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

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The research in this thesis was performed at the Center for Human and Clinical Genetics, the department of Pathology, Leiden University Medical Center (LUMC), and the Netherlands Foundation for the detection of Hereditary Tumors, the Netherlands.

The Dutch Digestive Diseases Foundation Grants funded the research described in this thesis (grant MWO 0355). Jan Ivo Foundation 2004.128

Publication of this thesis was financially supported by kind contributions from:

- Nederlandse Vereniging voor Gastroenterologie
- Dutch Digestive Foundation
- MRC Holland
- Polyposis Contactgroep

ISBN: 978-90-9025977-2
Cover photo: T. Luijendijk
Cover design and lay-out: Esther Beekman (www.estherontwerpt.nl/www.ideesther.nl)
Printed by: Ipskamp Groep

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PROEFSCHRIFT

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden

op gezag van de Rector Magnificus Prof.mr. P.F. van der Heijden,

volgens besluit van het College voor Promoties

te verdedigen op donderdag 10 maart 2011

klokke 16.15

door

Maartje Nielsen

Geboren te Amsterdam in 1975

Promotiecommissie

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Aim and outline of this thesis

Aim of the study

There is compelling evidence that colorectal carcinoma (CRC) has a benign requisite precursor, the colorectal polyp (in most cases an adenoma). One to two percent of all CRC patients have a genetic predisposition for multiple polyps, called polyposis. Two main responsible genes have been identified: i.e. *APC* in 1991 and *MUTYH* in 2002. Further elucidation of the clinical spectrum of heritable CRC and polyposis syndromes is relevant for estimating cancer risks, simplifying the diagnostic route and offering reliable tailor made surveillance. This thesis describes several clinical and molecular aspects of *MUTYH*-associated polyposis (MAP). MAP is found in up to a quarter of polyposis patients. In addition we aimed for the detection of less well known mutations in the *APC* gene in patients previously reported *APC* negative.

Outline of the thesis

I Introduction

In **chapter 1** an introduction on polyposis/CRC syndromes and an update on the latest research on *MUTYH*-associated polyposis are given.

- **1.1** General introduction on polyposis syndromes.
- **1.2** Update on *MUTYH*-associated polyposis.

II Clinical phenotype of MAP

In **chapter 2** the clinical phenotype of MAP in biallelic *MUTYH* carriers is presented, including the spectrum of extracolonic manifestations and genotype-phenotype correlations. Furthermore, the prevalence of *MUTYH* and *APC* mutations in well defined attenuated familial adenomatous polyposis (AFAP) families is determined.

- **2.1** Multiplicity in polyp count and extracolonic manifestations in 40 Dutch patients with *MUTYH*-associated polyposis coli (MAP).
- **2.2** Duodenal carcinoma in *MUTYH*-associated polyposis.
- **2.3** Expanded extracolonic tumour spectrum in *MUTYH*-associated polyposis.

- **2.4** Analysis of *MUTYH* genotypes and colorectal phenotypes in patients with *MUTYH*-associated polyposis.
- **2.5** Germline mutations in *APC* and *MUTYH* are responsible for the majority of families with attenuated familial adenomatous polyposis.

III MUTYH heterozygotes

There has been much debate on the possible risk to develop CRC for carriers of a single *MUTYH* mutation. Because of the high prevalence of *MUTYH* heterozygotes in the population (1-2%), an estimation of a possible elevated CRC risk is of clinical importance. Most studies estimating odds ratios are population based case and control studies. Another approach, namely family based, is presented in **chapter 3**. Also, the significant prevalence of *MUTYH* heterozygotes in the population has implications for the offspring of MAP patients. MAP can be transmitted to the next generation when the spouse of a MAP patient happens to be a *MUTYH* heterozygote. In **paragraph 2** a cost effectiveness study is presented determining whether partner screening for *MUTYH* is cost effective.

- **3.1** Increased colorectal cancer incidence in obligate carriers of heterozygous mutations in *MUTYH*.
- **3.2** Cost-utility analysis of genetic screening in families of patients with germline *MUTYH* mutations.

