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Association of TERT polymorphisms with colorectal polyps and colorectal cancer risk

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Abstract

The present diploma thesis is part of the project "Molecular Epidemiology of Colorectal Cancer", and intends to develop a polygenic model of biomarkers in order to identify high-risk individuals and offer new insights into the prevention and treatment of colorectal cancer (CRC). With more than 4500 new incidence cases a year CRC represents one of the most common cancers in Austria. The telomerase reverse transcriptase (TERT) gene is essential for telomere length maintenance and stability in cancer cells. Genetic polymorphisms in the TERT gene may contribute to interindividual differences in the chromosomal stability and as a result influence individual colorectal polyp and CRC susceptibility. CRC is a multifactorial disease, environmental factors and genetic predisposition contribute to its development. Genetic factors include rare, but highly penetrant mutations and the more common genetic polymorphisms. In a case-control study, consisting of 182 colorectal carcinoma patients, 332 high-risk polyp patients, 1065 low-risk polyp patients and 1822 colonoscopy negative controls, five selected single nucleotide polymorphisms (SNPs) within the TERT were genotyped using the 5' nuclease TaqMan® MGB Assay. No significant overall association was found for the genotyped SNPs and colorectal cancer risk. However, a significant increased risk for high-risk polyps was seen among homozygous carriers of the intronic SNP rs2075786. As a consequence, these results suggest potential involvement of genetic variation in susceptibility to colonic polyps. However, investigations in larger cohorts are required to establish rs2075786 as a possible biomarker for high-risk colonic polyps, and to further characterize the effect of multiple genetic polymorphisms within telomere stability genes on colonic polyps and CRC risk.

Zusammenfassung

Die vorliegende Diplomarbeit ist Teil des laufenden Projekts „Molekulare Epidemiologie des kolorektalen Karzinoms" mit dem Ziel, ein polygenetisches Modell zu entwickeln um Patienten mit hohen Risikoprofilen zu identifizieren und neue Ansätze in der Prävention und Therapie des kolorektalen Karzinoms (KRKs) zu entwickeln. Mit über 4500 Neuerkrankungen jährlich zählt das KRK zu den häufigsten Krebserkrankungen in Österreich. Das Telomerase Reverse Transkriptase (TERT) Gen ist verantwortlich für die Erhaltung und Stabilität der Telomere in Krebszellen. Genetische Polymorphismen im TERT Gen könnten die chromosomale Stabilität und folglich das Risiko für Kolonpolypen und dem KRK beeinflussen. Das KRK ist eine multifaktorielle Erkrankung. Neben umweltbedingte Risikofaktoren können auch genetische Prädispositionen zur Entstehung des Tumors führen. Zu den genetischen Faktoren zählen seltene, hochpenetrante Mutationen sowie häufig vorkommende genetische Polymorphismen. In einer Fall-Kontroll Studie, bestehend aus 182 KRK Patienten, 332 Patienten mit Hochrisiko-Polypen, 1065 Patienten mit Niedrigrisiko-Polypen und 1822 Teilnehmern mit einem negativen Koloskopiebefund wurden die Genotypen von fünf ausgewählten "Single Nucleotide Polymorphisms" (SNPs) im TERT Gen, mittels dem 5' Nuklease TaqMan® MGB Assay bestimmt. Es konnte kein Zusammenhang zwischen den genotypisierten SNPs und einem veränderten KRK Risiko nachgewiesen werden. Homozygote Träger des intronischen SNPs rs2075786 wurden jedoch mit einem signifikant erhöhten Risiko für Hochrisiko-Polypen assoziiert. Die Ergebnisse dieser Studie weisen auf einen möglichen Einfluss genetischer Varianten auf die Suszeptibilität für Kolonpolypen hin. Weitere Studien in größerem Ausmaß sind jedoch erforderlich um rs2075876 als Biomarker zu validieren und den Einfluss von multiplen Polymorphismen in Telomerstabilitäts-Genen auf das kolorektale Polypen und KRK Risiko zu untersuchen.

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Abbreviations

ACF	Aberrant crypt foci
APC	Adenomatous polyposis coli
BMI	Body mass index
BMP4	Bone morphogenetic protein 4
CDH1	Cadherin 1
CI	Confidence interval
CIN	Chromosomal instability
COX-2	Cyclooxygenase 2
CRC	Colorectal cancer
DCC	Deleted in colorectal carcinoma
FAP	Familial adenomatous polyposis
FOBT	Fecal occult blood test
FRET	Fluorescence resonance energy transfer
GWA	Genome-wide association
hMLH1	Human mutL homolog 1
hMSH2	Human mutS homolog 2
hMSH6	Human mutS homolog 6
HNPCC	Hereditary nonpolyposis colorectal cancer
hPMS1	Human postmeiotic segregation 1
hPMS2	Human postmeiotic segregation 2
HWE	Hardy-Weinberg equilibrium
LD	Linkage disequilibrium
LOH	Loss of heterozygosity
MAF	Minor allele frequency
MGB	Minor groove binder
MIN	Microsatellite instability
MTHFR	Methylenetetrahydrofolate reductase
NFQ	Non-fluorescent quencher
NSAID	Non-steroidal anti-inflammatory drugs
NTC	No template control

OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbons
POT1	Protection of telomeres 1
RHPN2	Rho GTPase binding protein
R_n	Normalized reporter
SD	Standard deviation
SDS	Sequence detection software
SMAD 7	Mother against decapentaplegic homolog 7
SNP	Single nucleotide polymorphism
TERC	Telomerase RNA component
TERF1	Telomeric repeat binding factor 1
TERF2	Telomeric repeat binding factor 2
TERF2IP	TERF2 interacting protein
TERT	Telomerase reverse transcriptase
TGF-β	Transforming growth factor beta
TINF2	TERF1 interacting nuclear factor 2
T_m	Melting temperature
TNKS	Tankyrase
TNM	Tumor-node-metastasis
tSNP	Tagging SNP

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1. Introduction

1.1 Epidemiology

Colorectal cancer (CRC) is one of the major malignancies in the Western World. Worldwide the incidence of CRC varies widely, with a 25-fold variation between different countries. High incidences are reported from developing countries like North America, Australia, New Zealand and Japan. In contrast, incidence rates are low in Africa and Asia, and moderate in Southern parts of South America. Incidence rates also vary considerably within Europe. Geographic patterns show high incidences in Western and Eastern Europe, with the highest risk areas in the Czech Republic and Hungary (Parkin DM, 2004). These geographic variations probably reflect the influence of external and sociocultural factors on developing CRC. Indeed, changes in incident patterns from several migrant studies support the major role of environmental exposure and lifestyle factors on the etiology of this disease. Migrants quickly adopt patterns of new communities and lose the risk associated with their original home community within one generation (Boyle and Langman, 2000; Flood et al, 2000).
With an estimated rate of 195.400 new incidence cases in 2006, CRC was the third most common cancer in European men and the second most common form of cancer in European women. Accounting for about 12.2% of total cancer deaths in 2006, CRC represents one of the leading causes of cancer death in Europe, distributed almost equally among men and women (Ferlay et al, 2007). However, favorable patterns for both genders are observed in most of the European countries, with the exception of some Mediterranean (Greece, Portugal, Spain) and Eastern European (Bulgaria, Poland, Romania) countries (Fernández et al, 2005). Furthermore, CRC survival in Eastern European countries is lower than the European average. These substantial differences are probably related to differences in the stage at diagnosis and to the quality of therapeutic approaches (Gatta et al, 2000; Coleman et al, 2003).

In Austria a total of 4857 CRC cases were diagnosed in 2006, wherein cumulative rates showed striking variations by geographic area, with a clear east-west divide and the highest incidence rates in the Province of Burgenland (Statistik Austria, 2009a). With

about 2349 cancer deaths, it was the second major cause of cancer death in 2006 in both genders, exceeded only by lung cancer (Statistik Austria, 2009b). Nevertheless, the first study on survival data concerning Austria showed an increased 5-year survival rate in colon and rectal cancer patients compared to earlier results, probably explained by the effect of earlier detection and better therapeutic treatment (Haidinger et al, 2006).

1.2 Histopathology of colorectal cancer

The vast majority (95%) of CRCs are adenocarcinomas arising from the columnar surface epithelium and showing in some cases a mucinous component. Further rare malignancies of the large bowel include signet-ring cell carcinomas, squamous carcinomas, melanomas and lymphomas. In contrast, carcinoid tumors are seen rather frequently and are most common in the appendix. The anatomic distribution of cancers throughout the colon varies, but the majority of neoplastic lesions are located distal to the splenic flexure (60-70%), particularly in the sigmoid and descending colon. However, the literature demonstrates a steady migration of CRCs from distal to more proximal sites. It is still unclear whether this represents a true biological effect or only a consequence of a wider use of colonoscopy (Ponz de Leon and Di Gregorio, 2001). In addition, several factors such as increased age, female gender and the presence of comorbid illness are associated with a greater likelihood of developing CRC in a proximal location. Finally, proximal cancers have a tendency to present at a more advanced stage and therefore have a poorer prognosis (Gonzalez et al, 2001).

Generally, the prognosis of CRC is directly related to the stage of the tumor at time of diagnosis. Although there are several different staging systems, one of the most widely used is the Dukes' classification system established in 1932. To assess the best possible prognosis and to determine the treatment of patients, the system involves four different stages (Table 1). The Dukes' Stage A refers to invasive cancer confined to the submucosa without the involvement of lymph nodes. Stage B lesions are subdivided, including tumors invading the muscularis mucosae and tumors extending into the surrounding serosa. Dukes' Stage C is defined by the presence of lymph nodes, subdivided into C1 and C2. Stage D is characterized by the presence of distant metastases. There are substantial differences in survival according the stage of disease. Patients with Dukes' A have an

excellent prognosis, their 5-year survival rate is close to 100%. 5-year survival´rates in Dukes' B is about 80-85%, dropping to 50-70% in Dukes' C. The age-adjusted 5-year survival rate for colorectal adenomas is merely about 14% for Dukes' D (Yamada T, 1998).

Table 1. Dukes' classification of colorectal tumors (based on Yamada T, 1998)

Stage	Depth of invasion	Frequency (%)	Age-adjusted 5 year-survival (%)
A	Submucosa	15	95-100
B1 B2	Muscularis propria Serosa	>31	80-85
C1 C2	1-4 lymph nodes > 4 lymph nodes	>23	50-70
D	Distant metastases	30	5-15

However, there has been a gradual move from using Dukes classification to using the tumor-node-metastasis (TNM) classification, describing and recording more accurately the anatomic extent of the cancer. The staging is based on four classifying individual elements: the primary site (T), usually divided into T1 to T4, the presence or absence of regional lymph nodes (N), and the presence or absence of metastasis beyond the regional lymph nodes (M) (Greene and Sobin, 2008).

The overall prognosis and treatment choice for CRC is also related to the degree of differentiation i.e. the degree of similarity of tumor architecture to the structure of the organ from which the tumor arose. Tumors are stratified into four different histological grades (well differentiated, moderately differentiated, poorly differentiated and anaplastic). While a well-differentiated tumor will have a relatively good prognosis, the anaplastic grade is typical of a rapidly growing malignant tumor (Taylor et al, 1999).

1.3 Histopathology of colorectal polyps

Some CRC may develop “de novo”, but substantial evidence supports the importance of colonic polyps as a precursor in the development of CRC (Ponz de Leon and Di Gregorio, 2001). Morphologically a polyp is defined as any elevation or protrusion of mucosal surface and in general colonic polyps are classified into neoplastic and non-neoplastic polyps (Table 2), wherein adenomatous and hyperplastic are the most commonly detected types (Markowitz and Winawer, 1997; Colucci et al, 2003).

The vast majority of CRCs arise from premalignant transformation of an adenomatous polyp with a prevalence of 35% in Europe (Midgley and Kerr, 1999). Adenomas are monoclonal expansions of mutated epithelial stem cells (Bond JH, 2000), resulting from disordered cell replication and differentiation in the upper portions of the crypt (Ponz de Leon and Di Gregorio, 2001). However, of the remaining adenomas, only a small proportion is estimated to become malignant. An adenoma can be considered malignant when neoplastic cells have invaded down through the muscularis mucosa into the submucosa (Markowitz and Winawer, 1997). Colorectal adenomas are observed in all tracts of the large bowel and are quite rare under the age of 30 years, becoming more common with increased age (Cannon-Albright et al, 1994). Due their different manifestations of abnormal tissue architecture, colonic adenomas are divided histologically into tubular, tubulovillous and villous adenomas (Table 2) (Markowitz and Winawer, 1997; Colucci et al, 2003).

