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

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Chapter 1

Introduction
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Hereditary nonpolyposis colorectal cancer

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. In typical HNPCC families, individuals from multiple generations are affected with early-onset colorectal cancer or extracolonic cancers, such as endometrial cancer.1

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).2,3 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. Mutations in the MMR genes MLH1, MSH2 and MSH6 account for 42-75% of families who are clinically diagnosed with HNPCC.4-6 As mutations in the MMR genes are the cause of - or better - initiate the development of HNPCC associated tumours, HNPCC is not only defined by clinical, but also by genetic criteria, namely the presence of a germline MMR gene mutation.

The mismatch repair system

The MMR system repairs replication errors that are not immediately corrected by DNA polymerases. It corrects not only single base-pair mismatches, but also small mispaired loops of DNA that result from replication errors of microsatellite tracts.7 Many MMR proteins are involved in mismatch repair, and different combinations of these proteins recognise different types of replication errors.

Together, the MSH2 and MSH6 proteins form the MutSα complex, a heterodimer which recognises single base substitutions and small insertion/deletion-loops.8 After binding to mismatched DNA, this complex recruits MutLα, a heterodimer of MLH1 and PMS2.7,9 The MutSα/MutLα complex is thought to move along the DNA until it encounters a strand break, where it can load exonuclease I, which is required for the degradation of the mismatch-containing strand, after which a polymerase can synthesise a new DNA strand.9,10 The MutSβ complex – a heterodimer of the MSH2 and MSH3 proteins – recognises larger insertion/deletion loops.11 This complex recruits MutLα or possibly a heterodimer of either MLH1 and PMS1, or MLH1 and MLH3. However, the participation of the latter two heterodimers in eukaryotic mismatch repair is not entirely clear.12,13

When the MMR system is deficient, replication errors in the genome are not repaired, and as a consequence, mutations accumulate throughout the genome. Microsatellite sequences – or
short tandem repeats – are particularly vulnerable for replication errors, and MMR deficiency results therefore in microsatellite instability.\textsuperscript{14,15} Replication errors not only occur in non-coding sequences, but also in genes essential for genome stability (e.g. DNA polymerases, DNA repair enzymes, DNA damage checkpoint control and chromosome segregation genes), and this results in a state of hyper-mutagenesis in the affected cells. The progenitor cancer cells express a so-called “mutator phenotype” which drives selection and evolution of cells to those with a strong growth potential.\textsuperscript{16,17} There is also some evidence that deficiency of MMR genes can lead to a disturbed signalling in apoptotic response after certain types of DNA damage, hereby contributing to tumour development.\textsuperscript{18}

Identifying patients with MMR gene mutations

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 colorectal cancer (CRC) and decreases overall mortality.\textsuperscript{19,20} 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 tumours from the HNPCC spectrum. 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 1990, the International Collaborative Group on HNPCC (ICG-HNPCC) formulated the Amsterdam Criteria to define HNPCC clinically in order provide a basis for uniformity in collaborative studies.\textsuperscript{21} These criteria were published in a revised form in 1999 (Amsterdam Criteria II).\textsuperscript{22} Several studies have shown that the Amsterdam criteria I and II are to stringent, when (inappropriately) used as selection criterion for mutation analysis in the MMR genes. A significant proportion of families carrying MMR germline mutations does not fulfil these criteria, and these families will be missed and excluded from cancer surveillance programs when using these criteria for selection for mutation analysis.\textsuperscript{23-28}

Presence of microsatellite instability (MSI) in tumour DNA has been put forward as a selection criterion for subsequent mutation analysis. This is based on the finding that the large majority of CRCs from patients with a \textit{MSH2} or \textit{MLH1} mutation exhibit this feature.\textsuperscript{14,15,29,30} In 1996 the Bethesda guidelines were formulated for the identification of tumours that should be tested for MSI in order to select patients for subsequent MMR gene mutation analysis.\textsuperscript{31} To ensure uniformity, a reference panel of five sensitive microsatellite markers was developed and validated to perform MSI analysis in CRC.\textsuperscript{32}
Several authors have suggested the use of immunohistochemistry (IHC) as a prescreening method for mutation analysis of the MMR genes.\textsuperscript{33-35} In 1996 Leach et al. and Thibodeau et al. first reported the use of monoclonal antibodies directed against MSH2 and MLH1 in the immunohistochemical analysis of CRCs.\textsuperscript{36,37} Studies in CRCs and endometrial cancers have shown the predictive value of MSH2, MLH1 and MSH6 protein staining. Significant correlations were found between the absence of staining of these proteins in tumours and the presence of a truncating germline mutation in the corresponding genes.\textsuperscript{33,36-41} In addition to these methods, which are useful for the identification of HNPCC patients, one might also identify patients with sporadic tumours. Several (epi)genetic changes have been identified that can distinguish between HNPCC CRCs and sporadic CRCs. For example, sporadic colorectal tumours often show somatic hypermethylation of the \textit{MLH1} promoter.\textsuperscript{42-45} These tumours also frequently harbour the \textit{BRAF}-Val600Glu mutation, which cause oncogenetic activation of the RAS/RAF pathway. Therefore, presence of this mutation in a tumour might be used as an exclusion criterion in the selection of patients for mutation analysis in the MMR genes.

