by microsatellite instability and immunohistochemical analysis in endometrial tumours from HNPCC patients.
WJF de Leeuw, JW Dierssen, HFA Vasen, JT Wijnen, GG Kenter, H Meijers-Heijboer, A Brocker-Vrieds, A Stormorken, P Møller, F Menko, CJ Corneliosse and H Morreau.
Journal of Pathology 2000; 192:328-335.

Instability of microsatellite repeat sequences has been observed in colorectal carcinomas and in extracolonic malignancies, predominantly endometrial tumours, occurring in the context of HNPCC.

This study showed that all the endometrial carcinomas (n=12) from carriers of *MLH1* and *MSH2* germline mutations demonstrated an MSI-high phenotype involving all types of repeat markers, while in endometrial carcinomas from *MSH6* mutation carriers, only 4 out of 11 demonstrated an MSI-high phenotype. Interestingly, an MSI-high phenotype was found in endometrial hyperplasia from *MSH2* mutation carriers, in contrast to hyperplasia from *MLH1* mutation carriers, which exhibited an MSI-stable phenotype. Instability of only mononucleotide repeat markers was found in both endometrial carcinomas and hyperplasias from *MSH6* mutation carriers. In 29 out of 31 endometrial tumour foci, combined MSI and immunohistochemical analysis of MLH1, MSH2 and MSH6 could predict the identified germline mutation. The observation of MSI in endometrial hyperplasia and of altered protein staining for the MMR genes supports the idea that inactivation of MMR genes is an early event in endometrial tumourigenesis.

Assessment of altered protein staining combined with MSI analysis of endometrial tumours might direct the mutational analysis of MMR genes.

V. Prediction of the outcome of genetic testing in HNPCC kindreds using the revised Amsterdam criteria and immunohistochemistry.

A Stormorken, W Müller, B Lemkemeyer, J Apold, JT Wijnen, R Fodde, G Möslein and P Møller.

Familial Cancer 2001; 1:169-173.

Fifty-six families that were previously tested for *MLH1, MSH2* and *MSH6* mutations were selected for a pilot study. All pedigrees were extended and verified and the families were scored according to the Amsterdam I and Amsterdam II criteria. The probabilities for *MLH1* and *MSH2* mutations were calculated by logistic regression. In addition, all available tumour material from indexed family members was examined by IHC for the presence of the three gene products.

Three out of seven families where the mutation could be identified complied with the Amsterdam criteria I, while seven met the Amsterdam criteria II. All families carrying an *MLH1* or *MSH2* mutation had >15% calculated probability of finding a mutation. Tumours from all seven mutation carriers lacked the immunohistochemical expression of the corresponding MMR gene.

The results indicate that the Amsterdam criteria II in combination with immunohistochemistry of the mismatch repair proteins in tumours may be a cost-effective approach to select families for mutation analysis.

VI. Immunohistochemistry identifies carriers of mismatch repair gene defects causing Hereditary nonpolyposis colorectal cancer.

A Stormorken, IM Bowitz-Lothe, T Norèn, E Kure, S Aase, JT Wijnen, J Apold, K Heimdal, P Møller

Journal of Clinical Oncology 2005; 23:4705-4712.

After encouraging results in the pilot study we decided to expand the study. Pedigrees from 250 families were extended, cancer diagnoses were verified and families were classified according to the Amsterdam and the Bethesda criteria. Tumour specimens

were examined for the presence of MLH1, MSH2 and MSH6 proteins. Mutation analyses were performed in blood samples from the same patients.

Blood samples from affected index persons in 181 families and tumour specimens from 127 of these were obtained. Thirty tumours lacked one or more gene products. Sensitivity of IHC to detect mutation carriers was 100%, specificity was 82% and positive predictive value was 85%. Sensitivities, specificities and positive predictive values for the Amsterdam criteria were 82%, 8% and 45%, respectively, and for the Bethesda criteria were 100%, 0% and 48%, respectively. Distribution of mutations was *MLH1* = 4, *MSH2* = 11 and *MSH6* = 4.

Wide clinical criteria to select HNPCC kindreds, followed by immunohistochemistry of tumour material from one affected in each family had high sensitivity and specificity to predict MMR mutations.

VII. *MUTYH* mutations do not cause HNPCC or late onset familial colorectal cancer.

A Stormorken , KM Heintz , PA Andresen, E Hovig, P Møller.

Hereditary cancer in clinical practice 2006;4(2):90-93.

Reports on *MYH* mutations were reviewed and compared to our local findings. Our findings were that heterozygous *MYH* mutations did not cause familial clustering of colorectal cancer, and one homozygous carrier in our series of CRC patients did not meet the criteria for FAP or AFAP.