Table 2. Classification of colorectal polyps (based on Colucci et al, 2003)

Histological classification	Polyp Type	Malignant potential
Non-neoplastic	Hyperplastic polyps Hamartomas Lymphoid aggregates Inflammatory polyps	No
Neoplastic (adenomas)	Tubular adenomas (0-25% villous tissue) Tubulovillous adenomas (25-75% villous tissue) Villous adenomas (75-100% villous tissue)	Yes

Data from the National Polyp Study and St. Mark's Hospital have demonstrated that approximately 75-87% of adenomas are primarily tubular in architecture, 8-15% tubulovillous, and the residual 5-10% are villous (Muto et al, 1975; O'Brien et al, 1990). All types of adenomas exhibit some degree of dysplasia, graded into mild, moderate and severe, and determined by the degree of cytological epithelial atypia and glandular architectural distortion (Markowitz and Winawer, 1997). The National Polyp Study has shown that about 86% of adenomas show mild, 8% moderate, and 6% severe dysplasia (O'Brien et al, 1990). A severe dysplasia in an adenoma is considered as a selective marker for an increased risk in cancer. Probably most of the CRCs evolve through stages of increasingly severe dysplasia before becoming invasive lesions (Ponz de Leon and Di Gregorio, 2001). Additionally, the risk of an adenoma harboring a high-grade lesion or invasive adenocarcinoma is associated with larger polyp size and increased patient age (O'Brien et al, 1990). Furthermore, the St. Marks Hospital Study demonstrated an association of villous histology with an increased risk of malignancy. 4.8% of tubular adenomas, 22.5% of tubulovillous and about 40.7% of villous adenomas were malignant (Muto et al, 1975).

In 2006, Winawer et al, proposed new guidelines for colonoscopy surveillance after polypectomy, stratifying patients into those at lower or higher risk for a subsequent, advanced neoplasia. While patients with an increased risk have either three or more adenomas, or high-grade dysplasia, or villous features, or an adenoma (≤1cm), patients with low risk include those with one or two small tubular adenomas (≤1cm) with no high-grade dysplasia.

1.4 Molecular genetics of colorectal tumorigenesis

A fundamental paradigm for the pathogenesis of CRC is the adenoma-carcinoma sequence. According to this proposed genetic model, progressive accumulation of various, genetic alterations including the inactivation of tumor suppressor genes and the activation of proto-oncogenes, provides the stepwise pathological transformation of premalignant adenomas to invasive carcinoma. Although the alterations often occur according to a preferred sequence, the total accumulation rather than their order of occurrence seems to be most important in the development of CRC. The molecular

events themselves provide the epithelial cells with a growth advantage, leading to a clonal expansion of neoplastic cells (Fearon and Vogelstein, 1990). However, the neoplastic transformation of normal epithelial cells to early adenomas to invasive cancer is extremely variable, and a significant proportion of cases may arise through an alternative molecular pathway to the Vogelstein model (Smith et al, 2006). Important molecular events and processes driving colorectal carcinogenesis are therefore continually refined. However, the loss of genomic instability, in the form of chromosomal (CIN) or microsatellite instability (MSI) seems to be a crucial event for the pathological progression in CRC carcinogenesis. It provides a permissive cellular environment and accelerates the neoplastic process, ensuring subsequent strategic mutations. There is sound evidence that most CRC cases develop via the CIN pathway, resulting in an accumulation of numerical or structural abnormalities and a frequent loss of heterozygosity (LOH) (Worthley et al, 2007).

It is believed that aberrant crypt foci (ACF) are the earliest histological lesions in this pathway, harboring important genetic alterations for the development of CRC. Dysplastic ACFs in particular show the highest malignant potential, harboring mutations in the tumor suppressor gene adenomatous polyposis coli (APC) (Smith et al, 1994; Takayama et al, 1998). As a result, heterogeneous mutations in the APC gene are one of the earliest genetic changes associated with CRC carcinogenesis, and probably represent important key events for its initiation (Powell et al, 1992). Beside somatic APC mutations in sporadic CRC cases, germline mutations in the APC locus are responsible for the rare, inherited familial adenomatous polyposis (FAP) syndrome. The APC gene has a gatekeeper function in the colonic cell proliferation, it encodes a large protein with several functional domains, playing a role in the Wnt signaling pathway and the intercellular adhesion (Kinzler and Vogelstein, 1996). Loss or mutation of the gene results in a truncated protein, inducing polyp formation as a result of the loss of orderly cell proliferation, apoptosis, cell migration and mitosis (Villa E, 2000). Whether mutations in the tumor suppressor gene APC are particularly crucial and probably sufficient for the initiation of CRC carcinogenesis, several other genetic events are involved in the further tumor progression (Powell et al, 1992; Kinzler and Vogelstein, 1996). Candidate genes include the proto-oncogene K-ras (on chromosome 12p), the tumor suppressor gene p53 (on chromosome 17p) as well as allelic losses on chromosome 18q, containing the deleted in colorectal carcinoma (DCC) gene. While K-

ras mutations are believed to occur early, allelic deletions on 17p and 18q seem to be late events in the neoplastic process (Fearon and Vogelstein, 1990).
Approximately 85% of all CRC cases develop via the chromosome stability pathway (Grady WM, 2004), while the remaining sporadic and most hereditary nonpolyposis colorectal cancer (HNPCC) cases follow a different pathway, the phenotype of MIN. MIN results from a failure in the mismatch repair system and is characterized by a dramatic increase in genetic errors, fuelling the process of oncogenesis (Hoeijmakers JH, 2001).

1.5 Molecular epidemiology

In 1982, Perera and Weinstein introduced the term "molecular cancer epidemiology" as a new multidisciplinary approach in cancer research and prevention, in which advanced laboratory methods are used in combination with analytical epidemiology, to determine at the biochemical or molecular level specific exogenous agents or host factors, playing a critical role in human cancer pathogenesis (Perera and Weinstein, 1982). The incorporation of a wide range of biomarkers into epidemiologic studies should thereby provide a useful tool, having enormous potential in identifying risk populations with greater susceptibility and improving the exposure assessment. The biomarkers were generally categorized as markers of (1) internal dose, (2) biologically effective dose, (3) preclinical biological effects and (4) markers of susceptibility (Perera and Weinstein, 1982; Perera and Weinstein, 2000). Biomarkers of internal dose take into account individual differences in the absorption, the metabolism or the bioaccumulation of the substance and indicate the actual level of the compound or its metabolites within body tissues or excretions. Examples include cotinine, resulting from cigarette smoking exposure or 1-hydroxpyrene urinary levels from exposure to polycyclic aromatic hydrocarbons (PAHs). However, markers of internal dose do not indicate the amount of which a given compound has interacted with critical cellular targets. Therefore assays have been developed to measure the biologically effective dose of a compound, i.e. the amount of the activated agent that has actually reacted with the critical cellular macromolecules, such as DNA, RNA or proteins. The next category includes marker of early biological effects resulting from exposure and measured directly in the target tissue or in a surrogate source of cells. Representative biomarkers assess various types of

genotoxicity, including chromosomal aberrations, gene mutations, the activation of oncogenes and the inactivation of certain tumor suppressor genes (Perera and Weinstein, 2000). For example, the p53 tumor suppressor gene is mutated in about 40-50% of lung, breast, colon and other common cancers (Perera FP, 1997). The fourth and last category of biomarkers includes those related to inherited or acquired variations in host susceptibility, modulating the individual response to environmental carcinogens. This category is a promising area of research, providing insights into interindividual variation in human cancer risk and the complex interactions between environment and susceptibility factors in the multi-step process of carcinogenesis (Perera and Weinstein, 2000). Genetic influence on carcinogenesis is quite variable and ranges from highly penetrant mutations, accounting for a small proportion of incidences, to other more common low-penetrance mutations that influence individual biological response to environmental carcinogens (Perera FP, 1997). The detection and identification of low-penetrance mutations in genetic predisposition is of great importance especially in those cancers associated with lifestyle factors, such as diet, tobacco and alcohol (Kotnis et al, 2005).

1.5.1 Single nucleotide polymorphism

The most abundant and simplest genetic variation in the human genome takes the form of a stable inherited substitution of a single base, termed single nucleotide polymorphism (SNP). By the traditional definition a SNP has a minor allele frequency (MAF) of 1% or more in at least one population (Kruglyak and Nickerson, 2001). SNPs are dispersed throughout the genome and can be found at least on every 0.3-1 kilobase, suggesting an estimated total number of more than 15 million available SNPs in the human genome (Schork et al, 2000; Salisbury et al, 2003). While most of them are silent and do not alter the function or expression of a gene, an estimated 50.000-250.000 of SNPs will actually confer small to moderate biological effects (Chanock S, 2001; Erichsen and Chanock, 2004). For example, SNPs in coding regions may directly impact the protein function, while a SNP in a promoter region can influence gene expression (Schork et al, 2000). Functional polymorphisms are expected to have lower allele

frequencies and in fact the vast majority of coding region SNPs causing an amino acid change have an allele frequency below 5 % (Kruglyak and Nickerson, 2001).
Individuals carrying a particular allele at one locus often predictably carry specific alleles at other nearby variant sites, known as linkage disequilibrium (LD) (International HapMap Consortium, 2005). Blocks of SNPs in the same chromosomal region are not inherited randomly, but as a set of polymorphism alleles (haplotye blocks). Systematic studies of common polymorphisms are facilitated by the analysis of markers inherited on a haplotype (Erichson and Chanock, 2004). This block-like structure of LD and the existence of areas of low or high recombination rate lead to the identification of tagging SNPs (tSNPs), SNPs that are correlated with and can therefore be used to predict with a relatively high probability the set of alleles at other co-segregating tSNPs (Beckmann et al, 2007).
The ongoing discovery of SNPs and the characterization of haplotypes in human populations are having a fundamental impact on cancer biology, being a remarkable tool to investigate the interindividual differences in treatment response and the outcome of specific cancers (Erichsen and Chanock, 2004; Rebbeck et al, 2004). In addition, SNP and haplotype analysis may provide new insights into the complex correlation of exposure and cancer, having future implications for primary preventive (lifestyle, chemoprevention) and early intervention strategies (Erichsen and Chanock, 2004).

1.5.2 Molecular epidemiology of colorectal cancer

Several lifestyle factors have been associated with an increased CRC risk. Indirect evidence based on international differences in incidence patterns as well as migrant studies supports the importance of environmental risk factors on the etiology of CRC (Boyle and Langman, 2000; Parkin DM, 2004).
Dietary components have been estimated to determine up to 90% of the international difference in CRC incidence (Willett WC, 1989). Their protective or predisposing effect on CRC was investigated in several epidemiologic studies. There is sound evidence that a high intake of dietary fat and meat increases CRC risk (Willett et al, 1990), although controversial results exist (Flood et al, 2003). Heterocyclic amines, formed during certain cooking practices may be important carcinogens, and the individual risk may be

modified by polymorphisms in their metabolizing enzymes (Lang et al, 1994; Cheng et al, 1998). On the contrary, the consumption of dietary fibre has often been proposed to have a protective effect on CRC risk, however epidemiologic studies reported differences in the effect of components. Based on their physiologic function, components are commonly classified into insoluble, non-degradable constituents (mainly in cereal fibre) and soluble, degradable constituents (mainly present in fruit and vegetables) (Boyle and Langman, 2000). Most of the studies found no protective effect of cereal fibre, but found an inverse association with the total fruit and vegetable consumption and CRC risk (Steinmetz et al, 1991; Terry et al, 2001).

Furthermore, evidence indicates that tobacco smoking is one important risk factor of non-dietary origin. Long-term heavy cigarette smokers show a 2-3 fold elevated risk of developing CRC (Giovannucci E, 2001). In contrast, inverse associations have been demonstrated with increased physical activity (Colditz et al, 1997) and endocrine factors, like menopausal hormones (Hébert-Croteau N, 1998). Additionally, evidence is strong that non-steroidal anti-inflammatory drugs (NSAIDs), like aspirin seem to have a protective effect on CRC risk (Rosenberg et al, 1998). This beneficial effect has been ascribed to the inhibition of the cyclooxygenase 2 (COX-2), an enzyme central to the prostaglandin synthesis (Ulrich et al, 2006).

Several other environmental risk factors for CRC have been postulated, while some of the associations are still controversial, in having either a protective or causative effect (Boyle and Leon, 2002).

CRC is a multifactorial disease, beside the environmental risk factors, genetic predispositions contribute to an increased risk of developing CRC (De Jong et al, 2002). Since 1982 the molecular epidemiology has contributed to growing awareness of the importance of common genetic and acquired susceptibility factors in modulating risks from environmental carcinogens (Perera and Weinstein, 2000). Furthermore, CRC is one of the cancers for which most is known about the genes affected by cancer-causing mutations, their normal functions and their effects on carcinogenesis when they are mutated (De la Chapelle A, 2004).