**Candidate genes and epigenetic causes of HNPCC**

As mentioned earlier, mutations in \textit{MLH1}, \textit{MSH2} and \textit{MSH6} account for only 42-75\% of families which fulfil the Amsterdam II Criteria.\textsuperscript{44} These percentages are based on mutations that definitely give a loss of function of the encoded protein. Mutations giving rise to a single amino-acid change, are often difficult to classify in terms of pathogenicity. If the pathogenicity of a mutation is not yet clear, it is often referred to as an unclassified variant (UV). In families suspected of HNPCC, which do not fulfil all the Amsterdam criteria, the proportion of detected pathogenic MMR gene mutations is even much lower. There are several explanations conceivable for the unexplained HNPCC (suspected) cases. First, part of the UVs in \textit{MLH1}, \textit{MSH2} and \textit{MSH6} may prove to be pathogenic as determined in functional assays, which test the various properties of mutated MMR proteins, such as binding capacity and MMR proficiency. Second, mutations in genes other than \textit{MLH1}, \textit{MSH2} and \textit{MSH6} may be responsible for a (larger) proportion of the unexplained HNPCC suspected cases. For example, in HNPCC suspected families, germline mutations have been reported in \textit{PMS1}, \textit{PMS2}, \textit{EXO1}, and \textit{MLH3}.\textsuperscript{46-50} Third, one should also take into account the possibility that combinations of low penetrant mutations in different genes may be the cause of the disease. Such combinations have been reported, although proof that the combinations indeed caused cancer development in the reported patients is limited.\textsuperscript{49,51,52} Finally, epigenetic events may also be responsible for a small fraction of the HNPCC suspected cases. For example,
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hypermethylation of the MLH1 promoter in lymphocytes – suggesting a germline defect - was reported in eight patients with CRC.53-56

To summarise the above, several issues regarding the genetic basis of HNPCC need to be addressed. One question to be answered is, who should be screened for mutations in the known HNPCC genes, MLH1, MSH2, and MSH6? In other words, how should patients with HNPCC-associated tumours be selected for mutation-analysis? Another subject is the identification of other mutation types or genes that may lead to an HNPCC phenotype. These two issues will be addressed in the next chapters of this thesis.

OUTLINE OF THE THESIS

In Chapter 2, various approaches to select HNPCC suspected patients for mutation analysis are reviewed in relation 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, a group of HNPCC suspected patients with either CRC before the age of 50 or with multiple HNPCC associated tumours is studied. For these patients the value of family history, microsatellite instability analysis, and MMR protein staining in the tumour, to predict presence of a MMR gene mutation in patients, is determined. In Chapter 4, the possibility of using the presence of the BRAF Val600Glu mutation as a marker for sporadic CRC, and thus as exclusion criterion for mutation analysis in the MMR genes, is investigated. In Chapter 5, the frequency of germline mutations in PMS2 is studied in HNPCC suspected patients without a mutation of MLH1, MSH2 or MSH6. These patients had a microsatellite instable tumour, and/or were from an Amsterdam Criteria II positive family. In Chapter 6, the hypothesis that the combination of a monoallelic MUTYH mutation with an MMR gene mutation increases cancer risk, is tested. The prevalences of monoallelic MUTYH mutations in carriers of germline truncating and missense MMR mutations are determined, and compared with the prevalence of monoallelic MUTYH mutations in a group of colorectal and endometrial cancer patients without an MMR gene mutation. In Chapter 7, the prevalence of MLH1 promoter hypermethylation in lymphocytes in a series of HNPCC suspected patients is investigated and the characteristics of tumours of patients with hypermethylation of the MLH1 promoter in lymphocytes are studied. In Chapter 8 the issues addressed in this thesis are generally discussed, a strategy is presented for the selection of
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HNPCC suspected patients for mutation analysis, and future perspectives are formulated. The results of the different studies reported in this thesis are summarised in Chapter 9.

REFERENCES