We conclude that *MYH*, when mutated, causes a rare recessively inherited disorder including colorectal- and duodenal cancers, which is phenotypically different from FAP. It is not verified that heterozygous carriers of *MYH* mutations have an increased risk of cancer.

VIII. Estimated prevalences of hereditary cancers and need for surveillance in a Norwegian county, Telemark.

A Stormorken, G Hoff, J Norstein, IM Bowitz-Lothe, E Hanslien, E Grindedal, P Møller.

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All persons attending the NORwegian Colorectal Cancer Prevention (NORCCAP) trial in Telemark, were interviewed about cases of cancer in the family. Diagnoses were verified, pedigrees constructed and families classified according to preset criteria aiming at identifying hereditary cancer. Mutation analyses were performed in kindreds at risk for breast cancers when possible. Immunohistochemistry of tumors in assumed inherited colorectal cancer families was undertaken.

The screening examination was attended by 7224 persons among whom 2866 had cancer in the family. Of these, 2479 had no suspicion of any known inherited cancer syndrome. Family information questionnaires were mailed to 387 persons and returned by 191. Sixty-four of these 191 met the clinical criteria for familial cancer by family history after verification of diagnoses. Observed prevalences for being at risk for HBOC or HNPCC were 2.8‰ and 0.77‰ respectively.

The obtained number of colonoscopies and mammograms per year to serve those who needed so, were limited and reduced from 2866 with a family history of cancer to 64 proven cases by clinical genetic work-up. Continued surveillance of an unnecessary high number leads to unjustified cancer worry, is costly and occupies health care facilities. Genetic work-up is a one-time job, reduces input numbers to surveillance programs, provides a starting point for mutation testing and is economically cost-beneficial if inherited cancers are prevented or cured by health care programs offered.

IX. Prevention of colorectal cancer by colonoscopic surveillance in families with hereditary colorectal cancer.

A Stormorken, N Clarke, E Grindedal, L Mæhle, P Møller

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Persons at risk for colorectal cancers (CRC) were prospectively followed with colonoscopies. In this paper we report the observed results of our activity over the last 15 years.

Altogether 1133 individuals were included and observed for a total of 3474 follow-up years from the first to the last colonoscopy initiated by our activity. MMR mutations

were demonstrated in 6.5%. A total of 1383 polyps were removed, 72% were less than 5mm in diameter. Findings were scored as hyperplastic (n=887), adenomas with mild to moderate dysplasia (n=460), adenomas with high grade dysplasia (n=30) and cancers (n=6). Two cancers were observed after first colonoscopy, compared to 2.6 expected by chance and more than 20 expected under a hypothesis of dominantly inherited diseases in the families. Observed annual incidence rates for adenomas were similar in all groups, while in the mutation carriers they more frequently had progressed to severe dysplasia or infiltrating cancer.

A simple explanation for the combined findings may be that all selected families had a similar tendency to produce adenomas, while mutation carriers more frequently demonstrated dysplasia/cancer in the adenomas. The low annual incidence rates for CRC indicated that the removal of adenomas may have prevented cancers.

5 GENERAL DISCUSSION

The papers support the views that

- Genotype-phenotype correlations might influence the need for a more tailor made surveillance protocol.
- Amsterdam criteria II in combination with immunohistochemistry of the mismatch repair proteins in tumours may be a cost-effective approach to select families for mutation analysis.
- *MUTYH* mutations do not cause HNPCC or late onset familial colorectal cancer.
- Genetic work-up reduces input numbers to surveillance programs.
- The low annual incidence rates for CRC in our study indicated that the removal of adenomas may have prevented cancers.

5.1 Genotype-phenotype correlations might influence the need for a more tailor made surveillance protocol.

In collaboration with the Leiden group in the Netherlands we demonstrated substantial differences in cancer risks between *MLH1, MSH2* and *MSH6* mutation carriers (Papers I and II). We found a difference in cumulative lifetime risk of colorectal carcinoma between men and women. The results may be biased in different ways. The initial criteria for referral were clustering of CRC, which might have led to an overestimation of the risk of developing colorectal cancer. On the other hand, this should not influence the observed differences in developing cancer between the various groups of mutation carriers. Only patients who survived were available for mutation analysis, which might have led to an overrepresentation of cancers with a favourable prognosis. Environmental factors and modifying genes may have an influence. The potential effect of surveillance on cancers in the urinary tract and the endometrium was not examined.

5.2 Amsterdam criteria II in combination with immunohistochemistry of the mismatch repair proteins in tumours may be a cost-effective approach to select families for mutation analysis.

