Introduction
In 1895, Aldred Warthin, a pathologist, described a family, family G, in which many relatives had a diagnosis of colorectal cancer. Family G is the first documented family with hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominantly inheriting disorder, predisposing affected individuals to the development of cancer. HNPCC is characterized by the development of colorectal and extra-colonic cancer, often at young age, accounting for 2-5% of all subjects with colorectal cancer and 1-3% of subjects suffering of endometrial cancer. 1

To achieve uniformity for purpose of investigational protocols, a set of diagnostic criteria was formulated at a meeting of the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. The so-called Amsterdam criteria are as follows: 1) at least three relatives in at least two successive generations should have histologically verified colorectal cancer; 2) one should be a first degree relative of the other two; 3) one of the colorectal cancers should be diagnosed under the age of 50 years; 4) familial adenomatous polyposis should be excluded.2 Some years ago, the criteria were modified to include extra-colonic tumors, i.e. endometrial, and small-bowel cancer and transitional cell cancer of the renal pelvis or ureter.3 HNPCC patients have a high rate of synchronous and metachronous tumor development. As can be derived from the criteria, HNPCC cancers are diagnosed at a relatively early age. Colorectal cancers occur predominantly in the proximal colon.

Almost a century after the first HNPCC family was recognized clinically, the genetic background of this disorder was largely unraveled. The first known susceptibility loci, originally identified in bacteria and yeast, were mapped to chromosome 2p16 (hMSH2) and chromosome 3p21 (hMLH1)4,5, accounting for the majority of reported HNPCC cases. To date, two other genes, MSH6 and PMS2, have been associated with (atypical) HNPCC. Three genes that were previously implicated as the cause of HNPCC in some families, MLH3, PMS1, and EXO1, have recently been shown to be unlikely causes of HNPCC.6-8 The HNPCC susceptibility genes, so called mismatch repair (MMR) genes, are part of a complex DNA repair system which is responsible for correction of mismatches, small insertions or deletions that arise by mis-incorporations or slippage of the DNA-polymerase during DNA replication. A hMSH2 heterodimer recognizes and binds directly to mismatched DNA sequences.9 The heterodimeric complex hMSH2-hMSH6 preferentially recognizes single mismatched base-pair while hMSH2-hMSH3 recognizes large insertions or deletions.10 A second heterodimeric
complex, of hMLH1 and PMS2, is then recruited and binds to the hMSH2-hMSH6 complex after ATP is bound, and together this group of four proteins target hEXO1 to actually do the repair. There is some redundancy to the system which helps explain why defects in MLH1 and MSH2 account for the majority of HNPCC. MLH1 can also form heterodimers with either MLH3 or PMS1 and MSH2 can bind to MSH3. MLH1 is always required for mismatch repair, but the redundancy of the proteins that can partner with MLH1 lead to modest phenotype, if any at all.

Short tandem-repeat sequences, microsatellites, are distributed throughout the human genome and typically consist of DNA repeats of up to six nucleotides, and the total length of the stretch is fewer than 100 base pairs. Microsatellites are prone to errors, “slippage”, during DNA replication, resulting in instability of the short repetitive base sequences or so called microsatellite instability (MSI) if not recognized and corrected by the mismatch repair system.\(^\text{11}\) Being due to a deficient MMR system, MSI is the molecular hallmark of hereditary nonpolyposis colorectal cancer.\(^\text{11,12}\) To facilitate uniformity, microsatellite instability was defined at a consensus conference at the National Cancer Institute using a reference panel of five microsatellite markers and was divided into the two phenotypes: MSI-high (instability at at least two markers) and MSI-low (instability at one marker).\(^\text{13}\) No instability at any of the analyzed markers is called a microsatellite-stable (MSS) phenotype. However, two large studies have reported that the distinction between MSI-L and MSS is unwarranted as if enough markers are studied across a large set of tumors, eventually all tumors show instability at at least one marker.\(^\text{14}\)

Genetic inactivation of a mismatch repair gene is generally associated with loss of immunohistochemical expression of the corresponding protein.\(^\text{15}\) Immunohistochemistry for MLH1 and MSH2 has a high sensitivity and specificity for screening for DNA mismatch repair defects caused by mutation in MLH1 and MSH2, respectively, and thus indirectly for identifying colorectal tumors of the MSI-high phenotype.\(^\text{16,17}\)

Cells deficient of MMR, show a 100-1000-fold increase in the rate of spontaneous mutations as compared to normal cells.\(^\text{18,19}\) Accumulation of replication errors, microsatellite instability, may occur within repeat sequences of coding genes relevant for growth control and differentiation, thereby enhancing malignant transformation. This form of genetic instability has been regarded as ‘microsatellite mutator phenotype’ and the tumorigenesis follows the so-
called microsatellite instability (MIN) or ‘mutator’ pathway. In the majority of sporadic colorectal cancers the carcinogenesis pathway is characterized by chromosomal instability (CIN) as evidenced by loss of heterozygosity and referred to as the ‘suppressor’ pathway. The two distinct genetic instability pathways follow a similar morphological pathway of colorectal carcinogenesis as described by Kinzler and Vogelstein, the adenoma-carcinoma sequence.20

The premalignant property of adenomas in HNPCC is clinically demonstrated by the beneficiary results of colonoscopy with polypectomy in the study of Jarvinen et al.21 The adenomas in the colon of HNPCC patients may occur on a sporadic basis but evidence also supports the theory that the adenomas are the consequence of dysfunctional DNA mismatch repair. Irrespective of the initiation process, adenomas in HNPCC seem to have an accelerated transformation rate to carcinomas. The change in morphology and histology is a reflection of progressive acquisition of a variety of genomic alternations and a disbalance in proliferation and apoptosis in neoplastic cells leading to malignancy. The repertoire of genes which, when mutated, contribute directly to the oncogenic properties and progression of hereditary nonpolyposis colorectal tumors are partially known. As mentioned above, instability may occur in coding microsatellites, resulting in frameshift mutations of corresponding genes, leading to truncated and thus useless proteins. Genes, such as TGFβRII and Bax, have been demonstrated to be specifically altered in MMR deficient colorectal cancer cells.22,23 The target gene mutation profile differs between colorectal and endometrial cancers with mismatch repair deficiency, illustrating the complexity of the disease. Beside the target genes, the differences between sporadic and HNPCC-specific mutated regulating genes leading to altered proliferation and apoptosis and subsequently to tumor progression remain to be clarified.

