Clinical and molecular aspects of MUTYH- and APC-associated polyposis

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Summary
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This PhD thesis describes several clinical and molecular aspects of adenomatous polyposis syndromes and focuses on *MUTYH*-associated polyposis (MAP).

In **Chapter 1,** an introduction on polyposis syndromes is given. Approximately 5% of CRC cases are assumed to be associated with a highly penetrant dominant or recessive inherited syndrome. Identifying such patients is important because it allows for improved prevention and care for those in the highest category of colorectal cancer (CRC) risk. These hereditary gastro-intestinal tumour syndromes include Lynch syndrome, *APC*-associated polyposis, *MUTYH*-associated polyposis (MAP), hamartomatous and (mixed) hyperplastic polyposis syndromes. Describing the phenotype in these polyposis syndromes is an ongoing project. In **paragraph 2** a review and update on the latest research on *MAP* is given. Most biallelic *MUTYH* patients develop between 10 and 500 polyps, although some have CRC and no or only a few polyps. The CRC risk in MAP patients is about 43% at age of 60 years and the life time risk is assumed to be close to 100%, in the absence of timely surveillance. The lifetime risk for duodenal cancer is 4%. Colonic surveillance should start at age 18-20 years and gastroduodenal screening at age 25-30 years.

In **chapter 2** the clinical phenotype of MAP, including extra-colonic manifestations and geno-phenotype associations is studied. A collaboration between research groups from Leiden, Bonn and Cardiff resulted in the largest MAP patient group (N=276) described until now. Findings show that duodenal adenomas and carcinoma are a common extracolonic manifestation in MAP patients and significant higher risks for ovarian, bladder and skin cancer were found. Furthermore we found apparent geno-phenotype associations. Patients with a homozygous p.G396D mutation present later with MAP and have a significantly lower hazard of developing CRC than patients with a homozygous p.Y179C mutation. The age at CRC diagnosis is 12 years later in homozygous p.G396D patients compared with homozygous p.Y179C patients.

In **paragraph 5** it is shown that using well described criteria for AFAP, constitutional *MUTYH* and *APC* defects can be found in 18 of 25 (72%) of patients with an attenuated form of FAP.

In **chapter 3** the possible CRC risk in *MUTYH* heterozygotes is studied using a family based approach. We identified a twofold increased CRC risk for *MUTYH* heterozygote family members (standard incidence ratio (SIR) 2.1), which is higher than that reported in population based studies (odds ratios between 1.11 and 1.27). The possible increased risk and age at presentation in *MUTYH* CRC heterozygotes does not justify further surveillance other than population based screening.

Carriership in the general population of heterozygous *MUTYH* mutations is estimated at 1:50. When, by chance, the partner of a MAP patient happens to be a *MUTYH* heterozygote, MAP can be inherited in next generation. We show (**paragraph 2)** that the costs per quality adjusted life year (QALY) of genetic screening in families of MAP patients are acceptable and should be discussed with and offered to MAP families in clinical genetic practice.

Chapter 4 (paragraph 1, 2 and 3) describes the histology and molecular aspects of *MUTYH*-associated carcinomas. The molecular and histopathology aspects of 42 MAP CRCs are analysed and compared with sporadic and MSI-high and Lynch CRCs. Also the genetic instability in 26 MAP CRCs is studied using SNP arrays. Results show that CRCs are often right-sided and multiple at presentation and a mucinous histotype and tumour infiltrating lymphocytes (TILs) are common. Since these features are also a hallmark in tumours from LYNCH patients, both MAP and LYNCH syndrome should be considered in a tumour with these features.

Microsatellite instability was not present in the CRCs studied by us; others found instability in a minority of MAP CRCs. A high prevalence of copy neutral loss of heterozygosity (cnLOH) detected in MAP carcinomas suggests a relationship between mitotic recombination and base excision repair (BER) deficiency, although further research is necessary. The *KRAS* c.34 G>T mutation is found in 60% of MAP carcinomas. In sporadic CRC cases this mutation is much less prevalent (8% of *KRAS* mutations). We show that using *KRAS* c.34G>T somatic pre-screening in CRC tissue enables the identification of MAP patients.

In **paragraph 4** a survival study in a European cohort of MAP patients is done. A significant better survival in MAP CRC patients is found compared with sporadic CRC cases. This better survival might be explained by more activated immune response in MAP carcinomas compared to sporadic CRCs. Consequently, a strong selective pressure can be expected to favour the outgrowth of tumour cell clones with an immune evasive phenotype. A method for tumour cells to evade the immune system, namely loss of HLA class I molecule expression (involved in presenting mutated peptides to the immune system), is indeed a frequent event in MAP carcinomas as shown by us in **paragraph 5**.

Finally, in **chapter 5, paragraph 1** a HNPCC family in which *MSH6* and *MUTYH* germline mutations co-segregate is studied. One patient carried a mutation in *MSH6* as well as biallelic mutations in *MUTYH*, and had an exceptionally mild phenotype (only few adenomas at age 56). This might indicate that abrogation of both *MSH6* DNA mismatch repair and base excision repair might be mutually exclusive in humans (e.g. loss of both repair systems may lead to apoptosis of early tumour cells).

In a substantial proportion of patients with an attenuated form of polyposis, and to a lesser extent with classic polyposis, the genetic predisposition remains to be elucidated. The possibility of germline mutations in other base excision repair (BER) genes is studied in **paragraph 2.** The conclusion was that other BER genes do not seem to cause CRC or polyposis.In **paragraph 3** *APC* deletions are found in 19 polyposis patients (without detectable point mutations) with multiplex ligation-dependent probe amplification (MLPA). These *APC* deletions represent 8% of all *APC* mutations found at our DNA diagnostic laboratory. We propose that MLPA should be included in routine germline *APC* mutation analysis. Finally, in **paragraph 4** we show that mosaic *APC* mutations represent 4% of all index patients with pathogenic *APC* mutations (10/242). Half of the mosaic *APC* mutation carriers have a mild form of polyposis. *APC* mosaism should be considered especially in apparently *de novo* polyposis patients.

Future research should be aimed at finding yet undiscovered mutations in (high and low) penetrance genes and already identified polyposis/CRC associated genes. These goals can be accomplished by means of genome wide association studies, analysing distinct (molecular) tumor profiles and using exome or whole genome sequencing and will enable detection of causative variants which are not easily detected with SNP-array studies. The variants identified will hopefully also reveal several biological pathways and generate hypotheses for further research. Mutations in low penetrance genes (or genetic modifiers) and environmental factors might furthermore explain part of the variation in phenotype for *MUTYH*- and *APC*-associated polyposis phenotypes.