Molecular aspects of HNPCC and identification of mutation carriers
Niessen, Renée Cecil

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Chapter 9

Summary
Hereditary nonpolyposis colorectal cancer (HNPCC) - also referred to as Lynch syndrome - is an autosomal dominantly inherited disorder of cancer susceptibility with high penetrance (80-85%). It is one of the most common inherited cancer syndromes identified in humans so far. In typical HNPCC families, individuals from multiple generations are affected with early-onset colorectal cancer (CRC) or other extracolonic cancers, such as endometrial or ovarian cancer. HNPCC is caused by germline mutations in DNA mismatch repair (MMR) genes (first hit), followed by somatic inactivation of the wild-type allele (second hit). When both alleles of a MMR gene are inactivated, the DNA mismatch repair (MMR) system - which maintains genomic stability during DNA-replication – loses its mismatch repair capacity. As a consequence, mutations accumulate throughout the genome. Microsatellite sequences – or short tandem repeats - are typically vulnerable for replication errors, and MMR deficiency results therefore in microsatellite instability (MSI). Spontaneous mutations not only occur in non-coding sequences, but also in genes essential for genome stability, and this results in a state of hyper-mutagenesis in the affected cells. It is this “mutator phenotype” which drives selection and evolution of cells to those with unlimited growth potential.

Mutations in the MMR genes MLH1, MSH2 and MSH6 account for 42-75% of families who are clinically diagnosed with HNPCC. As mutations in the MMR genes are the cause of - or better - initiate the development of cancer in HNPCC families, HNPCC should in fact not be defined by clinical, but rather by genetic criteria, namely the presence of a germline MMR gene mutation.

In this thesis, as introduced in chapter 1, several subjects regarding HNPCC were addressed. One of the questions we wanted to answer was: who should be screened for mutations in the MMR genes? In other words, how should HNPCC suspected patients be selected for mutation-analysis? Identification of carriers of mutations in MMR genes is important, as they are eligible for surveillance programs to detect neoplasms at the earliest possible stages. It has been shown that periodic colonoscopy reduces the risk of CRC and decreases overall mortality. However, mutation analysis is presently costly and labour-intensive and because of this, mutation analysis in the MMR genes can not be offered to all patients with CRC or endometrial cancer. Therefore, there is a need for sensitive and specific criteria to select patients for mutation analysis. Clinical as well as non-clinical criteria can be used for this selection. In chapter 2, various approaches to select HNPCC suspected patients for mutation analysis are reviewed with respect to sensitivity, specificity and predictive values for the presence of pathogenic MLH1, MSH2 or MSH6 mutations. Criteria to determine the pathogenicity of unclassified variants are discussed, as well as differences in design of
functional assays to assess pathogenicity. In Chapter 3, 281 HNPCC suspected patients with either CRC before the age of 50, or CRC and another HNPCC associated tumour, were investigated for germline mutations in MLH1, MSH2 and MSH6. For these patients, the value of family history, microsatellite instability analysis, and MMR protein staining in the tumour (immunohistochemistry; IHC), to predict presence of a MMR gene mutation in patients, was determined. In total, twenty-five pathogenic mutations (8 MLH1, 9 MSH2, 8 MSH6) were found. The prevalence of pathogenic germline MMR gene mutations in the patients with CRC before the age of 50 was 6% and in the group of patients with two or more HNPCC associated tumours 22%. Sensitivity of family history, MSI analysis and IHC for the presence of a mutation was 76, 82 and 88%, specificity was 64, 70 and 84% and positive predictive value 19, 23 and 38%, respectively. MSI analysis missed three, and IHC missed two (other) mutation carriers. In the group of patients with multiple HNPCC associated tumours, no mutation carriers were found among the 29 patients who were diagnosed with their first tumour above the age of 60. Our data suggested that preselection by means of IHC and/or MSI-analysis before mutation analysis is justified for patients with CRC before the age of 50, and patients with CRC and another HNPCC associated tumour.

In Chapter 4, we investigated the possible use of the BRAF-Val600Glu mutation in tumours as a selection tool for mutation analysis in the MMR genes, as it was recently shown, that the oncogenic activation of BRAF - a member of the RAS/RAF family of kinases - by the Val600Glu mutation is characteristic for sporadic colon tumours with microsatellite instability. Previously, it had been shown that somatic BRAF mutations are absent in tumours from HNPCC families with germline mutations in the MMR genes MLH1 and MSH2. However, there were no data for MSH6 mutation carriers available. Therefore, we screened exon 15 of BRAF in 38 tumours from HNPCC families, either with a germline mutation in MSH6, or in whom no MMR gene mutation had been detected. Thirty-seven tumours did not harbour the Val600Glu mutation, one tumour of an MSH6 mutation carrier with multiple tumours did. This finding was likely caused by the fact that the MLH1 promoter was hypermethylated in that tumour. These data, in combination with the BRAF mutation data in MLH1 and MSH2 mutation carriers, suggested a potential use of the BRAF-Val600Glu mutation in tumours as an exclusion criterion for HNPCC mutation analysis or as a molecular marker of sporadic cancer.