- *High-penetrance mutations*

The role of high-penetrance mutations in CRC pathogenesis is well understood. Although, only a small proportion of all CRC incidence can be ascribed to highly

penetrant causative mutations, showing a strong hereditary component, a high lifetime risk and little environmental influence. Rare genetic syndromes conferring a high lifetime risk for CRC include FAP and HNPCC also known as Lynch Syndrome (De la Chapelle A, 2004).

The autosomal dominant syndrome FAP has a penetrance of ~100% and is caused by germline mutations in the gatekeeper gene APC, wherein the vast majority are nonsense or truncating mutations. Clinically the syndrome is characterized by an abnormal number of adenomatous polyps in the colon and rectum and extra colonic features, appearing during the second and third decades of life (Kinzler and Vogelstein, 1996; De la Chapelle A, 2004).

The genetic basis for the second inherited form, the HNPCC is caused by germline mutations of mismatch repair genes. Mutations in the hMSH2 and hMLH1 are responsible for a large majority of HNPCC cases (Peltomäki and de la Chapelle, 1997), several other mutations (hMSH6, hPMS1 and hPMS2) account for most of the remaining cases (Potter JD, 1999). Most clearly clinical features of HNPCC include a tendency to early onset and the predisposition to cancers of at least seven other organs (endometrium, stomach, ovaries, small bowel, hepatobiliary epithelium, uroepithelial epithelium and brain). Together the hereditary syndromes account for less than 5% of all colon cancer cases (De la Chapelle A, 2004).

- *Low-penetrance mutations*

The inherited cause of about 20-30% of all CRC cases (Grady WM, 2003) and a two fold risk in first degree relatives suggests that low-penetrance genes are responsible for the remaining familiarity of CRC cases and also for a high proportion of sporadic cases (De Jong et al, 2002). In contrast to high-penetrance mutations, the understanding of their influence on colorectal carcinogenesis is far less advanced. It is believed that common genetic traits pose a low individual risk, but could be important determinants of the high population attributable risk. Thus, the effect of low-penetrance mutations on the variation of CRC susceptibility is essential for understanding disease etiology and also for the development of new diagnostic, therapeutic and preventive strategies (De la Chapelle A, 2004).

The identification of biologically meaningful and disease causing genetic variants is the key challenge for genetic association studies (Rebbeck et al, 2004). Low-penetrance

alleles of extremely variable genes are implicated in the susceptibility for CRC and their selection requires detailed knowledge of biochemical and physiological pathways that are involved in the process of carcinogenesis (Kotnis et al, 2005). The candidate gene approach examines those genes that fit a plausible understanding of biology (Erichsen and Chanock, 2004). A number of promising susceptibility polymorphisms for CRC have emerged through this strategy. For example, there is strong evidence for significant associations between polymorphic variants in proinflammatory cytokines (like Interleukin 8) and an increased CRC and adenoma risk, highlighting the importance of the inflammatory processes implicated in the susceptibility of this disease (Gunter et al, 2006; Küry et al, 2008). Despite inconsistent results, there is also some evidence that the methylenetetrahydrofolate reductase (MTHFR), implicated in the folate metabolism, may represent one of the key players in CRC susceptibility. A meta-analysis supported the different role of MTHFR polymorphisms (C677T and A1298C) in colorectal adenoma and CRC. The 677T allele showed a small but significant protective effect against CRC compared with the 677C allele for a worldwide population. In contrast, a significantly decreased CRC risk was reported for the 1298C allele (Huang et al, 2007). In addition, genes involved in DNA repair have an important role for protecting the genome from cytotoxic damage caused by endogenous and exogenous agents and are of crucial importance as suppressors of carcinogenesis (Ishikawa et al, 2001). Hence, their altered function probably affects CRC risk. However, partly inconclusive data suggests that the individual cancer risk depends not only on a single SNP, but on a joint effect of multiple polymorphisms within different genes or pathways (Naccarati et al, 2007).

Candidate genes represent only a subset of the actual genes with variants that affect cancer risk (Rothman et al, 2001). The promising approach of genome-wide association (GWA) studies makes it possible to identify further common variants implicated in CRC susceptibility, without having to rely on choosing *a priori* candidates (Rebbeck et al, 2004). A current meta-analysis of GWA studies identified genetic variation in the bone morphogenetic protein 4 preproprotein (BMP4), a member of the transforming growth factor beta (TGF-β) family, as a susceptibility allele for CRC. In addition, three other previously unreported polymorphisms influencing CRC susceptibility were identified. These include: cadherin 1 (CDH1), having an established role in CRC carcinogenesis, the Rho GTPase binding protein 2 (RHPN2), involved in the regulation of the actin cytoskeleton and cell motility, and the region 20p12.3, rich in genes or predicted protein-

encoding transcripts (Houlston et al, 2008). Moreover, a susceptibility locus on 11q23, tagged by rs3802842, seems to be associated with CRC risk (Tenesa et al, 2008). The functional role of genetic variation in the 8q24 locus, as a risk factor for CRC was demonstrated in several GWA studies (Haiman et al, 2007; Tomlinson et al, 2007; Zanke et al, 2007). Furthermore, there is strong evidence suggesting that polymorphisms in mother against decapentaplegic homolog 7 (SMAD7) involved in the TGF-β and Wnt signaling are associated with CRC (Broderick et al, 2007). Both risk loci (8q24 and 18q21) were additionally confirmed by a recent meta-association study (Curtin et al, 2009).

Although GWA studies make it possible to scan the entire genome for associations, the candidate gene approach remains viable and also critical to confirm possible susceptibility genes identified by genome-wide approaches. In fact, candidate gene approaches have the advantage of maximizing the inferences about biological plausibility and the causality of complex diseases, like cancer (Rebbeck et al, 2004). Representative and promising candidate genes for CRC association studies are involved in the inflammation pathway, in the carcinogen metabolism, the DNA repair and in cell senescence and mortality, like telomerase (Houlston and Tomlinson, 2001).

1.6 Telomerase

Telomeres at eukaryotic chromosome ends are specialized structures composed of simple, tandemly repeated sequences and specific proteins (Blackburn EH, 1991). Functional telomeres protect chromosomal ends from degradation, aberrant recombination and end-to-end fusion and are therefore crucial for maintaining genomic stability and integrity. Due the inability of conventional DNA polymerase to fully replicate the single 3' end overhang of the lagging strand (the end replication problem), loss of telomeric DNA occurs with each cell division. Thus, telomeres progressively shorten with each replication to a critical length, resulting in genomic instability and cellular senescence. In contrast to most somatic cells; germ cells, embryonic stem cells and cancer cells circumvent telomere-mediated senescence by activation or inactivation of alternative pathways (Cong et al, 2002). The vast majority of human cancers express

high levels of telomerase activity (Shay and Bacchetti, 1997), a ribonucleoprotein enzyme responsible for the replication of chromosomal ends, providing the molecular basis for unlimited cell proliferation, through the addition of simple TTAGGG repeats (Blackburn EH, 2000). In human cells this holoenzyme constitutes a template RNA component (TERC) (Feng et al, 1995) and a reverse transcriptase (TERT), representing the catalytic protein subunit (Weinrich et al, 1997). The TERT gene is located on chromosome 5, compromising 16 exons and 15 introns (Cong et al, 1999).

In contrast to the ubiquitous expression of the RNA subunit, TERT is only expressed in embryonic stem cells and germ cells. In about 90% of human cancers, activated telomerase is responsible for telomere maintenance (Mathieu et al, 2004), although alternative mechanisms of telomere lengthening exist (Bryan et al, 1997). The important role of telomere stabilization was first described by Hahn et al, 1999a. They demonstrated that ectopic expression of the catalytic subunit TERT, and two oncogenes (largeT, ras) is sufficient for the tumorigenic conversion of human epithelium and fibroblast cells. Moreover, inhibition of the telomerase enzyme activity limited the growth of human cancer cells in vitro and also their tumorigenic pathway in vivo (Hahn et al, 1999b).

Several studies demonstrated an association between a high telomerase activity and CRC (Chadeneau et al, 1995; Engelhardt et al, 1997) and proposed an increased level of telomerase activity as an independent prognostic indicator of poor outcome in patients with CRC (Tatsumoto et al, 2000). In addition to the influence of telomerase activity on colorectal carcinogenesis, prognostic potential of TERT expression (Gertler et al, 2002; Gertler et al, 2004) and telomere length was reported (Gertler et al, 2004).

Telomere stability genes, including TERT, show a limited degree of nucleotide diversity and have a vital function in chromosomal integrity. Thus, genetic variation in these genes may not be well tolerated (Savage et al, 2005). The functional, polymorphic sequence -1327 T/C in the promoter region of TERT was associated with higher transcriptional activity, longer telomere length and an increased telomerase activity (Matsubara et al, 2006). Previous studies identified polymorphisms in several components of the telomerase complex as risk factors associated with rare and complex disorders, like cancer. Functionally important mutations in the TERT gene may be risk factors for marrow failure, impairing the telomerase activity by haploinsufficiency (Yamaguchi et al, 2005). A recent study from Savage et al, (2007) demonstrated an

association between genetic polymorphisms (-1381C>T, -244C>T, Ex2-659G>A) in TERT with reduced breast cancer risk, in individuals with a family history of breast cancer (Savage et al, 2007).

1.7 Aim of the study

The aim of the present thesis was to investigate whether any of the five genotyped SNPs within the TERT gene are associated with colorectal polyps or CRC risk.

The study population was recruited within a large CRC screening project in the Province of Burgenland and three hospitals in Vienna, consisting of 182 colorectal carcinomas, 1397 colorectal polyps and 1822 controls. The genotypes of the colonoscopy negative control group have been compared with three different risk groups, namely colorectal carcinoma group, high-risk and low-risk polyp group.

The recent study is part of the project “Molecular Epidemiology of Colorectal Cancer” with the main purpose of developing a polygenetic model for CRC and the aim to identify high-risk individuals and to allow more effective prevention and treatment strategies.

2. Material and Methods

2.1 Study population

The study population was recruited within the area wide Austrian project "Burgenland gegen Dickdarmkrebs" initiated by Dr. Karl March (KH Oberpullendorf). This large screening project targeted female and male inhabitants of the Province of Burgenland, aged 40-80 years. Subjects received an annual fecal occult blood test (FOBT)-screening with the purpose of decreasing carcinom-specific morbidity and mortality and improving the early diagnosis of CRC. More than 135.000 participants were included in this screening per year, wherein 2.500-3.000 patients with positive FOBTs received extensive diagnostic- work up. Between 2003 and 2007 a total of 3471 study participants were recruited within this molecular epidemiology project. Eligible cases included participants with a histologically confirmed, previously untreated CRC, newly diagnosed in this screening project. Further CRC cases were recruited from three hospitals in Vienna (Division of Oncology, Medical University of Vienna; Department of Surgery, SMZ Süd and Department of Gastroenterology, KH Rudolfstiftung).

Demographic, anthropometric and lifestyle factors (tobacco use, dietary habits) were assessed by a short questionnaire. All participants provided written informed consent.

For statistical analysis a classification in high-risk and low-risk groups was performed, depending on the villous tissue within the polyp (Table 3). The polyp group consisted of 332 patients with high-risk polyps and 1065 patients with low-risk polyps. The control group included a total of 1822 individuals with a positive fecal occult blood test, who underwent colonoscopy without apparent pathological findings.

Table 3. Classification of polyps in high-risk and low-risk

Classification	Polyp Type
High-risk group	Adenomatous tubulovillous polyps Adenomatous villous polyps Adenomatous tubular and tubulovillous polyps
Low-risk group	Hyperplastic polyps Adenomatous tubular polyps

2.2 Genomic DNA isolation

The purification of human genomic DNA from peripheral blood was performed according the QIAamp® DNA Blood Midi Kit spin protocol. (QIAGEN, Hilden Germany). 2 ml of each blood sample was added to 200 µl of QIAGEN Protease respectively, mixed briefly and incubated with 2.4 ml of lysis Buffer AL in a water bath (70°C) for about 30 minutes. After addition of 2 ml ethanol (100%) and mixing to obtain a homogenous solution, half of the solution was transferred into the midi column and centrifuged at 1850xg for 5 minutes. The DNA bound to the membrane was washed in two centrifugation steps (4000 rpm for 2 minutes, 4000 rpm for 18 minutes). The use of two different wash Buffers AW1 and AW2 improved the purity of DNA. Purified DNA was eluted with Buffer AE, incubated for 5 minutes and centrifuged at 4000 rpm for about 7 minutes.