We went to Düsseldorf, Germany to learn more about IHC and to do a pilot study on IHC (Paper V). Results were encouraging, and we decided to validate screening of high risk groups with IHC on tumour tissue (Paper VI). The results show that applying the AMSII

increases the sensitivity and underlines that CRC can not be an obligatory requisite to define HNPCC. IHC is a functional test, demonstrating the transcription and translation of the gene as well as the presence of the gene product in proper concentration in the right place. Sensitivity and specificity is demonstrated to be good in high risk groups, but the method also has its obstacles. There is a logistical problem having to collect tumour blocks from various hospitals. We have reported on scoring problems, particularly in endometrial cancers (Paper IV). Old blocks might be fixated in ways that makes the quality of the staining poor. In our study, IHC of adenomas has not been a reliable tool to predict MMR mutations. Concurrent loss of MSH2 and MSH6 proteins makes distinction between underlying *MSH2* and *MSH6* defects impossible. *MLH1* promotor hypermethylation and loss of this protein creates a further problem. These problems might become more prominent if applying the method on sporadic cancers, but so far the method has not been validated in sporadic cancers in general. When mutation testing becomes more accessible or if there are populations with founder mutations one might choose to start directly with mutation analysis.

In a large family we demonstrated that the inframe mutation in the family and lack of MSH2 protein expression was associated with disease (Paper III). We had an extended pedigree and the possibility of examining several branches of the family. It is not clarified why an inframe deletion predicted to cause a protein short of one amino acid, is associated with the absence of the whole protein. It is possible that the protein was present, but not detected by the IHC method applied. Possibly more probable, the altered protein structure may have changed the functional properties of the protein, with the effect that it was not found in relevant concentrations where it should have been to exercise its function.

5.3 *MUTYH* mutations do not cause HNPCC or late onset familial colorectal cancer.

Families in our study without detected mutations in the MMR genes were tested to see if mutations in the *MUTYH* gene could explain the aggregation of cancers. (Paper VII). At the time we initiated our study, *MUTYH,* when mutated, was thought to cause a recessively inherited disorder including CRC. Later it has been debated whether heterozygous carriers of *MUTYH* may or may not have a slightly increased risk of CRC. It has also been demonstrated that the MSH2/MSH6 complex, active in MMR, is physically associated with MYH at an MSH6 binding site and is required to stimulate MYH activity. (Gu Y 2002, Bai H 2005). So much is still unknown regarding *MUTYH* that further studies are necessary.

5.4 Genetic work-up reduces input numbers to surveillance programs.

We demonstrated that estimated prevalences of hereditary cancer and need for surveillance were dramatically reduced by a proper clinical genetic work-up (Paper VIII). Our prevalences were lower than what others have reported. Some of these reports were based on consecutive cancer series and others were done in populations known to have a high prevalence of founder mutations. Major draw backs were that our patients were part of a research population with older probands who may have had a limited motivation to provide family history and that 48 persons were excluded due to previous CRC. Underreporting of cancers could not be addressed, as only claimed cases could (by legislation) initiate a search for conformation. On the other hand, persons at risk may be over-represented in screening programs.

5.5 The low annual incidence rates for CRC in our study indicated that the removal of adenomas may have prevented cancers

The observed results of follow-up colonoscopies done on our patients over the last 15 years indicated that the removal of adenomas may have prevented cancers (Paper IX).
The strengths of the study were the nation-wide selection of CRC families, and a large number of persons at risk included. The limitation was the low prevalence of mutation carriers demonstrated, but this may reflect a low prevalence in our population. In families without a demonstrated mutation, all first degree relatives of affecteds were offered surveillance. Assuming autosomal dominant inheritance this means that half of the first degree relatives probably had a risk close to the population risk which would influence the annual incidence rates. Perhaps the observation time of colonoscopy screening is not long enough. The low number of cancers demonstrated was a finding. The low power of demonstrating a protective effect of an intervention is a general problem in studies like this and might not be a limitation specific to our study.

5.6 Other comments

Based on international consensus meetings, national collaboration and results from our studies, The Norwegian group on inherited cancer have made recommendations on handling inherited colorectal cancer (Møller et al., 2006). The recommendations are realistic with regard to what can be done within the structure of the Norwegian health service and Norwegian legislation.

Under Norwegian legislation, persons at risk should make the initial contact with the proper health personnel, and not vice versa. Consequently, other families may contain different features than the cohort ascertained in this study.