Another issue we have tried to address in this thesis is, whether mutations in other genes, or combinations of mutations in MMR genes, can lead to an HNPCC phenotype. As mentioned
above, mutations in the MMR genes MLH1, MSH2 and MSH6 account for only 42-75% of families who are clinically diagnosed with HNPCC. Mutations in other genes may have caused part of the unexplained HNPCC cases. Recently, it was suggested that germline mutations in another mismatch repair gene, PMS2, do play a far more important role in HNPCC than initially thought. To explore this further, we determined in Chapter 5 the prevalence of pathogenic germline mutations in PMS2 in 73 HNPCC suspected patients. These patients had an MSI-high colorectal or endometrial tumour and/or were from an Amsterdam Criteria II positive family. Previously, they had been tested for germline mutations in MLH1, MSH2 and MSH6 with a negative outcome. Amongst the 73 HNPCC suspected patients, we identified three patients with a pathogenic PMS2 mutation (4.1%). IHC of PMS2 was informative in only one of the mutation carriers and in this case the tumour showed loss of PMS2 expression. IHC of MLH1 was negative in a tumour of one of the other two PMS2 mutation carriers. Our data confirm the finding of previous studies that PMS2 is more frequently involved in HNPCC than originally expected. We suggest that IHC for PMS2, and mutation analysis in PMS2 should be added to the screening protocols currently used to identify patients with MMR gene mutations.

All studies on HNPCC so far, have considered HNPCC as a clear Mendelian disease, one mutation causing the syndrome in an autosomal dominant way with incomplete penetrance. Non-Mendelian inheritance, especially in the group of patients we have been focusing on, can however not be excluded. It is conceivable that certain mutations are harmless on its own, but deleterious in combination with mutations in other genes. Thus, combinations of low penetrant mutations in different genes may cause a phenotype that resembles HNPCC. In Chapter 6, we investigated the hypothesis that the combination of a monoallelic MUTYH mutation with an MMR gene mutation increases cancer risk. The MUTYH protein is a base excision repair DNA glycosylase, which is involved in repair of oxidative DNA damage. This protein interacts with the MMR protein MSH6 which is part of the MSH2/MSH6 heterodimer (hMutSα). It was shown that hMutSα stimulates the DNA binding and glycosylase activities of MUTYH. To test our hypothesis, we investigated the prevalence of monoallelic MUTYH mutations in carriers of a germline MMR gene mutation: 40 carriers of a truncating mutation (group I) and 36 of a missense mutation (group II). These patients had been diagnosed with either colorectal or endometrial cancer. We compared their MUTYH mutation frequencies with those observed in a group of 134 Dutch colorectal and endometrial cancer patients without an MMR gene mutation (0.7%) and those reported for Caucasian controls (1.5%). In group I one monoallelic MUTYH mutation was found (2.5%). In group II five monoallelic
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Germline MUTYH mutations were found (14%), four of them in MSH6 missense mutation carriers (20%). Of all patients with an MMR gene mutation, only those with a missense mutation showed a significantly higher frequency of (mono-allelic) MUTYH mutations than the Dutch cancer patients without MMR gene mutations (p = 0.002) and the published controls (p = 0.001). Our results suggest that the presence of a monoallelic MUTYH mutation combined with a missense MMR gene mutation, of MSH6 in particular, leads to an increased cancer risk.

In addition to mutations in other genes, or combinations of mutations in genes, epigenetic events may be the cause of a small proportion of HNPCC cases. Recently, eight CRC patients were reported with generalised (germline) hypermethylation of the MLH1 promoter. In chapter 7, we investigated the prevalence of MLH1 promoter hypermethylation in lymphocytes in 40 HNPCC suspected patients without a germline mutation in MLH1, MSH2 or MSH6. Twenty-five of these patients had a tumour in which IHC had shown loss of the MLH1 protein; 15 patients had not been investigated with IHC, but had an MSI-high tumour. In one patient we found hypermethylation of the MLH1 promoter region in lymphocytes. Analysis of skin fibroblasts, a mouth wash and tumour material of the same patient showed methylation as well. The family history for HNPCC associated tumours was negative. The tumour showed MSI, loss of MLH1 staining and loss of heterozygosity. Further DNA analysis of the siblings, the mother, and a tumour of the deceased father showed no MLH1 promoter hypermethylation. In the tumour of the index patient a mutation in codon 12 of KRAS and methylation of two tumour suppressor genes (RARB and PTEN) was detected, suggesting that the tumour followed a similar pattern of development as sporadic tumours with a CpG island methylator phenotype. Our data showed that MLH1 promoter hypermethylation accounts for a small percentage of HNPCC suspected patients. Therefore, in patients with MLH1 hypermethylation in the tumour and a clinical suspicion of HNPCC, methylation analysis in lymphocytes should be considered.

In Chapter 8, the issues addressed in this thesis are discussed, and a strategy is presented for the selection of HNPCC suspected patients for mutation analysis. In this strategy, presence of the BRAF-Val600Glu mutation in the tumour, IHC and MSI are the most important selection tools. This strategy takes into account that a proportion of missense mutations may turn out to be pathogenic in functional assays. The design of such functional assays is one of the major challenges in future HNPCC research.