IV Pathology and tumour studies in MAP patients

The underlying genetic defect in MAP syndrome and Lynch syndrome, the most common inheritable CRC syndrome, *both* affect the DNA–repair system. Given the specific histology and molecular hallmarks in Lynch associated CRCs, the pathologist has an important role in identifying Lynch patients. So far, little pathognomonic features have been described for MAP CRCs. In **chapter 4** the molecular and histopathology aspects of MAP CRCs are analyzed and compared with sporadic, MSI-high and Lynch syndrome CRCs (**paragraph 1**). Also the genetic instability involved in the MAP carcinogenesis is studied (**paragraph 2**). Furthermore the feasibility of identifying MAP patients with less florid polyposis by *KRAS2* c.34G>T prescreening followed by *MUTYH* hotspot mutation analysis in formalin-fixed paraffin-embedded tissue (FFPE) is studied (**paragraph 3**).
Specific histological and molecular genetic features in MAP CRCs might influence tumour behaviour and survival. In theory, the disruption of the *MUTYH* function in MAP carcinomas might lead to a surplus of mutated peptides that activate the immune system and would thereby lead to a better survival of MAP than sporadic CRC cases. Because of the activated immune system a strong selective pressure can be expected, favouring

the outgrowth of tumour cell clones with an immune evasive phenotype. Defects in the human leukocyte antigen (HLA) class I expression are a well known mechanism for avoiding cancer cell recognition and thus immune evasion in colorectal cancers bearing mismatch repair (MMR) deficiencies. It was hypothesized that these HLA class 1 defects might also occur in MAP carcinomas. **Paragraph 4 and 5** show the results of a survival study in a European MAP patient's cohort and a study on the HLA class I expression in MAP carcinomas.

- **4.1** Colorectal carcinomas in *MUTYH*-associated polyposis display histopathological similarities to microsatellite unstable carcinomas.
- **4.2** High frequency of copy-neutral LOH in *MUTYH*-associated polyposis carcinomas.
- **4.3** Identification of patients with (atypical) *MUTYH*-associated polyposis by *KRAS2* c.34G>T pre-screening followed by *MUTYH* hotspot analysis in formalin-fixed paraffin-embedded tissue.
- **4.4** Survival of *MUTYH*-Associated Polyposis Patients With Colorectal Cancer and Matched Control Colorectal Cancer Patients.
- **4.5** *MUTYH*-associated polyposis carcinomas frequently lose HLA class I expression-a common event amongst DNA-repair-deficient colorectal cancers.

V Other causes of polyposis

A branch of a Lynch (HNPCC) family in which *MSH6* and *MUTYH* germline mutations co-segregate is studied in **chapter 5, paragraph 1**. This family demonstrates the clinical consequences of different combinations of base excision repair and mismatch repair defects.

In a number of polyposis patients the genetic predisposition remains to be elucidated. In **paragraph 2** it is analysed whether enzymes working together with *MUTYH* in protecting the DNA against oxidative damage (like OGG1, NUDT1, NTH1, NEIL1, 2 and 3) represent candidate polyposis genes. In **paragraph 3** *Multiplex Ligation-dependent Probe Amplification* (MLPA) is used to detect previously unnoticed deletions in the *APC* gene and in **paragraph 4** the prevalence of somatic mosaism of *APC* gene mutations is studied. We shown that *APC* gene deletions (paragraph 2) and mosaic *APC* mutations (**paragraph 3)** explain a tangible proportion of the so far unaccounted for polyposis patients.

- **5.1** The natural history of a combined defect in *MSH6* and *MUTYH* in a Dutch HNPCC family.
- **5.2** Inherited predisposition to colorectal adenomas caused by multiple rare alleles of *MUTYH* but not *OGG1, NUDT1, NTH1* or *NEIL 1, 2* or *3*.

- **5.3** *APC* Genotype-phenotype correlations in 19 Dutch cases with *APC* gene deletions and a literature review.
- **5.4** Somatic *APC* Mosaicism: An Underestimated Cause of Polyposis Coli.