6 CONCLUSIONS

Based on current knowledge, including the reports in the present thesis, the genetic work-up of families suspected to have HNPCC was reformatted and now starts with immunohistochemistry of MMR gene products in tumours from affecteds, followed by mutation analyses of the genes not having a demonstrated gene product in the tumours. If a causative MMR mutation is found, predictive genetic testing is used as basis for preventive modalities. If no lack of gene products is found in tumours, the probability for HNPCC is low and the families will have to conform strictly to the Amsterdam criteria to be subjected to colonoscopy every second year from early adulthood. This has the effect of reducing work-load for the health care system, reducing unsubstianted fear in the families, and providing rationale for better targeted preventive measures to the mutation carriers with respect to other expressions of the genetic defects, also considering the different expressions of the different genes when mutated.

The low annual incidence rates for CRC in our study indicated that the removal of adenomas may have prevented cancers. This is an argument needed to continue the surveillance program in the mutation carriers, it is an information asked for by every patient, and it is to be included in any genetic counselling session to the families in question.

A number of unresolved problems remain. A few of them are: Families without detected mutations indicate that there might be other genes involved that we still have not mapped. We have insufficient information on long-term strategies on how to handle the colon in those who have been prevented to die from colon cancer at an early age. We do not know whether or not continued surveillance is effective or if there should be a more systematic approach to undertake prophylactic colectomy. As more colorectal cancers are prevented or cured, we will obtain more information about the frequency and mortality of the other phenotypes. Surveillance, prevention and cure of these manifestations need to be evaluated. To gain knowledge on these subjects, we have to continue monitoring the families. Because of low numbers included at any clinical genetic centre, multi-centre collaboration to provide sufficient data sets for analyses is needed. Future handling of these patients calls for broad collaborative studies to arrive at more definite conclusions.

7 REFERENCES

Aaltonen L, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, et al. Clues to the pathogenesis of familial colorectal cancer. Science 1993;260:812-816.

Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214-218.

Akiyama Y, Sato H, Yamada T, Nagasaki H, Tsuchiya A, Abe R, et al. Germ-line mutation of the hMSH6/GTBP gene in an atypical hereditary nonpolyposis colorectal cancer kindred. Cancer Res 1997;57:3920-3923.

Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, et al. Inherited variants of MYH associated with somatic G:C→ T:A mutations in colorectal tumours. Nat Genet 2002;30:227-232.

Ames BN, Gold LS. Endogenous mutagens and the cause of aging and cancer. Mutat Res 1991;250:3-16.

Arason A, Jonasdottir A, Barkardottir RB, Bergthorsson JT, Teare MD, Easton DF, et al. A population study of mutations and LOH at breast cancer gene loci in tumors from sister pairs: two recurrent mutations seem to account for all BRCA1/BRCA2 linked breast cancer in Iceland. J Med Genet 1998;35(6):446-449.

Ashton KA, Meldrum CF, McPhillips ML, et al. Frequency of the common MYH mutations (G382D and Y165C) in MMR mutation positive and negative HNPCC patients. Hereditary cancer in clinical practice 2005;3(2):65-70.

Bai H, Jones S, Guan X, Wilson TM, Sampson JR, Cheadle JP, et al. Functional characterization of two human MutY homolog (hMYH) missense mutations (R227W and V232F) that lie within the putative hMSH6 binding domain and are associated with hMYH polyposis. Nucleic Acids Research 2005;33(2):597-604.

Bisgaard ML, Fenger K, Bülow S, Niebuhr, Mohr J. Familial adenomatous polyposis (FAP): frequency, penetrance and mutation rate. Hum Mut 1994;3:121-125.

Boland CR, Sato J, Appelman HD, Bresalier RS, Feinberg AP. Microallelotyping defines the sequence and tempo of allelic losses at tumour suppressor gene loci during colorectal cancer progression. Nat Med 1995;1(9):902-909.

Bonithon-Kopp C, Benhamiche AM. Are there several colorectal cancers? Epidemiological data. Eur J Cancer Prev 1999;8(1):3-12.

Bosman FT. Prognostic value of pathological characteristics of colorectal cancer. Eur J Cancer 1995;31:1216-1221.

Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 1999;91:1310-1316.

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homologue MLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368(6468):258-261.

Cancer in Norway. Cancer Registry of Norway. www.kreftregisteret.no

CAPP1/2. www.ncl.ac.uk/capp

Chang DK, Ricciardiello L, Goel A,Chang CL,Boland R. Steady-state Regulation of the Human DNA Mismatch Repair System. J Biol Chem 2000; 275(24):18424-18431.

Clark JC, Collan Y, Eide TJ, Esteve J, Ewen S, Gibbs NM, et al. Prevalence of polyps in an autopsy series from areas with varying incidence of large-bowel cancer. Int J Cancer 1985;36(2):179-186.

Claus EB, Schildcraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer 1996; 77: 2318-2324.

Council of Europe. European Public Health Committee. Screening as a tool of preventive medicine. Report by a working party (1972-1974). Strasbourg 1974.

Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ, et al. Hypermethylation of the hMLH1 promotor in colon cancer with microsatellite instability. Cancer Res 1998:58:5248-5257.

Douglas F, O'Dair L, Robinson M, Evans DGR, Lynch SA. The accuracy of diagnoses as reported in families with cancer: a retrospective study. J Med Genet 1999;36:309-312.

Dove-Edwin I, Boks D, Goff S, Kenter GG, Carpenter R, Vasen HF, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. Cancer 2002;94(6):1708-1712.

Dunlop MG, Farrington SM, Carothers AD, Wyllie AH, Sharp L, Burn J, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997;6:105-110.

Dunlop MG, Farrington SM, Nicholl I, Aaltonen L, Petersen G, Porteous M, et al. Population carrier frequency of hMSH2 and hMLH1 mutations. Br J Cancer 2000;83:1643-1645.

Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107(4):1183-1188.

Eide TJ, Stalsberg H. Polyps of the large intestine in Northern Norway. Cancer 1978;42(6):2839-2848.

Eng C. Cowden syndrome. J Genet Counselling 1997;6:181-191

Enholm S, Hienonen T, Suomalainen A, Lipton L, Tomlinson I, Kärjä V, et al. Proportion and phenotype of MYH-associated colorectal neoplasia in a population-based series of Finnish colorectal cancer patients. Am J Pathol 2003;163:827-832.

Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG. MLH1 promoter hypermethylation is associated with microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene 1998;17: 2413-2417.

Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-767.

Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75:1027-1038.

Fleischmann C, Peto J, Cheadle J, Shah B, Sampson J, Houlston RS. Comprehensive analysis of the contribution of germline MYH variation to early-onset colorectal cancer. Int J Cancer 2004;109:554-558.

Gatta G, Capocacia R, Sant M, Bell CM, Coebergh JW, Damhuis RA, et al. Understanding variations in survival for colorectal cancer in Europe: a EUROCARE high resolution study. Gut 2000; 47:533-538.

Gryfe G, Gallinger S. Microsatelite instability, mismatch repair deficiency, and colorectal cancer. Surgery 2001;130: 17-20.

Gu Y, Parker A, Wilson TM, Bai H, Chang DY, Lu AL. Human MutY Homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/Human MutS homolog 6. J Biol Chem 2002;277(13):11135-11142.

Guerette S, Wilson T, Gradia S, Fishel R. Interactions of Human hMSH2 with hMSH3 and hMSH2 with hMSH6:Examination of Mutations Found in Hereditary Nonpolyposis Colorectal Cancer. Mol and Cell Biol 1998;18(11):6616-6623.

Guerette S, Achyara S, Fishel R. The interaction of the human mutL homologues in hereditary nonpolyposis colon cancer. J Biol Chem 1999;274:6336-6341.

Halford SE, Rowan AJ, Lipton L, Sieber OM, Pack K, Thomas HJ, et al. Germline mutations but not somatic changes at the MYH locus contribute to the pathogenesis of unselected colorectal cancers. Am J Pathol 2003;162(5):1545-1548.

Hamilton SR, Aaltonen LA. Pathology and Genetics. Tumours of the Digestive System. WHO. 2000:126-129. ISBN 9789283224105.

Hendriks YMC, Wagner A, Morreau H, Menko F, Stormorken A, Quehenberger F, et al. Cancer risk in Hereditary nonpolyposis colorectal caner due to *MSH6* mutations: Impact on counseling and surveillance. Gastroenterology 2004; 127(1):17-25.

Hemminki A, Peltomaki P, Mecklin JP, Järvinen, Salovaara R, Nyström-Lahti M et al. Loss of the wild type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. Nature Genetics 1994; 8(4): 405-410.

Hodgson S, Maher ER. A practical guide to human cancer genetics. 1999: Cambridge University Press. ISBN 0521649617.

Holmberg M, Kristo P, Chadwicks RB, Mecklin JP, Järvinen HJ, de la Chapelle A, et al. Mutation sharing, predominant involvement of the MLH1 gene and description of four novel mutations in hereditary non-polyposis colorectal cancer. Hum Mutat 1998;11:482.

Houlston RS, Collins A, Slack J, Morton NE. Dominant genes for colorectal cancer are not rare. Ann Hum Genet.1992;56:99-103.

Houlston RS, Tomlinson IPM. Polymorphisms and colorectal tumour risk.

Gastroenterology 2001;121:282-301

http://www.insight-group.org/ (ICG-HNPCC)

Iino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, et al. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 2000;47(1):37-42.

Jass JR, Stewart SM. Evolution of hereditary non-polyposis colorectal cancer. Gut 1992;33(6):783-786.

Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, et al. High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. Cancer Res 1996; 56:3663-3665.