The DNA concentration was quantified spectrophotometrically at a wavelength of 260 nm using the NanoDrop ND-1000 Spectrophotometer (PEQLAB Biotechnologie GMBH, Erlangen Germany). Samples were diluted with buffer AE to a working dilution of 10 ng/µl and stored at -80°C.

2.3 SNP selection

Four tSNPs in TERT (rs2736098, rs4975605, rs2736100, rs2075786) with a MAF greater than 3.5% were picked out for the HapMap CEU population (www.hapmap.org).

An additional SNP in the promoter region of TERT (rs2736940) was selected from the publicly available SNP500Cancer database (www.snp500cancer.nci.nih.gov/home.cfm) because of its likely functional and phenotypic significance. As regards to the nomenclature we referred to the SNP500Cancer database.

2.4 Genotyping of TERT polymorphisms

Genotyping was performed on a 7500 Fast Real Time PCR System (Applied Biosystems, California US) using standard protocols and reagents.

The TaqMan® pre-designed SNP Genotyping assay (Applied Biosystems, California US) was conducted using 96-well reaction plates optimized for 10 µl reactions. The genotyping reaction mix for a 10 µl approach with 20 ng DNA consisted of 2.875 µl Aqua bidestillata sterilis (Fresenius Kabi, Austria), 5 µl TaqMan® Genotyping Master Mix (Applied Biosystems, California US) and 0.125 µl 40X TaqMan® SNP Genotyping Assay. The TaqMan® Genotyping Master Mix contained all components except primers and probes.

Standard cycling conditions were an initial denaturation step at 95°C for 10 minutes for the activation of the AmpliTaq Gold® Polymerase, followed by 40 cycles of 92°C for 15 seconds (denaturation) and 60°C for 1 minute (anneal/extend).

2.4.1 TaqMan® MGB probes and primers

The TaqMan® SNP Genotyping assay included predesigned sequence specific primers and TaqMan® minor groove binder (MGB) probes (Table 4). Final concentrations of both primers (forward and reverse) were 36 μM, and for the probes 8 μM, respectively.

Table 4. Predesigned TaqMan® MGB probes for genotyping TERT polymorphisms

	Component	5' dye	Context sequence	3' dye	Allele
rs2735940 -1381C>T	Probe 1	VIC®	GGATTAC[**A**/G]GGTCGCT	NFQ/MGB	T
	Probe 2	FAM™	GGATTAC[A/**G**]GGTCGCT	NFQ/MGB	C
rs2736098 Ex2-659G>A	Probe 1	VIC®	GGGGGCC[**C**/T]GCGTGGT	NFQ/MGB	G
	Probe 2	FAM™	GGGGGCC[C/**T**]GCGTGGT	NFQ/MGB	A
rs4975605 IVS6-3133G>T	Probe 1	VIC®	AAGAAAG[**A**/C]AAGCCTC	NFQ/MGB	T
	Probe 2	FAM™	AAGAAAG[A/**C**]AAGCCTC	NFQ/MGB	G
rs2736100 IVS2-3777G>T	Probe 1	VIC®	AAAGCTA[**A**/C]AGAAACA	NFQ/MGB	T
	Probe 2	FAM™	AAAGCTA[A/**C**]AGAAACA	NFQ/MGB	G
rs2075786 IVS10+269T>C	Probe 1	VIC®	AGGAGCC[**A**/G]GGTCACC	NFQ/MGB	T
	Probe 2	FAM™	AGGAGCC[A/**G**]GGTCACC	NFQ/MGB	C

2.4.2 Allelic detection with TaqMan® MGB probes

The TaqMan® Genotyping Assay uses dual-labeled fluorogenic MGB probes to determine specific PCR products during the amplification reaction. The homogenous assay is based on two principles:
the technology of fluorescence resonance energy transfer (FRET) and
the 5' nuclease activity of the AmpliTaq Gold® DNA Polymerase.

The TaqMan® MGB probes incorporate a non-fluorescent quencher (NFQ) dye at the 3' end and a fluorescent reporter dye (VIC® or FAM™) at the 5' base, along with a MGB moiety. The presence of two probes labeled with two different reporter dyes enables bi-allelic genotyping. In the intact probe the reporter dye emission is adequately absorbed by the quencher due to the process of FRET. Primary conditions include close proximity of the fluorophors and overlapping of the absorption spectrum of the quencher with the emission spectrum of the reporter. After hybridization of the probe to the template strand, the 5'–3' nuclease activity of the AmpliTaq Gold® DNA Polymerase cleaves the reporter dye from the probe. The distance dependent energy transfer is interrupted and the released reporter dye emits its characteristic fluorescence signal. Thus, the increase in fluorescence intensity indicates that the probe specific PCR product has been generated. Based on a substantial increase in the VIC® or FAM™ fluorescence signal, homozygous polymorphic genotypes can be detected. An increase in both signals correlates with a heterozygous genotype (Table 5) (Applied Biosystems, 2004).

Table 5. Correlation between fluorescence signals and genotypes (based on Applied Biosystems, 2006)

Substantial increase in:	Indicates:
VIC® fluorescence only	Homozygosity for Allele 1
FAM™ fluorescence only	Homozygosity for Allele 2
Both fluorescences	Heterozygosity for both Alleles

Even single base mismatches between probe and targets reduce the efficiency of probe hybridization and destruction (Figure 1) (Livak KJ, 1999).

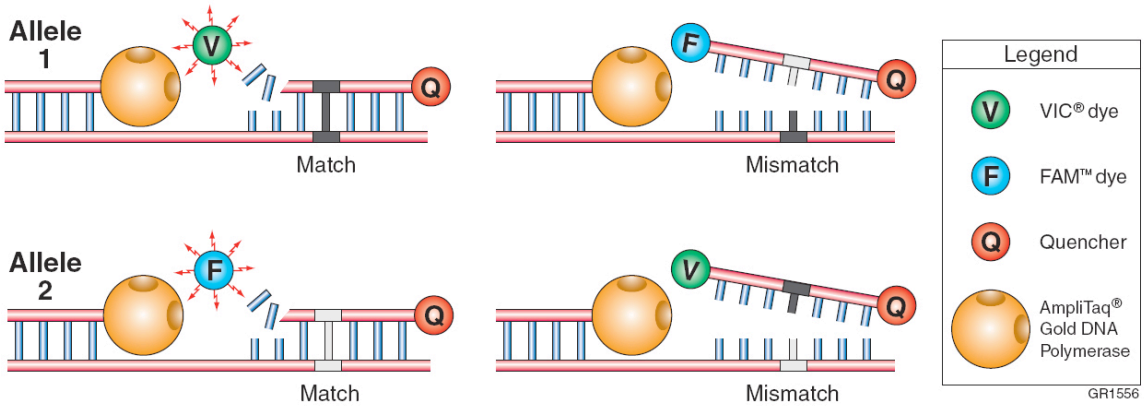

Figure 1. Principle of mismatch discrimination with TaqMan® 5' nuclease assay (Applied Biosystems, 2006)

Stable binding of perfectly matched probes compared to mismatches, improves allelic specific hybridization and the amount of reporter dye cleaved from a quencher. In addition, mismatched probes are more likely to be displaced from the target by the DNA Polymerase without degradation. Each of these factors contributes to a powerful mismatch discrimination and reduction of nonspecific fluorescence signals. Additional increase in mismatch discrimination is provided by the attachment of a MGB to the 3' end of the TaqMan® probe. MGB´s are relatively long, crescent-shaped molecules, which bind isohelically to the minor groove of DNA through van der Waals contacts, hydrophobic and electrostatic interactions. Conjugated MGBs enhance the stability of hybrid duplexes, causing an increased melting temperature (T_m), which allows shorter probe sequences (Afonina et al, 1997; Kutyavin et al, 1997). Shorter fluorogenic probes exhibit greater differences in T_m values between perfectly matched and mismatched probes, thus resulting in increased sequence specificity (Applied Biosystems, 2004)

Allelic discrimination is a multiplex assay, performed as an endpoint fluorescence reading using the 7500 Fast Real Time PCR System (Applied Biosystems, California US). The increase in fluorescence of the reporter dyes associated with the TaqMan® probes is measured by the sequence detection software (SDS). Corresponding normalized reporter (R_n) values are displayed on a scatter plot, grouped into four distinct genotype clusters:

homozygote for allele 1, homozygote for allele 2, heterozygote and no template controls (NTCs) to determine the background signal (Applied Biosystems, 2006).

2.5 Statistical analysis

Genotypic counts of controls were tested for Hardy-Weinberg equilibrium (HWE) using a χ^2 Test. Haplotype estimates were determined and the frequencies of the most common haplotypes were derived using the program FASTPHASE (Scheet and Stephens, 2006). LD statistics were computed using Haploview 4.0 (Barrett et al, 2005). Multiple logistic regression was applied to compare individuals of the control group against three different risk groups defined in Table 3.

Separate models were estimated where each of the five polymorphisms described in Table 4 was included as three-level-factor (homozygous wild-type, heterozygous, homozygous polymorph) and each haplotype was included as explanatory variable. Age and sex were used as confounders. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each polymorphism and haplotype; reference categories were wild-type and the most frequent haplotype, respectively.

Analysis of data was performed using the software R Ver 2.6.2. All p-values are 2-sided; p-values <0.05 were considered to be statistically significant.

3. Results

3.1 Study population

Demographic variables and lifestyle factors were analyzed in a study population of 3471 subjects. In total there were 1555 (44.8%) female and 1916 (55.2%) male probands, with significantly less male probands in the control group (Table 6; Figure 2).

Among the 3471 study subjects, 70 were excluded from TERT genotype analysis. 68 participants were without histological data and two participants without an indication of age. The remaining study population consisted of a total of 3401 eligible participants, consisting of 182 carcinoma patients, 332 high-risk polyp, 1065 low-risk polyp patients and 1822 colonoscopy negative controls.

Table 6. Sex distribution of the study population

Sex	Others[a]	Controls	Carcinomas	High-risk polyps	Low-risk polyps	Total
Female	24	972	74	115	370	1555
Male	44	851	108	218	695	1916
Male rate		0.47	0.59	0.65	0.65	
CI[b]		0.44-0.49	0.52-0.66	0.60-0.71	0.62-0.68	

[a] Participants without histology

[b] 95% confidence interval

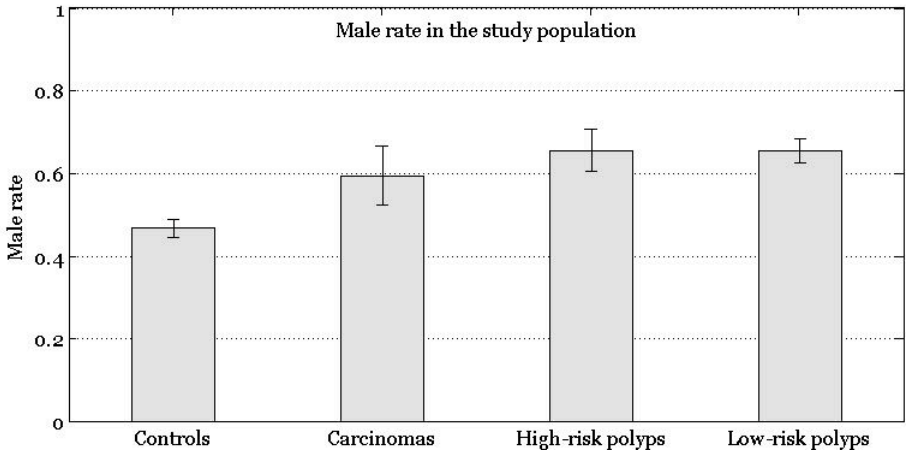

Figure 2. Proportion of male subjects in the study population with appropriate confidence intervals

The mean ages of the study participants are summarized in Table 7. Carcinoma patients (67.04±1.63 years) showed a significant higher mean age compared to the polyp and the control group. In contrast the mean age of the control group (61.35±0.52 years) was significant lower than the mean age in the three risk groups (Figure 3).