Preventing cancer in HNPCC is primarily focused on applying genetic screening and identifying subjects at risk for a variety of life-threatening or otherwise debilitating disease prior to onset of symptoms or other clinicopathologic manifestations of disease. To modify the cancer risk, these subjects can subsequently be included in screening programs. Due to the range of target organs at risk in HNPCC preventive interventions are trivial. Jarvinen et al have shown that colonoscopies with polypectomy at 3-year intervals and subsequent polypectomies result in a reduction in the incidence of colorectal tumors and in a significant survival advantage.21 With regard to screening for endometrial and ovarian cancer comparable
data are not available and the effectiveness has not been shown. The Dutch Working Party for Hereditary Cancer (STOET) has recommended annual gynecologic screening for all female HNPCC patients with vaginal ultrasound and serum CA125, starting at 25 years of age.

Persons with HNPCC are ideal candidates for cancer prevention strategies that attempt to modulate their risk; chemoprevention offers one such strategy. Pharmacologic prevention offers potential benefits: 1) modulation of the need for surgical prophylaxis toward a less extensive or delayed procedure, 2) less frequent demands for target organ surveillance, 3) greater personal control over cancer fates and interventive options for those at genetic cancer risk, 4) the potential for systemic or multi-site modulation of cancer risk. The nonsteroidal anti-inflammatory drug (NSAID) sulindac, inhibitor of cyclooxygenase (COX), has been shown to reduce the risk of developing adenocarcinoma of the colon, induce regression and prevent the development of adenomatous polyps in the colorectum in subjects with familial adenomatous polyposis (FAP). How sulindac or other NSAIDs exert these effects is not fully understood. Sulindac has been reported to display profound antiproliferative effects, to alter the cell cycle distribution, and to induce apoptosis in cell lines and in vivo.24-26 Potential biomarkers to predict treatment outcome are besides mucosal prostaglandin levels (result of inhibition of COX) epithelial apoptosis and proliferation.

Outline of this thesis

The first part of this thesis concentrates on further elucidating the carcinogenesis of HNPCC-related colorectal and endometrial cancer. The second part addresses the care of HNPCC patients by evaluating an existing endometrial screening program and studying the possible role for chemopreventive treatment with NSAID sulindac.

As mentioned above colorectal cancers in HNPCC have a right predominance, the majority occurring proximal of the splenic flexure. In Chapter 2 we investigated whether this proximal preponderance is due to a proximal preponderance of adenomas or (also) due to differences in transformation rates from adenoma to cancer in the distal and proximal colon. In Chapter 3 the clinical consequences of simultaneous inheritance of two gene mutations are reported. The altered carcinogenesis is explored and the complexity of the preventive and curative care in
this unfortunate situation is addressed. Adenomas are considered the premalignant lesions of colorectal cancer in HNPCC, complying with the Vogelstein adenoma-carcinoma sequence of tumorigenesis. Recently, it was shown that a small percentage of hyperplastic polyps exhibit microsatellite instability and it is suggested that these are the precursor lesion for microsatellite instable sporadic colorectal cancers. Whether hyperplastic polyps are possible premalignant lesions in HNPCC is examined and documented in **Chapter 4**.

The tumorigenesis in HNPCC differs from sporadic colorectal cancer at an early stage. In **Chapter 5**, we studied whether this difference could be explained from disparities in expression of several cell cycle and apoptosis-related proteins in relation to proliferation and apoptosis in HNPCC and sporadic adenomas. Furthermore, we studied endometrial cancers, in **Chapter 6**, in a similar immunohistochemical manner to identify differences in the carcinogenetic pathways of HNPCC and sporadic endometrial cancers. In HNPCC, women have an increased cumulative life time risk for endometrial and ovarian cancer. Therefore female members of HNPCC-families are offered a gynecologic examination, a transvaginal ultrasound and serum level CA 125 analysis to be performed annually. However, unlike colonoscopy, the effectiveness of gynecologic surveillance procedures has not been shown in either prospective or retrospective studies. **Chapter 7** describes a study which evaluates our 10 years experience in endometrial and ovarian cancer screening in women belonging to HNPCC-families to determine whether our present screening method achieves the aspired prevention or early detection of gynecologic cancers.

With the increased risk of cancer and the early age at which tumors are diagnosed a search for more than early detection, namely (primary) prevention is ongoing. The ultimate goal of the present thesis was- with the knowledge of the carcinogenesis of HNPCC colorectal cancers- to explore the potential role of sulindac in HNPCC chemoprevention. So the effects of sulindac were evaluated in HNPCC patients using surrogate end-points for cancer risk including epithelial cell proliferative activity, degree of apoptosis and expression in normal colonic epithelium of proliferation-, apoptosis- and cell cycle-involved genes. The results of a randomised, double-blind, placebo-controlled cross-over study in ascertained MMR gene mutation carriers and subjects with more than 50% risk to be MMR gene mutation carriers is described in **chapter 8**. Finally the results of the thesis are summarized and discussed in **chapter 9**.
References