Jones S, Emmerson P, Maynard J, Best JM, Jordan S, Williams GT, et al. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. Hum Mol Genet 2002;11:2961-2967.

Jänne PA, Mayer RJ. Chemoprevention of colorectal cancer. N Eng J Med 2000;342:1960-1968.

Järvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomäki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyoposis colorectal cancer. Gastroenterol 2000;118:829-834.

Kahl G. Dictionary of gene technology. 1995. VCH publishers. ISBN 3-527-30005-8.

Kairupan CF, Meldrum CJ, Crooks R, Milward EA, Spigelman AD, Burgess B, et al. Mutation analysis of the MYH gene in an Australian series of colorectal polyposis patients with or without germline APC mutations. Int J Cancer 2005;116:73-77.

Kambara T, Whitehall VLJ, Spring KJ, Barker MA, Arnold S, Wynter CV, et al. Role of inherited defects f MYH in the development of sporadic colorectal cancer. Genes, chromosomes and cancer 2004;40:1-9.

Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159-170.

Knudson Jr AG. Hereditary cancer, oncogenes and antioncogenes. Cancer Res 1986; 45:1437-1443.

Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomaki P, et al. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. Am J Pathol 2000;156:1773-1779.

Lalloo F, Kerr B, Friedman J, Evans G. Risk assessment and management in cancer genetics. Oxford University Press. 2005. ISBN 978-0-19-852960-6

Lanspa SJ, Lynch HT, Smyrk TC, Strayhorn P, Watson P, Lynch JF, et al. Colorectal adenomas in the Lynch syndromes. Results of a colonoscopy screening program. Gastroenterology 1990;98:1117-1122.

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993;75(6):1215-1225.

de Leeuw WJF, Dierssen JW, Vasen HFA, Wijnen JT, Kenter GG, Meijers-Heijboer H, et al. Prediction of a mismatch repair gene defect by microsatellite instability and imunohistochemical analysis in endometrial tumors from HNPCC patients. J Pathol 2000;192:328-335.

Lieberman DA. Cost-effectiveness model for colorectal cancer screening. Gastroenterology 1995;109:1781-1790.

www. Lovdata.no. Bioteknologiloven

Lynch HT, Krush AJ. Cancer family “G” revisited: 1895-1970.Cancer 1971; 27:1505-1511.

Lynch H, Harris R. Management of Hereditary site specific colon cancer. Arch Surg 1977:170-174.

Lynch HT, Smyrk T. Colorectal cancer, survival advantage, and hereditary nonpolyposis colorectal carcinoma. Gastroenterology 1996;110:943-954.

Lynch HT, de la Chapelle A. Genetic susceptibilty to non-polyposis colorectal cancer. J Med Genet 1999;36(11):801-818.

Marra G, Iaccarino I, Lettierei T, Roscilli G, Delmastro P, Jiricny J. Mismatch repair deficiency associated with overexpression of the MSH3 gene. Biochemistry 1998;95(15): 8568-8573.

Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997;17(3):271-272.

Møller P. Genetic cancer: a challenge and a possible strategy. Journal of Cancer Care 1993;2: 94-99

Møller P, Evans G, Haites N, Vasen H, Reis MM, Anderson E, et al. Guidelines for follow-up of women at high risk for inherited breast cancer: Consensus statement from Biomed 2 demonstration programme on inherited breast cancer. Dis Markers 1999;15:207-211.

Møller P, Heimdal K, Apold J, Fredriksen Å, Borg Å, Hovig E, et al. Genetic epidemiology of *BRCA1* mutations in Norway. European Journal of Cancer 2001; 37:2428-2434.

Møller P, Borg Å, Evans G, Haites N, Reis MM, Vasen H, et al. Survival in prospectively ascertained familial breast cancer: analysis of a series stratified by tumor characteristics, BRCA mutations and oophorectomy. Int J Cancer 2002;101:555-559.

Møller P, Stormorken A, Apold J. Handling of hereditary intestinal cancer in Norway. Tidsskr Nor Lægeforen 2006; 15:55-59 (Norwegian)

NHMRC 1999. National Health and Medical Research council, Guidelines for prevention, early detection and management of colorectal cancer. Canberra.

NCBI.nlm.nih.gov/entrez//

Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, et al. Mutation of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994;371:75-80.

Norwegian Gastrointestinal Cancer Group (NGICG). Norwegian. http://ngicg.no

Nyström-Lathi M, Kristo P, Nicolaides NC, Chang S-Y, Aaltonen LA, Moisio A-L, et al. Founding mutations and Alu-mediated recombination in hereditary cancer. Nat Med 1995;1:1203-1206.