Table 7. Age distribution of the study population

Age (Years)	Others[a]	Controls	Carcinomas	High-risk polyps	Low-risk polyps	Total
≤50	5	356	15	45	143	564
≤60	14	442	38	70	240	804
≤70	25	585	53	126	394	1183
≤80	24	418	58	87	273	860
>80		21	18	4	15	58
Mean	65.32	61.35	67.04	63.24	63.19	62.47
SD[b]	9.80	11.23	11.14	10.06	10.30	10.91
CI[c]		60.83-61.86	65.41-68.67	62.15-64.32	62.58-63.81	

[a] Participants without histology

[b] Standard deviation

[c] 95% confidence interval

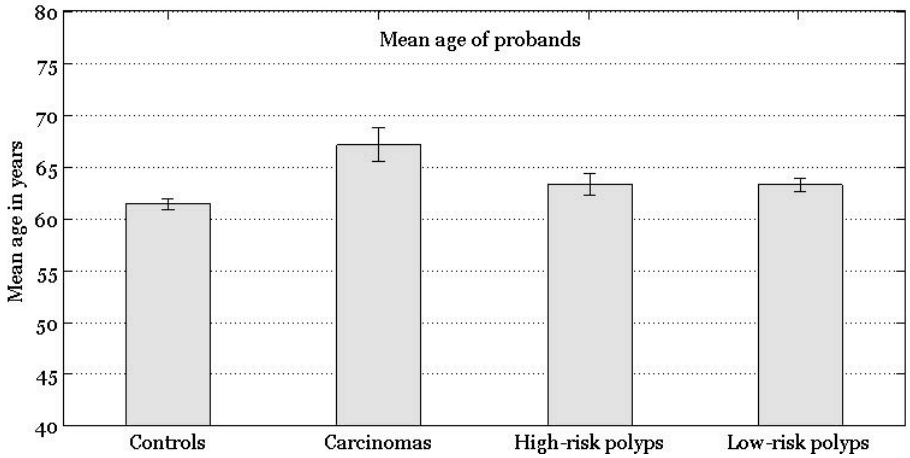

Figure 3. Histogram with age patterns and associated confidence intervals in the study population

The study population consisted of 1815 non-smokers, 555 current and 1021 former smokers (Table 8), with a slightly lower proportion of smokers seen in the control group (Figure 4). 80 participants were without an indication of their smoking status.

Table 8. Smoking status of study participants

Smoking Status	Others[a]	Controls	Carcinomas	High-risk polyps	Low-risk polyps	Total
Current	7	245	29	62	212	555
Former	23	492	71	104	331	1021
Never	34	1042	80	158	501	1815
Smoker rate[b]		0.41	0.56	0.51	0.52	
CI[c]		0.39-0.44	0.48-0.63	0.46-0.57	0.49-0.55	

[a] Participants without histology
[b] Former and current smokers
[c] 95% confidence interval

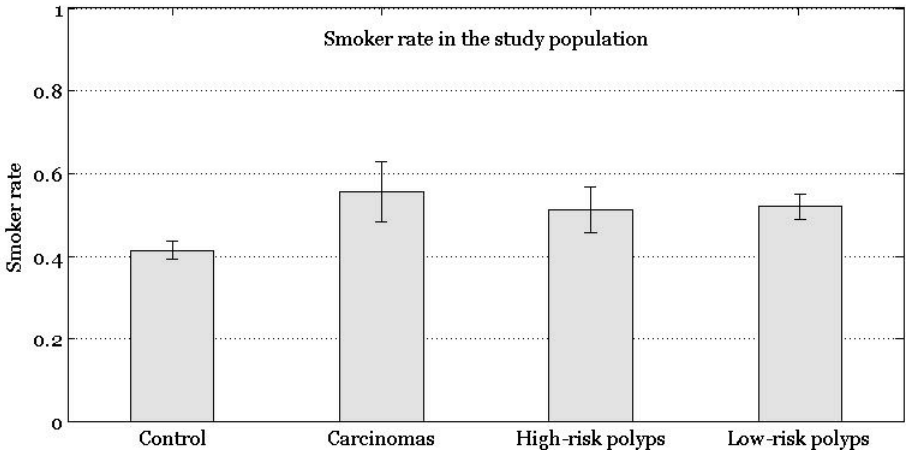

Figure 4. Rate of smokers among the study population

The mean body mass index (BMI) assessed for this study population was 28.68 kg/m^2. Data analysis showed no statistically significant differences between the BMIs in the high-risk groups and the controls.

The intake of meat and the education of the study participants were not significantly different between the controls, the carcinomas and the two polyp groups.

3.2 TERT genotypes

TERT genotypes (rs2735940, rs2736098, rs4975605, rs2736100, rs2075786) were determined using TaqMan® MGB probes (Applied Biosystems, California US). One probe was labeled with a VIC® dye and the other probe was labeled with a FAM™ reporter dye at the 5´base, respectively.

An allelic discrimination was performed on a post PCR product. Corresponding R_n values were displayed as points on a scatter plot, grouped into four different clusters corresponding to three different specific genotypes based on the graph location: homozygote wild-type, homozygote polymorphic, heterozygote and the NTCs. Figure 6 shows a representative example for a rs2735940 allelic discrimination plot. The PCR reaction included a VIC® labeled probe for the A/T allele (x-axis) versus a FAM™ labeled probe for the G/C allele (y-axis) of rs2735940.

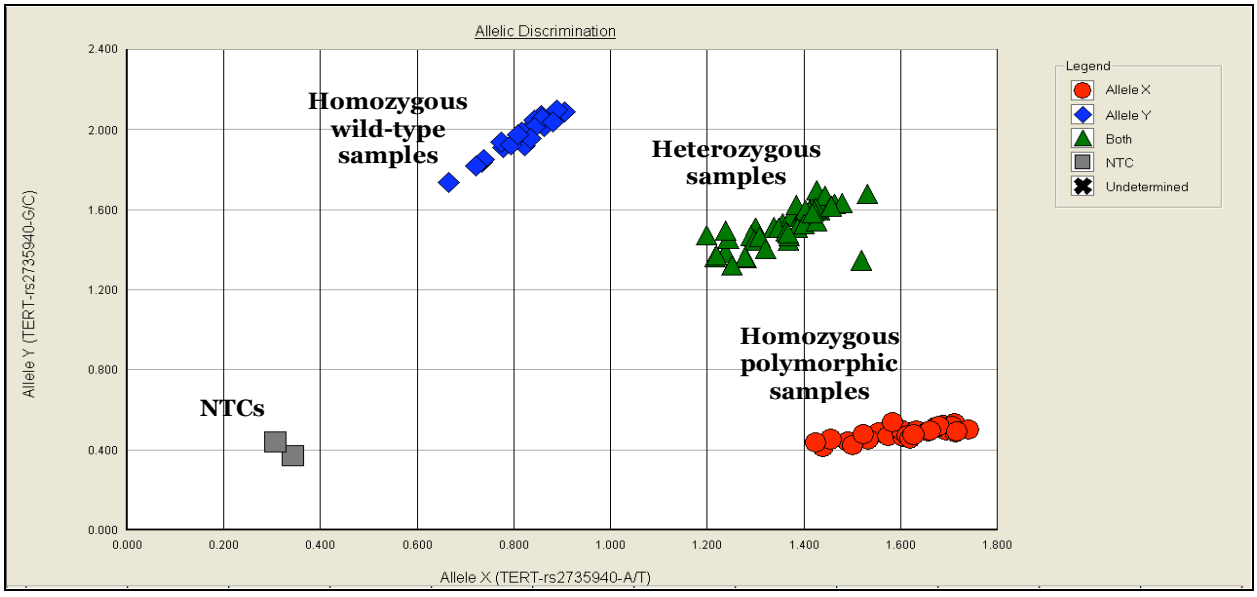

Figure 5. Example for a TERT rs2735940 allelic discrimination assay plot

The genotype distributions of the five analyzed SNPs in this study population were found to be in HWE. All ORs were calculated relative to the subjects with the polymorphic wild-type, i.e. homozygous for the major allele.

Multiple logistic regression was applied to compare the individuals of the control group against three different risk groups: carcinomas, high-risk polyp group, low-risk polyp group. The frequency distributions of the five genotyped SNPs are given in Table 9.
The data analysis showed no statistically significant association between the investigated TERT SNPs and CRC risk. However, for the polymorphism rs2075786 an association with an altered risk for high-risk polyps was found. The genotype distribution of the polymorphism in the study population was as follows: CC, 40% (1362); TC, 46.1% (1569) and TT, 13.8% (470), respectively. Carriers of the TT genotype were associated with an increased risk (OR: 1.44; 95% CI: 1.01-2.06) for high-risk polyps.

Table 9. Distribution of the TERT genotypes in association with colonic polyp and CRC risk

Genotype	Controls	Carcinomas			High-risk polyps			Low-risk polyps		
	n	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
rs2735940										
CC	475	56	1	0	100	1	0	281	1	0
CT	920	86	0.79	0.55-1.13	147	0.77	0.58-1.02	530	0.99	0.82-1.20
TT	427	40	0.80	0.52-1.24	85	0.96	0.70-1.33	254	1	0.81-1.25
rs2736098										
GG	1012	107	1	0	189	1	0	586	1	0
GA	681	63	0.84	0.61-1.18	123	0.97	0.75-1.24	406	1.03	0.88-1.21
AA	129	12	0.80	0.42-1.51	20	0.83	0.50-1.36	73	0.91	0.67-1.25
rs4975605										
GG	598	65	1	0	109	1	0	322	1	0
GT	867	92	0.96	0.69-1.36	162	1.03	0.79-1.34	543	1.16	0.97-1.38
TT	357	25	0.62	0.38-1.01	61	0.94	0.66-1.32	200	1.02	0.82-1.28
rs2736100										
TT	480	45	1	0	89	1	0	292	1	0
TG	925	99	1.14	0.79-1.67	154	0.91	0.68-1.21	535	0.96	0.80-1.16
GG	417	38	0.97	0.61-1.54	89	1.18	0.85-1.63	238	0.94	0.76-1.17
rs2075786										
CC	747	69	1	0	121	1	0	425	1	0
TC	833	87	1.10	0.79-1.55	157	1.18	0.91-1.53	492	1.05	0.89-1.24
TT	242	26	1.18	0.73-1.91	54	**1.44**	**1.01-2.06**	148	1.10	0.87-1.40

The MAFs in this study population were in concordance with those reported in the SNP500Cancer database (Table 10), with the exception of the intronic polymorphism rs2075786.

Table 10. MAF distribution for the TERT SNPs in the control group and in the SNP500Cancer database

SNP	MAF (Control group)	MAF (SNP500Cancer database)[a]
rs2735940 -1381C>T	0.487	0.452
rs2736098 Ex2-659G>A	0.258	0.258
rs4975605 IVS6-3133G>T	0.434	0.467
rs2736100 IVS2-3777G>T	0.483	0.483[b]
rs2075786 IVS10+269T>C	0.361	0.306

[a] Caucasian subpopulation
[b] Failed HWE in the SNP500Cancer population

To investigate multilocus associations, haplotype analyses were performed for the four tSNPs (rs2736098, rs4975605, rs2736100, rs2075786) within TERT. The most frequent haplotype (1113) was applied as a reference category. Corresponding adjustments for variables with potential confounding effects, like age and sex, were conducted.
The haplotypes were similarly distributed among the colonoscopy negative controls and the three different risk groups, obviously the evaluated combination had no influence on the development of colorectal polyps or CRC (Table 11).

Table 11. Distribution of the TERT haplotypes

Haplotype	Controls	Carcinomas			High-risk polyps			Low-risk polyps		
	n	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
1[a]113[b]	971	92	1	0	172	1	0	580	1	0
1111	198	20	1.03	0.61-1.73	41	1.17	0.80-1.70	107	0.92	0.71-1.19
1131	559	65	1.30	0.92-1.83	103	1.05	0.80-1.37	318	0.96	0.81-1.15
1133	122	6	0.50	0.21-1.17	24	1.10	0.68-1.76	67	0.92	0.66-1.27
1311	536	62	1.23	0.87-1.73	96	1.02	0.77-1.34	325	1.03	0.86-1.23
1313	79	10	1.38	0.68-2.79	11	0.79	0.41-1.52	54	1.12	0.77-1.62
1331	223	20	0.94	0.56-1.58	47	1.24	0.86-1.77	115	0.90	0.70-1.16
3131	292	30	1.02	0.66-1.59	38	0.71	0.48-1.03	162	0.89	0.71-1.11
3133	101	7	0.71	0.31-1.59	14	0.84	0.47-1.51	61	1.08	0.77-1.52
3331	201	21	1.17	0.70-1.94	47	1.40	0.97-2.01	131	1.13	0.88-1.45
3333	244	24	0.95	0.59-1.54	52	1.20	0.85-1.70	145	0.97	0.77-1-23
Rest	118	7	0.64	0.29-1.42	19	0.89	0.53-1.49	65	0.89	0.64-1.24

[a] Wild-type
[b] Polymorph

4. Discussion

The present diploma thesis examined five allelic variants within the telomere-related TERT gene and their implications on colonic polyp and CRC risk in an Austrian population. Overall, the results demonstrated no significant influence of the five genotyped polymorphisms on CRC susceptibility. However, polymorphic homozygous carriers of the intronic SNP (rs2075786) were associated with an increased risk for high-risk polyps.