Nyström-Lathi M, Wu Y, Moiso A-L, Hofstra RMW, Osinga J, Mecklin J-P, et al. DNA mismatch gene mutations in 55 kindreds with verified or putative hereditary non-polyposis colorectal cancer. Hum Mol Genet 1996;5:763-769.

Oxford dictionary of biochemistry and molecular biology. Oxford University Press 1997. ISBN 0198547684

Palli D. Epidemiology of gastric cancer: an evaluation of available evidence. J Gastroenterol 2000;35(12):84-89.

Peltomaki P, Vasen HFA, the International Collaborative group on hereditary nonpolyposis colorectal cancer. Mutations predisposing to hereditary nonpolyposis colorectal cancer:database and results of a collaborative study. Gastroenterology 1997;113:1146-58.

Papadopoulos N, Nicolaides NC, Wei YF, Ruben S, Carter KC, Rosen CA,et al. Mutation of a mutL homolog in hereditary colon cancer. Science 1994;(263):1625-1629.

Passarge E. Color atlas of genetics. Thieme 2001. ISBN 0-86577-958-9.

Ponz de Leon M, Benatti P, Percesepe A, et al. Clinical features and genotype phenotype correlations in 41 Italian families with Adenomatosis Coli. Ital J Gastrenterol Hepatol 1999; 31: 850-60.

Ponz de Leon M. Colorectal Cancer. Springer Verlag. 2002. ISBN 3-540-43047-4

Rex DK, Smith JJ, Ulbright TM, Lehman GA. Distal colonic hyperplastic polyps do not predict proximal adenomas in asymptomatic average-risk subjects. Gastroenterology 1992; 102:317-319.

Rijcken FEM, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. Gut 2002;50:382-386.

Rijcken FEM, van der Sluis T, Hollema H, et al. Hyperplastic polyps in hereditary non-polyposis colorectal cancer. Am J Gastronterol 2003;98(10):2306-2311.

Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on hereditary nonpolyposis colorectal cancer syndrome:meeting highlights and Bethesda guidelines. J Natl Cancer Inst. 1997;89:1758-1762.

Rutten A, Burgdorf W, Hugel H, Kutzner H, Hosseiny-Malayeri HR, Friedl W, et al. Cystic sebaceous tumours as marker lesions for the MUIR Torre syndrome: a histopathologic and molecular genetic study. Am J Dermatopathol 1999; 21:405-413.

Salovaara R, Loukola A, Kristo P, Kaariainen H, Ahtola H, Eskelinen M, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. J Clin Oncol 2000;18:2193-2200.

Sanchez-Cespedes M, Esteller M, Hibi K, Cope FO, Westra WH, Piantadosi S, et al. Molecular detection of neoplastic cells in lymph nodes of metastatic colorectal cancer patients predicts recurrence. Clin Cancer Res 1999;5:2450-2454.

Sankila R, Aaltonen LA, Jarvinen HJ, Mecklin JP. Better survival rates in patients with MLH-associated hereditary colorectal cancer. Gastroenterol 1996;110:943-947.

Schweizer P, Moiso AL, Kuismanen SA, Truninger K, Vierumaki R, Salo R, et al. Lack of MSH2 and MSH6 characterizes endometrial but not colon carcinomas in hereditary nonpolyposis colorectal cancer. Cancer Res 2001;61:2813-2815

Sedwick WD, Markowitz SD, Veigl ML. Mismatch repair and drug responses in cancer. Drug Resist Update 1999;2(5):295-306.

Shashidharan M, Smyrk T, Lin KM, Ternent CA, Thorson AG, Blatchford GJ, et al. Histologic comparison of hereditary nonpolyposis colorectal cancer associated with MSH2 and MLH1 and colorectal cancer from the general population. Dis Colon Rectum 1999;42:722-726.

Shimodaira H, Filosi N, Shibata H, Suzuki T, Radice P, Kanamuru R, et al. Functional analysis of human MLH1 mutations in Saccaharomyces cerevisiae. Nature Genet 1998;19:384-389.

Shureiqi I, Reddy P, Brenner DE. Chemoprevention: general perspective. Critical reviews in Oncology/Hematology 2000;33:157-167.

Sijmons RH, Kiemeney LA, Witjes JA, Vasen HF. Urinary tract cancer and hereditary nonpolyposis colorectal cancer: Risks and screening options. J Urol 1998;160:466-470.

Stormorken AT, Müller W, Lemkemeyer B, Apold J, Wijnen J, Fodde R, et al. Prediction of the outcome of genetic testing in HNPCC kindreds using the revised Amsterdam criteria and immunohistochemistry. Fam Cancer 2001;1:169-173.

Stormorken A, Müller W, Lindblom A, Heimdal K, Aase S, Bowitz-Lothe IM, et al. The inframe MSH2 codon 596 deletion is linked with HNPCC and associated with lack of MSH2 protein in tumors. Fam Cancer 2003;2(1):9-13.

Stormorken A, Bowitz-Lothe IM, Norén T, Kure E, Aase S, Wijnen J, et al. Immunohistochemistry identifies carriers of mismatch repair gene defects causing HNPCC. J Clin Oncol 2005;23(21):4705-4712.

Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL. Natural History of untreated colonic polyps. Gastroenterology 1987;93(5):1009-1013.

Syngal S, Weeks JC, Schrag D, Garber JE, Kuntz KM. Benefits of colonoscopic surveillance and prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer mutations. Ann Intern Med 1998;129(10):786-96.

Thibodeau SN, Bren G, Schaid D: Microsatellite instability in cancer of the proximal clon. Science 1993;260:816-819.

Thibodeau SN, French AJ, Roche PC, Cunningham JM, Tester DJ, Lindor NM, et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 1996;56:4836-4840.

Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. Cancer Res 1998;58: 1713-1718.

Thiis-Evensen E, Hoff GS, Sauar J, Majak BM, Vatn MH. Flexible sigmoidoscopy or colonoscopy as a screening modality for colorectal adenomas in older age groups? Findings in a cohort of the normal population aged 63-72 years. Gut 1999;45:834-839.

Trimbath JD, Petersen GM, Erdman SH, Ferre M, Luce MC, Giardiello FM. Cafe-au-lait spots and early onset colorectal neoplasia: a variant of HNPCC? Fam Cancer 2001;1:101-105.

Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96:261-268.

Vasen HFA, Mecklin J-P, Meera Khan P, Lynch HT. The International Collaborative Group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). Dis Colon Rectum 1991;34: 424-425.

Vasen HF, Taal BG, Nagengast FM, Griffioen G, Menko FH, Kleibeuker JH, et al. Hereditary nonpolyposis colorectal cancer: results of long-term surveillance in 50 families. Eur J Cancer 1995;31A(7-8):1145-1148.

Vasen FA, Watson P, Mecklin J-P, Lynch HT, ICG-HNPCC. New clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the international collaborative group on HNPCC.Gastroenterology 1999;116:1453-1456.

Vasen HFA, Stormorken A, Menko F, Nagengast FM, Kleibeuker JH, Griffioen G, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: A study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol. 2001;19(20):4074-4080.

Vatn MH, Stalsberg H. The prevalence of polyps of the large intestine in Oslo: an autopsy study. Cancer 1982;49(4):819-825.

Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, et al. Biallelic inactivation of MLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc Natl Acad Sci USA 1998; 95(15):8698-8702.

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319(9):525-532.

Wagner A, Hendriks Y, Meijers-Heijboer EJ, de Leuw WJ, Morreau H, Hofstra R, et al. Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree. J Med Genet 2001;38:318-322.

Warthin AS. Heredity with reference to carcinoma as shown by the study of cases examined in the pathological laboratory of the University of Michigan. Arch Int Med 1913;12:546-555.

Watson P, Lynch HT. The tumour spectrum in HNPCC. Anticancer Res 1994;14(4B):1635-1639.

Wattenberg LW. Chemoprevention of cancer. Cancer Res 1985; 45:1-8.

Wheeler JMD, Loukola A, Aaltonen LA, Mortensen NJ, Bodmer WF. The role of hypermethylation of the hMLH1 promotor region in HNPCC versus MSI+ sporadic colorectal cancers. J Med Genet 2000; 37:588-592.

Wijnen J, van der Klift H, Vasen, Khan PM, Menko F, Tops C, et al. MSH2 genomic deletions are a frequent cause of HNPCC. Nat Genet 1998;20:326-328.

Wijnen JT, Vasen HFA, Khan PM, Zwinderman AH, van der Klift H, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Eng J Med 1998;339:511-518.

Wijnen JT, Leeuw Wd, Vasen H, van der Klift H, Møller P, Stormorken A et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nature Genetics 1999; 23, 142-144.

Wilcox GM, Beck JR. Early invasive cancer in adenomatous colonic polyps (”Malignant polyps”). Evaluation of the therapeutic options by decision analysis. Gastroenterology 1987;92:1159-1168.

Williams AR, Balasooriya BA, Day DW. Polyps and cancer of the large bowel: a necropsy study in Liverpool. Gut 1982;23:835-842.

Wilson JN, Jungner F. Principles and practices of screening for disease. Geneva: World Health Organization. Public papers (number 34), 1968.

Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, et al. Prevention of colorectal cancer by colonoscopic polypectomy. N Engl J Med 1993;329:1977-1981.