Telomeres are crucial for genomic stability and integrity and their maintenance has emerged as a key biochemical and critical process in the regulation of cancer (Callén and Surrallés, 2004). The activation of telomerase has thereby been proposed as an essential step in the immortalization and progression of cancer cells (Meyerson M, 2000). Data suggests that in about 90% of human cancers activated telomerase is responsible for telomere maintenance (Shay and Bacchetti, 1997), whereas TERT is the catalytic subunit and the reverse transcriptase component of the holoenzyme, located at the 5p15.33 locus (Cong et al, 2002; Ducrest et al, 2002).
So far, several epidemiologic studies have investigated the impact of high telomerase activity on CRC (Chadeneau et al, 1995; Engelhardt et al, 1997; Brümmendorf TH, 2005) and proposed an increased level of telomerase activity as an independent prognostic indicator of poor outcome in patients with CRC (Tatsumoto et al, 2000). In addition, a prognostic potential of telomere length (Gertler et al, 2004) and TERT expression (Gertler et al, 2002; Gertler et al, 2004) was reported. On the contrary, less is known about how genetic variants within TERT contribute to an interindividual CRC susceptibility. Telomere stability genes (TERT, POT1, TNKS, TERF1, TINF2, TERF2 and TERF2IP) are highly conserved in sequence between species, showing a limited degree of nucleotide diversity. Hence, genetic variations in these genes, vital for chromosomal integrity may have a profound effect (Savage et al, 2005). In addition, given the relevance of this genomic region to cancer biology, TERT seems to be a plausible candidate for cancer association studies (Rafnar et al, 2009).

To capture most of the common genetic variation within TERT and to allow an economical interrogation of genotypes, the study focused on representative tSNPs, including non-coding (rs4975605, rs2736100, rs2075786) and coding SNPs (rs2736098). Moreover, an additional SNP in the promoter region (rs2735940) was selected due to the fact that functional SNPs may reside within regulatory elements. Another criterion for selecting SNPs was a relatively high MAF in Caucasians. The MAFs in this study population were in concordance with those reported in the SNP500Cancer database, except for the polymorphism rs2075786, which showed a slight difference. In addition, the intronic polymorphism rs2736100 failed the HWE in the SNP500Cancer database. With the exception of the polymorphism in intron 6 (rs4975605), all of the investigated allelic variants have previously shown to be associated with cancer risk (e.g. lung and breast cancer), having either a protective or predisposing effect. To my knowledge this is the first study investigating SNPs within the telomerase gene as possible susceptibility factors for colorectal polyps and CRC risk.

In the present study population polymorphic carriers of the promoter polymorphism rs2735940 (-1381C<T), were found to be associated with a decreased CRC risk compared to the wild-type, even though this effect was statistically not significant. The frequency distribution of the -1381T/T genotype in the control group (23.44%) was similar to that reported for Caucasian control subjects (21%-24.3%) in previous studies (Nordfjäll et al, 2007; Savage et al, 2007). Moreover, in a Japanese study conducted by Matsubara et al, 2006 the T/T genotype distribution among healthy individuals was only 15.2%. The T-sequence was associated with a higher transcriptional activity, lack of age-dependent telomere shortening and telomerase activity. However, due to the small sample size (46 subjects), the obtained results may be a consequence of chance. Nevertheless, findings from Savage et al, 2007 supports the functional role of this promoter SNP, suggesting that variants in TERT could have an effect in individuals already at increased genetic risk of breast cancer, although the number of individuals with a family history of breast cancer was small. Interestingly, there are some ambiguous references regarding the nucleotide numbering of this polymorphism, corresponding to the reference number rs2735940. Matsubara et al, 2006 used the nucleotide numbering according to Horiwaka et al, 1999, though the SNP is located at nucleotide -1327, upstream of the transcription-starting site of TERT. The same nucleotide numbers were used by

Nordfjäll et al, 2007 for analyzing the promoter length in myocardial infarction patients and controls. In contrast, Savage et al, 2007 indicated the polymorphism with the nucleotide numbering -1381C>T, congruent with the information in the SNP500Cancer database.

Referring to the rs2736098 polymorphism in exon 2 our results did not support any statistically significant association with CRC or colonic polyp risk. Nevertheless, carriers of the A/A genotype showed a slightly decreased risk for CRC compared to the wild-type, even though not significant. The genotype frequency of the variant allele (7.08%) in the control group was similar to that reported in a previous breast cancer study in Caucasians (6%) (Savage et al, 2007). Savage et al, 2007 revealed a protective effect of this coding SNP for homozygous carriers of the less common A-allele (OR: 0.57; 95% CI: 0.39-0.84), having a positive family history. Functional significance of this SNP was additionally supported by a GWA study conducted by Rafnar et al, 2009. The rs2736098 polymorphism was associated with a number of serious cancer types, including basal cell carcinoma, lung cancer, bladder cancer and prostate cancer.

Beside the promoter polymorphism (rs2735940) and the coding SNP (rs2736098), three intronic SNPs (rs4975605, rs2736100, rs2075786) were investigated in this molecular epidemiologic study. Malkinson and You, 1994 hypothesized that introns of genes, whose products influence tumor development may affect cancer incidence. Additionally, it was assumed that intronic SNPs could influence splicing (Schork et al, 2000). But so far only a few studies concerning intronic polymorphism within TERT and cancer risk have been published (Savage et al, 2007; McKay et al, 2008; Hosgood 3rd et al, 2009). Recently, the less common allele of rs2736100 was detected as disease marker (OR: 1.29; 95% CI: 1.17-1.43) for lung cancer in a GWA study (McKay et al, 2008). Homozygous carriers of the rs2736100 polymorphism showed a decreased risk for CRC, even though not significant.

None of the investigated polymorphisms in this study was significantly associated with CRC susceptibility. However, the +269T/T genotype of the rs2075786 polymorphism was significantly associated with a 1.44-fold risk of high-risk polyps when compared to the wild-type. The MAF for the rs2075786 polymorphism in our control group (0.361) was somewhat lower than the MAF indicated in the SNP500Cancer database for the

Caucasian subpopulation (0.404), but somewhat higher than in the HapMap Caucasian subpopulation (0.306). The slight differences may be a consequence of the relatively small group size in SNP500 and HapMap, compared to the present study population.

However, the genotype frequency of +269T/T in our control group (13.28%) was in accordance with the frequencies found in other Caucasian populations (HapMap= 14%; SNP500Cancer database= 12.9%). Furthermore, it was similar to that reported for healthy Caucasians (12%) in a previous breast cancer study (Savage et al, 2007). In contrast, the variant genotype frequency was much lower in a Chinese population (6.4%). The homozygous carriers of the polymorphic allele were associated with a decreased lung cancer risk (Hosgood 3rd et al, 2009). Contrary to these findings, Savage et al, 2007 reported no significant interaction between the polymorphism and breast cancer risk.

In general, associations found between the polymorphism and overall cancer risk could be due to chance or be attributed to etiologic and ethnical differences. SNPs that display different associations between populations may be the marker, rather than the functional polymorphisms, because they are in different LD blocks or occur on different haplotypes in different populations (King et al, 2005). In addition, the lung cancer association study conducted by Hosgood 3rd et al, 2009 included a relatively small number of study subjects (110 controls, 120 cases). Thus, further investigations with larger sample sizes are needed to obtain reliable results.

These data reflect the critical issue of a large sample size, important for the sufficient identification of common genetic polymorphisms involved in cancer susceptibility. According to Rothman et al, 2001, the sample size needed to study a particular genotype with an OR of 1.5 and a prevalence of about 10% in the general population would be about 900 cases and controls. Lango and Weedon, 2008 even propose thousands of subjects for a reliable detection of polygenic variants with small effects. Beside an inadequate study design such as a small sample size, ethnically mixed study populations, selection of controls, control for confounding factors and statistical methods can be potential sources of bias. One of the major strengths of this case-control study was the large sample size, although as expected for a screening project, predominantly consisting of patients with colonic polyps and polyp free participants. In spite of screening a certain age group, the mean age of the control group was significantly lower than the mean age in the three risk groups. In addition, carcinoma patients showed a significantly higher

mean age compared to the polyp and control group. The difficulty of selecting an appropriate control group was resolved by a hospital-based control group, involving polyp and cancer free participants, who had undergone colonoscopy. Using a control group which did not undergo colonoscopy could include some undiagnosed participants with colonic polyps or CRC, because in this age group (40-80 years) those conditions are relatively common. Furthermore, to adjust different malignant potential of the colonic polyps diagnosed in this screening project, polyps were classified into a high and a low-risk group, according to their villous architecture. Patients with villous histology are at a greater risk to develop CRC and were therefore assigned to the high-risk group. The St. Marks Hospital Study supported the association of villous histology with an increased risk of malignancy; 4.8% of tubular adenomas, 22.5% of tubulovillous and 40.7% of villous adenomas were malignant (Markowitz and Winawer, 1997). However, implications of this histological classification were a lower sample size of high-risk polyps (n= 333) compared to the low-risk polyp group (n= 1065). Another limitation in the present study design is the relatively small number of carcinoma cases within the study population, which could be a potential explanation for the nonexistent significant associations between TERT SNPs and CRC risk. According to Wacholder et al, 2004 an increased number of cases and control subjects would additionally reduce a false-positive report probability substantially. In some extent, this was resolved by the incorporation of further cases from three hospitals in Vienna. Furthermore, the number of investigated SNPs in this study population was too small to allow a definitive conclusion. As the diploma thesis is part of the project "Molecular Epidemiology of Colorectal Cancer", additional polymorphisms will be sought in an effort to draw definitive conclusions about genetic variations within telomere stability genes as prognostic markers for CRC risk.

With regard to the rs2075786 polymorphism, the significant positive association between homozygous carrier of the T-allele and an increased high risk polyp risk should be cautiously interpreted until these results are replicated, since observed associations with particular SNPs may be due to another SNP in LD (Hosgood 3rd et al, 2009). In addition, according to Lango and Weedon, 2008 statistical significance levels in case-control association studies need to be interpreted with much more caution.

In conclusion, this diploma thesis has shown no statistically significant associations of the five genotyped polymorphisms in TERT and CRC risk. Thus, the role of genetic variation within TERT in the susceptibility to CRC remains unclear. However, a significant association for the homozygous carrier of the intronic SNP rs2075786 and an increased risk of developing high-risk polyps was demonstrated. Due to the fact that patients with high-risk polyps are at greater risk to develop CRC, this polymorphism may be an indirect molecular marker of CRC. But this must be confirmed in further studies.

The risk for a complex and multifactorial non Mendelian disease, like CRC may be modulated not only from a SNP within one gene, but also as a joint effect of multiple polymorphisms within different genes and pathways. Therefore, further investigations in larger studies are needed to combine effects of polymorphisms within telomere biology genes and to develop a polygenic model of CRC risk. Positional cloning and the application of linkage analysis has been extremely useful in the identification of genes responsible for monogenetic traits, but has a limited success for complex human diseases without obvious Mendelian inheritance, with often disappointing and inconsistent results (Altmüller et al, 2001). The method of choice and a promising field in the identification of common variants predisposing to complex diseases are GWA studies, accompanied by new high throughput genotyping technologies, such as microarrays (Lango and Weedon, 2008). Microarrays allow genotyping of a large number of SNPs across the genome simultaneously, raising the possibility that genetics will find a major role at the clinic distinguishing between individuals at low to high risk of cancer (Schork et al, 2000; Lango and Weedon, 2008).

A further research project using the genome wide SNP Array 6.0 will be started, in order to identify additional susceptibility markers for high-risk colonic polyps and CRC.

5. References

Afonina I, Zivarts M, Kutyavin I, Lukhtanov E, Gamper H, Meyer RB. (1997) Efficient priming of PCR with short oligonucleotides conjugated to a minor groove binder. *Nucleic Acids Research* 25: 2657-2660

Altmüller J, Palmer LJ, Fischer G, Scherb H, Wjst M. (2001) Genomewide scans of complex human diseases: true linkage is hard to find. *American Journal of Human Genetics* 69: 936-950

Applied Biosystems (2004) TaqMan® SNP Genotyping Assay Protocol. www.appliedbiosystems.com

Applied Biosystems. (2006) Allelic Discrimination Getting Started Guide. www.appliedbiosystems.com

Barrett JC, Fry B, Maller J, Daly MJ. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265

Beckmann JS, Estivill X, Antonarakis SE. (2007) Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *Nature Reviews Genetics* 8: 639-646

Blackburn EH. (1991) Structure and function of telomeres. *Nature* 350: 569-573

Blackburn EH. (2000) Telomere states and cell fates. *Nature* 408: 53-56

Bond JH. (2000) Polyp guideline: diagnosis, treatment, and surveillance for patients with colorectal polyps. Practice Parameters Committee of the American College of Gastroenterology. *The American Journal of Gastroenterology* 95: 3053-3063

Boyle P, Langman JS. (2000) ABC of colorectal cancer: Epidemiology. *BMJ* 321: 805-808

Boyle P, Leon ME. (2002) Epidemiology of colorectal cancer. *British Medical Bulletin* 64: 1-25

Broderick P, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, Rowan A, Lubbe S, Spain S, Sullivan K, Fielding S, Jaeger E, Vijayakrishnan J, Kemp Z, Gorman M, Chandler I, Papaemmanuil E, Penegar S, Wood W, Sellick G, Qureshi M, Teixeira A, Domingo E, Barclay E, Martin L, Sieber O; CORGI Consortium, Kerr D, Gray R, Peto J, Cazier JB, Tomlinson I, Houlston RS. (2007) A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. *Nature Genetics* 39: 1315-1317

Brümmendorf TH. (2005) Telomerase activity-a prognostic factor in colorectal cancer? *Onkologie* 28: 550-551

Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. (1997) Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nature Medicine* 3: 1271-1274

Callén E, Surrallés J. (2004) Telomere dysfunction in genome instability syndromes. *Mutation Research* 567: 85-104

Cannon-Albright LA, Bishop DT, Samowitz W, Di Sario JA, Lee R, Burt RW. (1994) Colonic polyps in an unselected population: prevalence, characteristics and associations. *American Journal of Gastroenterology* 89: 827-831. Quoted in Ponz de Leon M, Di Gregorio C. (2001) Pathology of colorectal cancer. *Digestive and Liver Disease* 33: 372-388

Chadeneau C, Hay K, Hirte HW, Gallinger S, Bacchetti S. (1995) Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Research* 55: 2533-2536

Chanock S. (2001) Candidate genes and single nucleotide polymorphisms (SNPs) in the study of human disease. *Disease Markers* 17: 89-98. Quoted in Rebbeck TR, Ambrosone CB, Bell DA, Chanock SJ, Hayes RB, Kadlubar FF, Thomas DC. (2004) SNPs, haplotypes, and cancer: applications in molecular epidemiology. *Cancer Epidemiology, Biomarkers & Prevention* 13: 681-687

Cheng J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, Hennekens CH, Kelsey KT, Hunter DJ. (1998) A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. *Cancer Research* 58: 3307-3311

Colditz GA, Cannuscio CC, Frazier AL. (1997) Physical activity and reduced risk of colon cancer: implications for prevention. *Cancer Causes Control* 8: 649-667

Coleman MP, Gatta G, Verdecchia A, Estève J, Sant M, Storm H, Allemani C, Ciccolallo L, Santaquilani M, Berrino F and the EUROCARE working group. (2003) EUROCARE-3 summary: cancer survival in Europe at the end of the 20th century. *Annals of Oncology* 14 Suppl 5: v128-149

Colucci PM, Yale SH, Rall CJ. (2003) Colorectal polyps. *Clinical Medicine & Research* 1: 261-261

Cong YS, Wen J, Bacchetti S. (1999) The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. *Human Molecular Genetics* 8: 137-142

Cong YS, Wright WE, Shay JW. (2002) Human telomerase and its regulation. *Microbiology and Molecular Biology Reviews* 66: 407-425

Curtin K, Lin W, George R, Katory M, Shorto J, Cannon-Albright LA, Bishop DT, Cox A, Camp NJ, Colorectal Cancer Study Group. (2009) Meta association of colorectal cancer confirms risk alleles at 8q24 and 18q21. *Cancer Epidemiology, Biomarkers & Prevention* 18: 616-621

De Jong MM, Nolte IM, te Meerman GJ, van der Graaf WT, de Vries EG, Sijmons RH, Hofstra RMW, Kleibeuker JH. (2002) Low-penetrance genes and their involvement in colorectal cancer susceptibility. *Cancer Epidemiology, Biomarkers & Prevention* 11: 1332-1352

De la Chapelle A. (2004) Genetic predisposition to colorectal cancer. *Nature Reviews* 4: 769-780

Ducrest AL, Szutorisz H, Lingner J, Nabholz M. (2002) Regulation of the human telomerase reverse transcriptase gene. *Oncogene* 21: 541-552

Engelhardt M, Drullinsky P, Guillem J, Moore MA. (1997) Telomerase and telomere length in the development and progression of premalignant lesions to colorectal cancer. *Clinical Cancer Research* 3: 1931-1941

Erichsen HC, Chanock SJ. (2004) SNPs in cancer research and treatment. *British Journal of Cancer* 90: 747-751

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

Feng J, Funk WD, Wang SS, Weinreich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, et al. The RNA component of human telomerase. *Science* 269: 1236-1241. Quoted in Weinreich SL, Pruzan R, Ma L, Quellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. (1997) Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nature Genetics* 17: 498-502

Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. (2007) Estimates of the cancer incidence and mortality in Europe in 2006. *Annals of Oncology* 18: 581-592

Fernández E, La Vecchia C, González JR, Lucchini F, Negri E, Levi F. (2005) Converging patterns of colorectal cancer mortality in Europe. *European Journal of Cancer* 41: 430-437

Flood A, Velie EM, Sinha R, Chaterjee N, Lacey JV, Jr, Schairer C, Schatzkin A. (2003) Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. *American Journal of Epidemiology* 158: 59-68

Flood DM, Weiss NS, Cook LS, Emerson JC, Schwartz SM, Potter JD. (2000) Colorectal cancer incidence in Asian migrants to the United States and their descendants. *Cancer Causes and Control* 11: 403-411

Gatta C, Capocaccia R, Sant M, Bell CMJ, Coebergh JWW, Damhuis RAM, Faivre J, Martinez-Garcia C, Pawlega J, Ponz de Leon, M, Pottier D, Raverdy N, Williams EMI, Berrino F. (2000) Understanding variations in survival for colorectal cancer in Europe: a EUROCARE high resolution study. *Gut* 47: 533-538

Gertler R, Rosenberg R, Stricker D, Werner M, Lassmann S, Ulm K, Nekarda H, Siewert JR. (2002) Prognostic potential of the telomerase subunit human telomerase reverse transcriptase in tumour tissue and nontumorous mucosa from patients with colorectal carcinoma. *Cancer* 95: 2103-2111

Gertler R, Rosenberg R, Stricker D, Friedrichs J, Hoos A, Werner M, Ulm K, Holzmann B, Nekarda H, Siewert JR. (2004) Telomere length and human telomerase reverse transcriptase expression as markers for progression and prognosis of colorectal carcinoma. *Journal of Clinical Oncology* 22: 1807-1814

Giovannucci E. (2001) An updated review of the epidemiological evidence that cigarette smoking increases risk of colorectal cancer. *Cancer Epidemiology, Biomarkers & Prevention* 10: 725-731

Gonzalez EC, Roetzheim RG, Ferrante JM, Campbell R. (2001) Predictors of proximal vs. distal colorectal cancers. *Diseases of Colon and Rectum* 44: 251-258

Grady WM. (2003) Genetic testing for high-risk colon cancer patients. *Gastroenterology* 124: 1574-1594

Grady WM. (2004) Genomic instability and colon cancer. *Cancer Metastasis Review* 23: 11-27

Greene FL, Sobin LH. (2008) The staging of cancer: a retrospective and prospective appraisal. *CA: A Cancer Journal for Clinicians* 58: 180-190

Gunter MJ, Canzian F, Landi S, Chanock SJ, Sinha R, Rothman N. (2006) Inflammation-related gene polymorphisms and colorectal adenoma. *Cancer Epidemiology, Biomarkers & Prevention* 15: 1126-1131

Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. (1999a) Creation of human tumour cells with defined genetic elements. *Nature* 400: 464-468

Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JHM, Meyerson M, Weinberg RA. (1999b) Inhibition of telomerase limits the growth of human cancer cells. *Nature Medicine* 5: 1164-1170

Haidinger G, Waldhoer T, Hackl M, Vutuc C. (2006) Survival of patients with colorectal cancer in Austria by sex, age, and stage. *Wiener Medizinische Wochenschrift* 156: 549-551

Haiman CA, Le Marchand L, Yamamoto J, Stram DO, Sheng X, Kolonel LN, Wu AH, Reich D, Henderson BE. (2007) A common genetic risk factor for colorectal and prostate cancer. *Nature Genetics* 39: 954-956

Hébert-Croteau N. (1998) A meta-analysis of hormone replacement therapy and colon cancer in women. *Cancer Epidemiology, Biomarkers & Prevention* 7: 653-659

Hoeijmakers JH. (2001) Genome maintenance mechanisms for preventing cancer. *Nature* 411: 366-374

Horiwaka I, Cable PL, Afshari C, Barrett JC. (1999) Cloning and characterization of the promoter region of human telomerase reverse transcriptase gene. *Cancer Research* 59: 826-830

Hosgood HD 3rd, Cawthon R, He X, Chanock S, Lan Q. (2009) Genetic variation in telomere maintenance genes, telomere length, and lung cancer susceptibility. *Lung Cancer* 12: ahead of print

Houlston RS, Tomlinson IPM. (2001) Polymorphisms and colorectal tumor risk. *Gastroenterology* 121: 282-301

Houlston RS, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, Chandler I, Vijayakrishnan J, Sullivan K, Penegar S; Colorectal Cancer Association Study Consortium, Carvajal-Carmona L, Howarth K, Jaeger E, Spain SL, Walther A, Barclay E, Martin L, Gorman M, Domingo E, Teixeira AS; CoRGI Consortium, Kerr D, Cazier JB, Niittymäki I, Tuupanen S, Karhu A, Aaltonen LA, Tomlinson IP, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Cetnarskyj R, Porteous ME, Pharoah PD, Koessler T, Hampe J, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Chang-Claude J, Hoffmeister M, Brenner H, Zanke BW, Montpetit A, Hudson TJ, Gallinger S; International Colorectal Cancer Genetic Association Consortium, Campbell H, Dunlop MG. (2008) Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. *Nature Genetics* 40: 1426-1435

Huang Y, Han S, Li Y, Mao Y, Xie Y. (2007) Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *Journal of Human Genetics* 52: 73-85

International HapMap Consortium. (2005) A haplotype map of the human genome. *Nature* 437: 1299-1320

Ishikawa T, Ide F, Qin X, Zhang S, Takahashi Y, Sekiguchi M, Tanaka K, Nakatsuru Y. (2001) Importance of DNA repair in carcinogenesis: evidence from transgenic and gene targeting studies. *Mutation Research* 477: 41-49

King CR, Yu J, Freimuth RR, McLeod HL, Marsh S. (2005) Interethnic variability of ERCC2 polymorphisms. *The Pharmacogenomics Journal* 5: 54-59

Kinzler KW, Vogelstein B. (1996) Lesson from hereditary colorectal cancer. *Cell* 87: 159-170

Kotnis A, Sarin R, Mulherkar R. (2005) Genotype, phenotype and cancer: role of low penetrance genes and environment in tumour susceptibility. *Journal of Biosciences* 30: 93-102

Kruglyak L, Nickerson DA. (2001) Variation is the spice of life. *Nature Genetics* 27: 234-236

Kutyavin IV, Lukhtanov EA, Gamper HB, Meyer RB. (1997) Oligonucleotides with conjugated dihydropyrroloindole tripeptides: base composition and backbone effects on hybridization. *Nucleic Acids Research* 25: 3718-3725

Küry S, Buecher B, Robiou-du-Pont S, Scoul C, Colman H, Le Nell T, Le Houérou C, Faroux R, Ollivry J, Lafraise B, Chupin L, Sébille V, Bézieau S. (2008) Low penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer* 8: 326:1-14

Lang NP, Butler MA, Massengill J, Lawson M, Stotts CR, Hauer-Jensen M, Kadlubar FF. (1994) Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiology, Biomarkers & Prevention* 3: 675-682

Lango H, Weedon MN. (2008) What will whole genome searches for susceptibility genes for common complex disease offer to clinical practice? *Journal of Internal Medicine* 263: 16-27

Livak KJ. (1999) Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genetic Analysis: Biomolecular Engineering* 14: 143-149

Malkinson AM, You M. (1994) The intronic structure of cancer-related genes regulates susceptibility to cancer. *Molecular Carcinogenesis* 10: 61-65

Markowitz AJ, Winawer SJ. (1997) Management of colorectal polyps. CA: *A Cancer Journal for Clinicians* 47: 93-112

Mathieu N, Pirzio L, Freulet-Marrière MA, Desmaze C, Sabatier L. (2004) Telomeres and chromosomal instability. *Cellular and Molecular Life Sciences* 61: 641-656

Matsubara Y, Murata M, Yoshida T, Watanabe K, Saito I, Miyaki K, Omae K, Ikeda Y. (2006) Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT. *Biochemical and Biophysical Research Communications* 341: 128-131

McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, McLaughlin J, Shepherd F, Montpetit A, Narod S, Krokan HE, Skorpen F, Elvestad MB, Vatten L, Njølstad I, Axelsson T, Chen C, Goodman G, Barnett M, Loomis MM, Lubiński J, Matyjasik J, Lener M, Oszutowska D, Field J, Liloglou T, Xinarianos G, Cassidy A; EPIC Study, Vineis P, Clavel-Chapelon F, Palli D, Tumino R, Krogh V, Panico S, González CA, Ramón Quirós J, Martínez C, Navarro C, Ardanaz E, Larrañaga N, Kham KT, Key T, Bueno-de-Mesquita HB, Peeters PH, Trichopoulou A, Linseisen J, Boeing H, Hallmans G, Overvad K, Tjønneland A, Kumle M, Riboli E, Zelenika D, Boland A, Delepine M, Foglio M, Lechner D, Matsuda F, Blanche H, Gut I, Heath S, Lathrop M, Brennan P. (2008) Lung cancer susceptibility locus at 5p15.33. *Nature Genetics* 40: 1404-1406

Meyerson M. (2000) Role of telomerase in normal and cancer cells. *Journal of Clinical Oncology* 18: 2626-2634

Midgley R, Kerr D. (1999) Colorectal cancer. *The Lancet* 353: 391-399

Muto T, Bussey HJR, Morson BC. (1975) The evolution of cancer of the colon and rectum. *Cancer* 36: 2251-2270. Quoted in Markowitz AJ, Winawer SJ. (1997) Management of colorectal polyps. *CA: A Cancer Journal for Clinicians* 47: 93-112

Naccarati A, Pardini B, Hemminki K, Vodicka P. (2007) Sporadic colorectal cancer and individual susceptibility: A review of the association studies investigating the role of DNA repair genetic polymorphisms. *Mutation Research* 635: 118-145

Nordfjäll K, Osterman P, Melander O, Nilsson P, Roos G. (2007) hTERT (-1327)T/C polymorphism is not associated with age-related telomere attrition in peripheral blood. *Biochemical and Biophysical Research Communications* 358: 215-218

O'Brien MJ, Winawer SJ, Zauber AG, Gottlieb LS, Sternberg SS, Diaz B, Dickersin GR, Ewing S, Geller S, Kasimian D et al. (1990) The National Polyp Study: Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. *Gastroenterology* 98: 371-379. Quoted in Markowitz AJ, Winawer SJ. (1997) Management of colorectal polyps. *CA: A Cancer Journal for Clinicians* 47: 93-112

Parkin DM. (2004) International variation. *Oncogene* 23: 6329-6340

Peltomäki P, de la Chapelle A. (1997) Mutations predisposing to hereditary nonpolyposis colorectal cancer. *Advances in Cancer Research* 71: 93-119. Quoted in Potter JD. (1999) Colorectal cancer: molecules and populations. *Journal of the National Cancer Institute* 91: 916-932

Perera FP. (1997) Environment and cancer: who are susceptible? *Science* 278: 1068-1073

Perera FP, Weinstein IB. (1982) Molecular epidemiology and carcinogen DNA adduct detection: new approaches to studies of human cancer causation. *Journal of Chronic Diseases* 35: 581-600
Perera FP, Weinstein IB. (2000) Molecular epidemiology: recent advances and future directions. *Carcinogenesis* 21: 517-524

Ponz de Leon M, Di Gregorio C. (2001) Pathology of colorectal cancer. *Digestive and Liver Disease* 33: 372-388

Potter JD. (1999) Colorectal cancer: molecules and populations. *Journal of the National Cancer Institute* 91: 916-932

Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW. (1992) APC mutations occur early during colorectal tumorigenesis. *Nature* 359: 235-237

Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, Jakobsdottir M, Helgadottir H, Thorlacius S, Aben KK, Blöndal T, Thorgeirsson TE, Thorleifsson G, Kristjansson K, Thorisdottir K, Ragnarsson R, Sigurgeirsson B, Skuladottir H, Gudbjartsson T, Isaksson HJ, Einarsson GV, Benediktsdottir KR, Agnarsson BA, Olafsson K, Salvarsdottir A, Bjarnason H, Asgeirsdottir M, Kristinsson KT, Matthiasdottir S, Sveinsdottir SG, Polidoro S, Höiom V, Botella-Estrada R, Hemminki K, Rudnai P, Bishop DT, Campagna M, Kellen E, Zeegers MP, de Verdier P, Ferrer A, Isla D, Vidal MJ, Andres R, Saez B, Juberias P, Banzo J, Navarrete S, Tres A, Kan D, Lindblom A, Gurzau E, Koppova K, de Vegt F, Schalken JA, van der Heijden HF, Smit HJ, Termeer RA, Oosterwijk E, van Hooij O, Nagore E, Porru S, Steineck G, Hansson J, Buntinx F, Catalona WJ, Matullo G, Vineis P, Kiltie AE, Mayordomo JI, Kumar R, Kiemeney LA, Frigge ML, Jonsson T, Saemundsson H, Barkardottir RB, Jonsson E, Jonsson S, Olafsson JH, Gulcher JR, Masson G, Gudbjartsson DF, Kong A, Thorsteinsdottir U, Stefansson K. (2009) Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nature Genetics* 41: 221-227

Rebbeck TR, Ambrosone CB, Bell DA, Chanock SJ, Hayes RB, Kadlubar FF, Thomas DC. (2004) SNPs, haplotypes, and cancer: applications in molecular epidemiology. *Cancer Epidemiology, Biomarkers & Prevention* 13: 681-687

Rosenberg L, Louik C, Shapiro S. (1998) Nonsteroidal antiinflammatory drug use and reduced risk of large bowel carcinoma. *Cancer* 82: 2326-2333

Rothman N, Wacholder S, Caporaso NE, Garcia-Closas M, Buetow K, Fraumeni JF Jr. (2001) The use of common genetic polymorphisms to enhance the epidemiologic study of environmental carcinogens. *Biochimica et Biophysica Acta* 1471: C1-C10

Salisbury BA, Pungliya M, Choi JY, Jiang R, Sun XJ, Stephens JC. (2003) SNP and haplotype variation in the human genome. *Mutation Research* 526: 53-61

Savage SA, Stewart BJ, Eckert A, Kiley M, Liao JS, Chanock SJ. (2005) Genetic variation, nucleotide diversity, and linkage disequilibrium in seven telomere stability genes suggest that these genes may be under constraint. *Human Mutation* 26: 343-350

Savage SA, Chanock SJ, Lissowska J, Brinton LA, Richesson D, Peplonska B, Bardin-Mikolajczak, Zatonski W, Szeszenia-Dabrowska N, Garcia-Closas M. (2007) Genetic variation in five genes important in telomere biology and risk for breast cancer. *British Journal of Cancer* 97: 832-836

Scheet P, Stephens M. (2006) A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *American Journal of Human Genetics* 78: 629-644

Schork NJ, Fallin D, Lanchbury S. (2000) Single nucleotide polymorphisms and the future of genetic epidemiology. *Clinical Genetics* 58: 250-264

Shay JW, Bacchetti S. (1997) A survey of telomerase activity in human cancer. *European Journal of Cancer* 33: 787-791

Smith AJ, Stern HS, Penner M, Hay K, Mitri A, Bapat BV, Gallinger S. (1994) Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. *Cancer Research* 54: 5527-5530

Smith D, Ballal M, Hodder R, Selvachandran SN, Cade D. (2006) The adenoma carcinoma sequence: an indoctrinated model for tumorigenesis, but it is always clinical reality. *Colorectal Disease* 8: 296-301

Statistik Austria a. Österreichisches Krebsregister (Stand: 24.02.2009). http://www.statistik.at/web_de/statistiken/gesundheit/krebserkrankungen/dickdarm_enddarm/index.html (letzte Änderung: 17.03.2009)

Statistik Austria b. Österreichische Todesursachenstatistik (Erstellt am 13.03.2009). http://www.statistik.at/web_de/statistiken/gesundheit/krebserkrankungen/dickdarm_enddarm/index.html (letzte Änderung: 17.03.2009)

Steinmetz KA, Potter JD. (1991) Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 2: 427-442. Quoted in Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, Wolk A. (2001) Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *Journal of the National Cancer Institute* 93: 525-533

Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H, Niitsu Y. (1998) Aberrant crypt foci of the colon as precursors of adenoma and cancer. *The New England Journal of Medicine* 339: 1277-1284

Tatsumoto N, Hiyama E, Murakami Y, Imamura Y, Shay JW, Matsuura Y, Yokoyama T. (2000) High telomerase activity is an independent prognostic indicator of poor outcome in colorectal cancer. *Clinical Cancer Research* 6: 2696-2701

Taylor I, Garcia-Aguilar J, Goldberg SM. (1999) *Fast Facts Colorectal Cancer*. Oxford: Health Press

Tenesa A, Farrington SM, Prendergast JG, Porteous ME, Walker M, Haq N, Barnetson RA, Theodoratou E, Cetnarskyj R, Cartwright N, Semple C, Clark AJ, Reid FJ, Smith LA, Kavoussanakis K, Koessler T, Pharoah PD, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Schmidt CO, Hampe J, Chang-Claude J, Hoffmeister M, Brenner H, Wilkening S, Canzian F, Capella G, Moreno V, Deary IJ, Starr JM, Tomlinson IP, Kemp Z, Howarth K, Carvajal-Carmona L, Webb E, Broderick P, Vijayakrishnan J, Houlston RS, Rennert G, Ballinger D, Rozek L, Gruber SB, Matsuda K, Kidokoro T, Nakamura Y, Zanke BW, Greenwood CM, Rangrej J, Kustra R, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. (2008) Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. *Nature Genetics* 40: 631-637

Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, Wolk A. (2001) Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *Journal of the National Cancer Institute* 93: 525-533

Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, Penegar S, Chandler I, Gorman M, Wood W, Barclay E, Lubbe S, Martin L, Sellick G, Jaeger E, Hubner R, Wild R, Rowan A, Fielding S, Howarth K; CORGI Consortium, Silver A, Atkin W, Muir K, Logan R, Kerr D, Johnstone E, Sieber O, Gray R, Thomas H, Peto J, Cazier JB, Houlston R. (2007) A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. *Nature Genetics* 39: 984-988

Ulrich CM, Bigler J, Potter JD. (2006) Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nature Reviews Cancer* 6: 130-140

Villa E. (2000) Molecular screening for colon cancer detection. *Digestive and Liver Disease* 32: 173-177

Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. (2004) Assessing the probability that a positive report is false: an approach for a molecular epidemiology studies. *Journal of the National Cancer Institute* 96: 434-442

Weinreich SL, Pruzan R, Ma L, Quellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. (1997) Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nature Genetics* 17: 498-502

Willett WC (1989) The search for the causes of breast cancer and colon cancer. *Nature* 338: 389-394

Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE (1990) Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *New England Journal of Medicine* 323: 1664-1672. Quoted in Boyle P, Langman JS. (2000) ABC of colorectal cancer: Epidemiology. *BMJ* 321: 805-808

Winawer SJ, Zauber AG, Fletcher RH, Stillman JS, O´Brien MJ, Levin B, Smith RA, Liebermann DA, Burt RW, Levin TR, Bond JH, Brooks D, Byers T, Hyman N, Kirk L, Thorson A, Simmang C, Johnson D, Rex DK. (2006) Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society. *CA: A Cancer Journal for Clinicians* 56: 143-159

Worthley DL, Whitehall VL, Spring KJ, Leggett BA. (2007) Colorectal carcinogenesis: road maps to cancer. *World Journal of Gastroenterology* 13: 3784-3791

Yamada T. (1998) *Handbook of Gastroenterology*. Ed. DH Alpers, WL Hasler, C Owyang, DW Powell, FE Silverstein, WM Tierney, PG Traber. Philadelphia: Lippincott-Raven Publishers

Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, Lansdorp PM, Young NS. (2005) Mutation in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *The New England Journal of Medicine* 352: 1413-1424

Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, Prendergast J, Olschwang S, Chiang T, Crowdy E, Ferretti V, Laflamme P, Sundararajan S, Roumy S, Olivier JF, Robidoux F, Sladek R, Montpetit A, Campbell P, Bezieau S, O'Shea AM, Zogopoulos G, Cotterchio M, Newcomb P, McLaughlin J, Younghusband B, Green R, Green J, Porteous ME, Campbell H, Blanche H, Sahbatou M, Tubacher E, Bonaiti-Pellié C, Buecher B, Riboli E, Kury S, Chanock SJ, Potter J, Thomas G, Gallinger S, Hudson TJ, Dunlop MG. (2007) Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. *Nature Genetics* 39: 989-999

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